Efficacy and Safety Assessment of Nasal Spray Containing Sodium Hyaluronate and Methylsulfonylmethane (MSM) in Otolaryngological Disorders

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Abstract: Otolaryngological disorders encompass a broad spectrum of conditions that affect the ear, nose, and throat. These conditions often lead to significant discomfort, a reduced quality of life, and the necessity for effective and well-tolerated therapeutic interventions. This article aims to provide an efficacy and safety assessment of VIARINOX®, a patented technology designed to remove mucus residue from nasal passages in both children and adults, targeting nasal congestion. The study focused on evaluating the antioxidant and anti-inflammatory capacities and the barrier effect of the patented technology. This evaluation involved chemical assays centered on free radical inhibition and assays conducted on reconstructed respiratory epithelium inserts, where damage was induced using LPS. The results obtained indicate that, owing to the presence of sodium hyaluronate, methylsulfonylmethane, and N-acetyl cysteine, this technology could serve as a pharmacological tool for treating otolaryngological disorders and as a preventive measure against the risk of microbial contamination.

Keywords: otolaryngological disorders; nasal spray; anti-inflammatory; antioxidant; barrier effect

1. Introduction

One of the most important physiological defense mechanisms is the integrity of the nasal mucosa. Nasal secretions primarily derive from anterior serous glands, goblet cells, sinus secretions, and small seromucous glands. The passage of water into the nasal lumen is facilitated by a network of subepithelial capillaries [1,2]. For a variety of reasons, this delicate balance can be disrupted, leading to nasal dryness by compromising both the epithelial barrier and mucociliary transport [3].

The most common symptoms range from mild burning, itching, and nasal congestion to a reduced sense of smell, persistent nasal discharge, and visible scabs on the nose. Dry rhinitis is one of the various forms of non-allergic rhinitis where the symptoms are independent of any allergic or infectious factors. Depending on the underlying disease, this condition may be nasal only or may be associated with other symptoms such as dry mouth [4–6]. Other causes of nasal dryness include climatic factors, workplace conditions, advancing age, allergic rhinitis (house dust mites and molds), bacterial infections, obstructive sleep apnea (OSA), or even specific side effects of medications. Of course, one of the first symptoms of a cold infection is the so-called “runny nose” [7–9].
Maintaining the nasal mucosa with continuous topical application of nasal sprays maintains good humidification and hydration and is an important therapy to counteract dry nose symptoms. Saline nasal sprays are commonly used, and there are several supposedly superior formulations on the market, such as those containing hyaluronic acid (HA), N-acetylcysteine (NAC), and Methylsulfonylmethane (MSM).

Hyaluronic acid is a physiological component of the nasal mucosa and belongs to the class of bioadhesive polymers. It increases the bioavailability and absorption of some compounds due to its high viscosity and mucoadhesiveness. One of its main functions is to moisturize the surface of the nasal pits to restore normal hydration [10–12]. It also intervenes in the regulation of vasomotor tone and glandular secretion, contributing to the defense of the nasal mucosa [13].

The mucolytic action of N-acetylcysteine is attributed to its ability to cleave the disulfide bridges that characterize many mucoproteins, which, once the bonds are broken, disintegrate into smaller units of lower viscosity, allowing excess mucosal mass to be thinned and facilitating subsequent expulsion. Some studies suggest that NAC stimulates glutathione biosynthesis by neutralizing free radicals and acting as an antioxidant [14,15]. In other studies, NAC has been found to have anti-inflammatory activity through the inhibition of nuclear factor NF-κB and modulation of pro-inflammatory cytokine synthesis [16,17].

Methylsulfonylmethane (MSM) is an organosulfur compound found naturally that has gained recognition in the realm of complementary and alternative medicine [18]. Over the years, several studies have highlighted that MSM has many effects (antioxidant and anti-inflammatory effects, anticholinesterase activity, and the capacity to induce histamine release from mast cells) [19] that allow it to cover a wide range of applications ranging from stress reduction, pain alleviation, and the treatment of parasitic infections, to claims of energy enhancement, metabolism acceleration, circulation improvement, and wound-healing promotion [20–25].

