

1 A Phytotherapeutic Formulation for Allergic Rhinitis
2 in Autistic Children

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17 **ABSTRACT**
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Aim: In this study, the efficacy of a nasal spray based on lemon pulp extract for the treatment of allergic rhinitis in autistic subjects was evaluated by nasal cytology. The lemon pulp extract was obtained using an innovative solid-liquid extraction technology using a Naviglio Extractor or a Rapid Solid-Liquid Dynamic Extractor (RSLDE) that extracts vegetable matrices from different species by using different types of solvents. Due to the pressurising and depressurising of this technique and using water as a solvent, it is possible to extract both the hydrophilic and lipophilic substances contained in vegetables in a heterogeneous solution.

Objectives: We used a nasal spray based