The purpose of this article is to investigate the efficacy and safety of VIARINOX®, a new patented technology suitable for the treatment of otolaryngological disorders. The efficacy of VIARINOX® is due to the main components present in the formulation: sodium hyaluronate, methylsulfonylmethane (MSM), and N-acetylcysteine. The innovation of the technology under study is the presence of the three active ingredients in a single formulation, which gives it the ability to protect the nasal mucosa and to exert antioxidant and anti-inflammatory effects at the same time.

2. Materials and Methods

2.1. Materials

Sodium nitroprusside, Griess’ reagent (1% sulfanilamide, 2% H3PO4, 0.1% naphthylethylenediamine dichlorhydrate), disodium hydrogen phosphate (Na2HPO4), sodium dihydrogen phosphate (NaH2PO4), sulfuric acid (H2SO4), ammonium heptamolybdate ((NH4)6Mo7O24), sodium phosphate (Na3PO4), DPPH (2,2′-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)). Fluorescein, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), AAPH (2,2′-Azobis(2-methylpropionamide) dihydrochloride), Biotinylated concanavalin A from Canavalia ensiformis (Con-A), disodium hydrogen phosphate, sodium dihydro-gen phosphate, sodium chloride, streptavidin peroxidase, hydrogen peroxide, and o-phenylenedi amine dihydrochloride (o-pd) were purchased from Sigma-Aldrich (Sigma Chemical Co., St Louis, MO, USA). All of the solvents were reagent-grade or HPLC-grade, and were used without further purification. MucilAirTM nasal mucosa and MucilAirTM human airway epithelium were purchased from Epithelix (Geneva, Switzerland) IL-8 kit ELISA (Invitrogen, Carlsbad, CA, USA), IL-6 kit ELISA (Abcam, Cambridge, UK). VIARINOX® was provided by Neilos s.r.l (Neilos s.r.l, Via Bagnulo, 95, 80063 Piano di Sorrento, NA, Italy).
2.1.1. Cell Cultures

The test system used was L-929 mouse fibroblast cells (ATCC CCL 1, NCTC clone 929 of strain L, Ref. ISO 10993-5; established cell lines preferred listed in Note 3). Primary supplier: Istituto Zooprofilattico Sperimentale dell’Emilia-Romagna, Via A. Bianchi, 9 25124 BRESCIA (Italy). The culture medium used was Minimum Essential Medium (MEM) with Earle’s salts and 5% fetal bovine serum, 1% L-glutamine, 0.6% penicillin/streptomycin and 0.3% fungizone (complete MEM).

2.1.2. Animals

New Zealand White rabbits, nulliparous and non-pregnant females and/or males were purchased from Giuseppe Bettinardi Via Cascinini 9—28015 Momo (NO), Italy. Hartley guinea pig nulliparous and non-pregnant females and/or males were from Charles River Laboratories Italia s.r.l. Via Indipendenza, 11–23885 Calco (Lecco), Italy. The housing conditions of the animals are reported in Tables S1 and S2 of the supplementary conditions.

2.2. Safety Assessment

2.2.1. Cytotoxicity Test

The study adhered to the guidelines outlined in ISO 10993-5: 2009 [26] and ISO 10993-12: 2012 [27]. Cell cultures were allowed to grow in plates until they formed a nearly confluent monolayer. Each sample was tested on three separate sets of cell culture plates. Additionally, three sets were allocated for the negative control, three for the positive control, and three for the liquid control during the extraction process.

In the plates designated for treatment with the sample, the culture medium was meticulously replaced with the sample extract. After 24 and 48 h of incubation with the extract, the cell cultures were subjected to microscopic examination to ascertain the presence of potential cytotoxic effects stemming from the sample extract. The assessment included the evaluation of general cell morphology, the presence of vacuolization, detachment, cell lysis, and membrane integrity. Any deviations from the normal cell morphology, as evidenced by the negative control, were assigned a score ranging from 0 to 4 (0 = none, 1 = light, 2 = slight, 3 = moderate, and 4 = severe reactivity). Additional details are provided in the Supplementary Materials (Table S3).

2.2.2. Irritation Tests—Irritation Tests on Animals

The test was conducted according to ISO 10993-10:2010 [28] and ISO 10993-12:2012 [27]. Three female albino rabbits, each weighing 2–4 kg and in good health, were used.

Approximately 24 h prior to analysis, a sufficiently large area of the back of each animal was shaved on both sides of the vertebral column. Test and control materials were then applied to the designated skin sites on the back of each rabbit and covered with an occlusive dressing for at least 4 h. Skin reactions at the treated sites were scored for erythema and edema 24, 48, and 72 h after the removal of the dressing, following the scoring system reported in the Supplementary Materials in Table S4.

Subsequently, the primary irritation index was derived. This index (Table S5) is characterized by a number and a description, in which 0–0.4 = negligible, 0.5–1.9 = mild, 2–4.9 = moderate, and 5–8 = severe.

2.2.3. Skin Sensitization Tests—Guinea Pig Maximization Tests (GPMTs)

The experiment was conducted following the standards set forth in ISO 10993-10:2010 [28] and ISO 10993-12:2012 [27]. Hartley-type guinea pigs, both male and female, weighing between 300 and 400 g, were used: ten animals for the test sample, five for the negative control, and ten for the positive control. Following a two-stage induction involving complete Freund’s adjuvant and sodium lauryl sulfate, the sample extract or the sample itself was applied to a swab, which was then placed on the skin of the guinea pigs. After 24 h, the swabs were removed, and the skin was examined for potential allergic reactions. The intensity of these reactions was evaluated with a score from 0 to 3 (Table S6) at 24 and 48 h after the removal
of the swab. Any animal displaying a reaction at 24 or 48 h with a score equal to or greater than one for erythema was classified as sensitized.

2.3. Efficacy Assessment: Anti-Inflammatory, Antioxidant and Mucoprotective Capacities

2.3.1. Scavenger Activity on Nitric Oxide—Anti-Inflammatory Action

The scavenger action against nitric oxide was determined according to the Griess method, which is commonly used to detect the presence of nitrite and measure its concentration spectrophotometrically [29,30].

The reaction involved Griess’ reagent, a mixture of sulfanilamide and 1-naphthylethylenediamine, in an acidic environment. Under these conditions, nitrite tends to form nitrous acid (HNO2), reacting with sulfanilamide to produce a diazonium salt through a diazotation reaction. This salt, in turn, undergoes a coupling reaction with 1-naphthylethylenediamine, creating a violet-colored azo dye that displays its maximum absorption peak at a wavelength of 540 nm.

A 0.1 mL sample aliquot was mixed in a vial with 2.5 mL of 5 mM sodium nitroprusside in phosphate buffer (pH 7.4) and was then subjected to UV irradiation for 3 h. Following this, 0.5 mL of the vial’s solution was taken and diluted with 0.5 mL of Griess’ reagent (1% sulfanilamide, 2% H3PO4, and 0.1% naphthylethylenediamine dihydrochloride). After 5 min, the absorbance of the chromophore was measured at 540 nm. The experiment also included a sample control prepared simultaneously, lacking the sample but containing an equivalent amount of distilled water. The scavenger activity toward nitric oxide was calculated using Equation (1):

$$
\text{% of inhibition} = \frac{A_0 - A_1}{A_0} \times 100
$$

(1)

where $A_0$ represents the absorbance of the control and $A_1$ is the absorbance of the analyzed sample.

2.3.2. Determination of Total Antioxidant Activity—Molybdate Assay

The total antioxidant activity of VIARINOX® was evaluated using the method reported in the literature [31]. The assay is based on a redox reaction, representing a direct index of the substance’s redox power. It relies on the reduction of Mo (VI) to Mo (V), leading to the formation of a green-colored phosphate-Mo(V) complex with an absorption maximum at 695 nm under acidic pH conditions.

For the assay, 10 µL of the sample was added to a reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. A control sample was prepared under identical conditions but without the sample. Subsequently, the vials were incubated at 90 °C in a thermostat bath for 150 min. Following cooling to room temperature, absorbance was measured at 695 nm. Total antioxidant activity was expressed as milligram equivalents of ferulic acid (FA) per gram of sample (mg eq FA/g) by comparing the obtained data with the calibration curve of the reference compound. This curve was generated using 5 different standard solutions of FA.

2.3.3. Evaluation of Antioxidant Activity: Scavenger Action on the DPPH Radical

The DPPH (2,2′-diphenyl-1-picrylhydrazyl) is an organic free radical with a maximum absorption peak between 515 and 528 nm, allowing for the evaluation of antioxidant activity in various compounds through spectrophotometric measurements. The reaction involves the reduction of the DPPH radical by antioxidant molecules, forming a yellow compound known as diphenylpicrylhydrazine. The extent of this reduction reaction depends on the molecules’ capability to act as electron donors.

To assess the antioxidant properties of the test samples, the scavenger activity against DPPH radicals was determined using a method reported in the literature [32]. The test involved adding 0.1 mL of the sample to 9.9 mL of a DPPH solution (200 µM) prepared in
ethanol. The absorbance was measured after 15 min at 517 nm. Data were expressed as percent inhibition, calculated according to Equation (1).

2.3.4. Determination of Antioxidant Activity: Scavenger Action on the ABTS Radical

ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) is a hydrophilic radical that displays its absorption peak around 734 nm [33]. Once again, the reaction relies on the antioxidant’s ability to donate hydrogen atoms. The scavenger action on the ABTS radical was studied by measuring the absorbance of the radical cation (ABTS•+) using a method reported in the literature, suitably modified.

This method is based on the capacity of antioxidants to act on the ABTS radical cation, characterized by a blue-green chromophore with distinctive absorption at 734 nm. ABTS•+ was generated by reacting an aqueous solution of ABTS (7 mM) with 2.45 mM potassium persulfate (K2S2O8). The resulting mixture was left in the dark at room temperature and, after 16 h, diluted with distilled water to achieve an absorbance of 0.999 ± 0.030 at 734 nm.

To assess the antioxidant properties of the samples, 0.1 mL of the sample was added to 1 mL of the final ABTS solution and to 0.9 mL of distilled water. A control was prepared under the same conditions, substituting the sample with distilled water. After 6 min, the absorbance was measured at 734 nm. The data obtained are expressed as the percentage of radical inhibition, calculated according to Equation (1).

2.3.5. ORAC Assay

The ORAC assay was conducted following the procedure reported in the literature after being suitably modified [34]. An aliquot of 20 mL from each sample underwent centrifugation at 14,000 rpm for 15 min. The resulting supernatant was appropriately diluted in phosphate buffer and analyzed. All solutions utilized for the assay were prepared in phosphate buffer pH 7.4 (75 mM), with fluorescein and AAPH solutions at concentrations of 200 nM and 150 mM, respectively.

Each well of a 96-well multi-well plate received 25 µL of buffer solution (blank), the sample for analysis (multiple concentrations were used), or a standard solution of Trolox with a known concentration. Subsequently, 150 µL of the fluorescein solution was added to each well, and the plate was incubated at 37 °C inside the spectrofluorometer employed for the assay (Synergy H1, Hybrid Reader, BioTek purchased from Agilent Technologies, Santa Clara, CA, USA). After 15 min, 25 µL of the previously prepared AAPH solution was introduced. Fluorescence was measured through excitation at 485 nm and emission at 535 nm every minute for 1 h and 30 min.

The area under the curve (AUC) was calculated according to the following Equation (2):

$$AUC = 1 + f_1/f_0 + f_2/f_0 + f_3/f_0 + \ldots + f_n/f_0$$ (2)

where f0, f1, f2, f3, …, fn represent the fluorescence intensity values at different times.

Standard solutions of Trolox were used to construct the calibration line where the values of net AUC were expressed as a function of Trolox concentration. The net AUC values for Trolox standard solutions and samples were calculated according to Equation (3):

$$\text{net AUC} = \text{AUCsample} - \text{AUCblank}$$ (3)

The antioxidant capacity of the samples analyzed was then expressed as micromole equivalents of Trolox per milliliter of sample (µmol TE/mL).

2.3.6. Mucoadhesion Assay

In vitro reconstructed nasal mucosa inserts (MucilAir™) were treated with 30 µL of VIARINOX®; a volume of 30 µL of 0.9% NaCl was added to the negative control. Incubation continued for 60 min in an incubator (37 °C, 5% CO₂). After performing 3 washes with PBS (Phosphate Buffered Saline), the tissues underwent incubation at 37 °C for 60 min with a solution containing Con-A at a concentration of 50 µg/mL. Subsequently, following
an additional 3 washes with PBS, the inserts were incubated at 37 °C for 30 min with streptavidin peroxidase. After 3 washes with PBS, o-fenylenediamine (o-pd) in 0.05 M citrate phosphate and H2O2 were added to the tissues, and after 5 min, the reaction was stopped with 1 M H2SO4. Finally, the absorbance values for the individual determinations were read using a spectrophotometer.

Mucoadhesive capacity (Table 1) is expressed as the percentage of inhibition of glycoprotein–lectin binding, or rather, as the percentage of mucoadhesion of the product according to the following Equation (4):

\[ \text{Percentage mucoadhesion of product} = (1 - \text{abs sample/abs control}) \times 100 \] (4)

Table 1. Evaluation of mucoadhesivity capacity based on percentage of mucoadhesivity obtained.

<table>
<thead>
<tr>
<th>% Mucoadhesivity</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–20%</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>20–40%</td>
<td>Discrete</td>
</tr>
<tr>
<td>40–80%</td>
<td>Good</td>
</tr>
<tr>
<td>80–100%</td>
<td>Excellent</td>
</tr>
</tbody>
</table>

2.3.7. Morphological Analysis

The assay involves the morphological and structural evaluation of tissue inserts comprising in vitro reconstructed human airway epithelium (MucilAirTM human airway epithelium), following the induction of insult by the inflammatory agent lipopolysaccharide (LPS). The epithelium is inserted and adhered to a support made of a collagen-based matrix, maintaining an air–liquid interface. Each epithelium insert is placed in a Transwell cell culture system within a 24-well plastic plate.

To simulate an irritant and inflammatory state, 3D epithelial models were initially treated with a solution of LPS at a concentration of 50 µg/mL for 3 h. Following PBS washing, the models were treated with the test compounds for 24 h to assess their protective or soothing abilities against the LPS-induced irritant insult. Post-incubation, each culture was fixed in 4% formalin for histological analysis. Morphological examination of the airway epithelium was conducted using hematoxylin and eosin staining observed under a light microscope.

2.3.8. IL-6 and IL-8 Quantification

The release of pro-inflammatory interleukins by MucilAirTM human airway epithelium, induced by the inflammatory agent lipopolysaccharide (LPS), was also evaluated. Concentrations of interleukins, specifically IL-6 and IL-8, were assessed after 24 h of treatment. For this assessment, 100 µL samples were extracted from the supernatant of each well. Interleukin quantification was performed using Enzyme-Linked Immunosorbent Assay (ELISA) with predefined measuring ranges: IL-6 (7.8–500 pg/mL) and IL-8 (15.6–1000 pg/mL).

The quantification of interleukin concentrations utilized human recombinant IL-6 and IL-8 as standards, following the manufacturer’s ELISA protocols. To standardize the data, interleukin concentrations in the supernatant of each well were normalized to the cell protein concentration of the respective well. Absorbance was measured at 450 nm using the Synergy H1, Hybrid Reader, BioTek spectrofluorometer.

2.4. Data Analysis

Obtained results were subjected to statistical analysis by means of the Student t-test. The variations versus negative control are considered significant at \( p < 0.05 \).
3. Results

3.1. Safety Assessment

3.1.1. Cytotoxicity Test

Cells treated with the undiluted and 1:5 diluted samples after 24 and 48 h of incubation exhibited some changes compared to the normal morphology observed in the negative control. However, cells treated with the 1:10 diluted sample after both 24 and 48 h of incubation showed no changes compared to the negative control. Specifically, the undiluted sample displayed strong reactivity after both 24 and 48 h of incubation. The 1:5 diluted sample exhibited moderate reactivity after 24 h of incubation and strong reactivity after 48 h. Conversely, the 1:10 diluted sample showed no reactivity. These observations were based on the reactivity scores recorded at 24 and 48 h. The results are shown in Figure 1.

![Figure 1](image_url)

**Figure 1.** Results after 24 h and 48 h (0 = none, 2 = light, 2 = slight, 3 = moderate, and 4 = severe reactivity).

3.1.2. Irritation Tests—Irritation Tests on Animals

The results presented in Figure 2 showed that the sample showed a negligible irritation response under the experimental conditions used for each animal tested.

3.1.3. Skin Sensitization Tests—Guinea Pig Maximization Tests (GPMT)

The results presented in Figure 3 show that the sample did not have a sensitizing effect under the experimental conditions that were used.

3.2. Efficacy Assessment: Anti-inflammation, Antioxidant and Mucoprotective Capacity

3.2.1. Scavenger Activity on Nitric Oxide—Anti-Inflammatory Action

The results reported in Table 2 show that VIARINOX® has anti-inflammatory properties due to the inhibition of NO enhanced by the presence, in the formulation, of MSM.

**Table 2.** Results of scavenger activity on nitric oxide.

<table>
<thead>
<tr>
<th></th>
<th>% Inhibition NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIARINOX®</td>
<td>44.1%</td>
</tr>
<tr>
<td>VIARINOX® WITHOUT MSM</td>
<td>33.2%</td>
</tr>
</tbody>
</table>
Figure 2. Results of the macroscopical evaluation (0 = absence of erythema, 1 = slight erythema, 2 = well-defined erythema, 3 = moderate erythema, and 4 = severe erythema or eschar formation) for rabbit number 69 (a), 70 (b) and 71 (c) after 24 h, 48 h and 72 h.

**Figure 3.** The results presented in Figure 3 show that the sample did not have a sensitizing effect under the experimental conditions that were used.
3.2. Efficacy Assessment: Anti-Inflammatory, Antioxidant and Mucoprotective Capacity

3.2.1. Scavenger Activity on Nitric Oxide—Anti-Inflammatory Action

The results reported in Table 2 show that VIARINOX® has anti-inflammatory properties due to the inhibition of NO enhanced by the presence, in the formulation, of MSM.

3.2.2. Determination of Total Antioxidant Activity—Molybdate Assay

Results reported in Table 3 show that VIARINOX® has antioxidant activity enhanced by the presence, in the formulation, of MSM.

Table 3. Results of determination of total antioxidant activity.

<table>
<thead>
<tr>
<th></th>
<th>Total Antioxidant Activity (mg eq FA/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIARINOX®</td>
<td>214 mg eq FA/g</td>
</tr>
<tr>
<td>VIARINOX® WITHOUT MSM</td>
<td>203 mg eq FA/g</td>
</tr>
</tbody>
</table>

3.2.3. Evaluation of Antioxidant Activity: Scavenger Action on the DPPH Radical

The results reported in Table 4 showed that VIARINOX® has antioxidant activity due to the inhibition of DPPH enhanced by the presence, in the formulation, of MSM.
Table 4. Results of determination of scavenger action on the DPPH radical.

<table>
<thead>
<tr>
<th></th>
<th>% Inhibition DPPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIARINOX®</td>
<td>62.7%</td>
</tr>
<tr>
<td>VIARINOX® WITHOUT MSM</td>
<td>56.9%</td>
</tr>
</tbody>
</table>

3.2.4. Determination of Antioxidant Activity: Scavenger Action on the ABTS Radical

The results reported in Table 5 show that VIARINOX® has antioxidant activity not due to the inhibition of ABTS influenced by the presence, in the formulation, of MSM.

Table 5. Results of determination of scavenger action on the ABTS radical.

<table>
<thead>
<tr>
<th></th>
<th>% Inhibition ABTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIARINOX®</td>
<td>100%</td>
</tr>
<tr>
<td>VIARINOX® WITHOUT MSM</td>
<td>100%</td>
</tr>
</tbody>
</table>

3.2.5. ORAC Assay

Figures 4 and 5 show the observed fluorescence decay curves for the samples analyzed, which were obtained by plotting the fluorescence intensity, evaluated at the wavelength of the emission maximum, as a function of time.

Figure 4. Fluorescence decay curves of VIARINOX®. Time is expressed as hours: minutes: seconds.

Figure 5. Fluorescence decay curves of VIARINOX® without MSM. Time is expressed as hours: minutes: seconds.
From the various curves, it is evident that the presence of the antioxidant keeps the fluorescence emission constant for a certain period. Subsequently, the radicals initiate the degradation of fluorescein, leading to a rapid decline in the emitted fluorescence intensity. Furthermore, the data obtained (shown in Table 6) underscored the fact that the antioxidant capacity is heightened by the presence of MSM in the formulation.

Table 6. Antioxidant capacity measured by ORAC assay.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antioxidant Capacity (µmol di TE/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIARINOX®</td>
<td>2905.50 ± 1.87</td>
</tr>
<tr>
<td>VIARINOX® WITHOUT MSM</td>
<td>2265.79 ± 1.95</td>
</tr>
</tbody>
</table>

3.2.6. Mucoadhesion Assay

The results obtained, shown in Table 7, demonstrate that VIARINOX® exerts a mucoadhesion percentage of about 38.01%. This percentage value, when compared with the mucoadhesive capacity rating scale in Table 1, results in DISCRETE mucoadhesion of the test sample.

Table 7. Results of absorbance values and mucoadhesion percentage. Values are expressed as mean ± st.dev. (**p < 0.001 Student t-test vs. negative control).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorbance l = 450 nm</th>
<th>Mucoadhesivity (%)</th>
<th>DEV. ST.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL (NaCl 0.9%)</td>
<td>I: 3.88, II: 3.75, III: 3.71, MEAN: 3.78</td>
<td>0.00%</td>
<td>0.08</td>
</tr>
<tr>
<td>VIARINOX®</td>
<td>I: 2.48, II: 2.22, III: 2.56, MEAN: 2.42 **</td>
<td>38.01%</td>
<td>0.17</td>
</tr>
</tbody>
</table>

3.2.7. Morphological Analysis

Histological evaluation (Figure 6 and Table 8) was performed on inserts treated with Sodium Hyaluronate alone, MSM alone, and Sodium Hyaluronate + MSM. The results revealed that the combination of these compounds in the formulation offers significant protective capabilities for the test samples. It notably reduces tissue damage, including cell degeneration, epithelial erosion, and necrosis, subsequent to an inflammatory insult with LPS for 3 h, when compared to the positive control. Additionally, similar studies were conducted on inserts treated with other compounds to select the best ingredients for inclusion in the formulation.

Figure 6. Morphological evaluation of the tissue inserts treated.
Table 8. Morphological evaluation of the treated tissue inserts. -: absent (0%); ++: moderate (>10 to 40%); +++: serious (>40%). The percentage indicates the cell counts of three different sections of three different experiments performed by two independent operators.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Negative Control</th>
<th>Positive Control (LPS 50 µg/mL)</th>
<th>Sodium Hyaluronate</th>
<th>MSM</th>
<th>Sodium Hyaluronate + MSM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular Degeneration</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Necrosis</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Erosion</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

In addition, the same study was conducted on inserts treated with other ingredients in order to choose the best to insert.

3.2.8. Il-6 and IL-8 Quantification

The results obtained are shown in Figure 7a for the quantification of human IL-6, and in Figure 7b for the quantification of human IL-8.

![Human IL-6](image1.png) ![Human IL-8](image2.png)

Figure 7. Quantification of Human IL-6 (a) and Human IL-8 (b). Values are shown as mean ± st.dev.

In both cases, an important reduction in the release of interleukins was observed after treatment with MSM alone and sodium hyaluronate alone in relation to the positive control, and a synergistic effect in the case of treatment with MSM and sodium hyaluronate together.

4. Discussion

Maintaining the integrity of the nasal mucosa stands as a fundamental physiological defense mechanism. Dry rhinitis can manifest with various symptoms, ranging from mild sensations like burning, itching, and nasal congestion to more severe indications such as a reduced sense of smell, persistent nasal discharge, and visible scabs on the nose. Among the different forms of non-allergic rhinitis, dry rhinitis stands out as it occurs independently of allergic or infectious triggers. Depending on the underlying condition, nasal dryness may solely affect the nasal passages or be accompanied by symptoms like dry mouth [35]. Other contributing factors to nasal dryness encompass climatic conditions, workplace environments, natural aging processes, allergies (e.g., reactions to house dust mites and molds), bacterial infections, obstructive sleep apnea (OSA), or the specific side effects of medication. It is important to note that a “runny nose” often emerges as one of the initial symptoms of a cold infection [10].
To alleviate the discomfort associated with nasal dryness, continuous topical application of nasal sprays remains a pivotal therapeutic approach.

In addition to saline solutions, nasal formulations leverage the activities of various active ingredients known for their impact on otolaryngological disorders [36–39].

Hyaluronic acid (HA) has shown promise in experimental models of chronic respiratory conditions. Its water-retaining ability helps hydrate and shield the respiratory passages, safeguarding them from harm. Moreover, HA exhibits anti-inflammatory properties, as observed in its effectiveness in reducing inflammation in the airways of mice with cystic fibrosis and human airway cells treated through nebulization. This indicates its potential as an anti-inflammatory agent [40,41].

Since the 1980s, beyond its established uses as a mucolytic and antidote for acetaminophen overdose, N-acetylcysteine (NAC) has been explored for conditions where oxidative stress is significant. A panel of experts convened in January 2019 to review NAC’s clinical applications, summarizing its pharmacology, role in combating oxidative stress, established uses in respiratory diseases and acetaminophen poisoning, and emerging potential in conditions like central nervous system disorders, cardiovascular disease, contrast-induced nephropathy, and certain eye conditions such as retinitis pigmentosa [42].

Methylsulfonylmethane (MSM), a natural sulfur-containing compound found in the body, fruits, vegetables, grains, and animals, is used orally and topically to alleviate chronic pain, osteoarthritis, exercise-induced muscle damage, and conditions like hemorrhoids and rosacea. While its benefits in treating allergic rhinitis and sinusitis have been less studied, some reports highlight its potential advantages in these allergic conditions [43–45].

Our study aimed to investigate how a formulation containing all three components could encompass the recognized beneficial properties of each active ingredient and their potential use in otolaryngological conditions.

To this end, the study initially assessed the formulation’s anti-inflammatory and antioxidant capacity through instrumental analysis. VIARINOX displayed excellent anti-inflammatory properties due to its nitric oxide-scavenging activity. The antioxidant capacity was confirmed through three evaluations: determining the total antioxidant activity (Molibdate assay), scavenging the DPPH radical, and scavenging the ABTS radical.

Subsequently, mucoadhesion and mucoprotection tests were conducted on reconstructed nasal mucosa tissue in vitro to demonstrate the technology’s efficacy on the nasal mucosa. For the evaluation of the mucoprotective capacity, the technique simulated an external insult to the nasal mucosa through LPS exposure. The efficacy of the sample was tested on reconstructed tissue after treatment with sodium hyaluronate, MSM, and sodium hyaluronate + MSM. The results revealed that both individual components and their combination had the ability to protect the tissue by reducing necrosis, cell degeneration, and erosion.

The mucoadhesion assay, performed on reconstructed nasal mucosa in vitro, demonstrated that the sample possessed mucoadhesive capacities, enabling it to exert the aforementioned mucoprotective actions.

5. Conclusions

Our research has unveiled promising outcomes regarding the efficacy and safety of VIARINOX®. The incorporation of pivotal components like sodium hyaluronate, methylsulfonylmethane (MSM), and N-acetylcysteine contributes significantly to its potential as a valuable pharmacological tool in managing otolaryngological disorders. Specifically, the product has demonstrated noteworthy antioxidant and anti-inflammatory capacities, pivotal in addressing inflammation associated with otolaryngological conditions. Furthermore, our studies indicate that VIARINOX® Spray exhibits barrier-enhancing properties, potentially pivotal in preventing microbial contamination. This extends its benefits beyond mere symptomatic relief. The unique formulation of this product has enabled the creation of a medical device with distinctive features, encompassing filmogenic, mucolytic, mucosal protective, and anti-inflammatory actions within a single product.
6. Patents

VIARINOX® is a technological patent approved by the Ministry of Enterprises and Made in Italy, and marketed as a medical device called RINOAIR® available in four formulations (ISO, 3, 5 and 7%).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app132413159/s1. Table S1. Housing conditions for animals used in Tests—Irritation Tests on animals; Table S2. Housing conditions for animals Skin sensitization tests—guinea pig maximization tests (GPMT); Table S3. Scoring System in Cytotoxicity test; Table S4. Scoring System in Irritation tests; Table S5. Scoring System in Irritation Index Irritation tests; Table S6. Scoring System in Skin sensitization tests—guinea pig maximization test (GPMT).

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Conflicts of Interest: Authors Marisa Francesca Motta, Giuseppe Pulitano and Fabio Amone were employed by the company Nutratech S.r.l. Maria Potenza is a Neilos s.r.l employee. Giampaolo Buriani was employed by the company Biochem S.r.l. Umberto Di Maio was employed by the company Shedir Pharma Group S.p.A. Vincenzo Nobile was employed by the company Complife Italia S.r.l. This does not alter the author’s adherence to all the journal policies on sharing data and materials. The other author declares no conflict of interest. The authors declare that this study received funding from Neilos S.r.l. The funder had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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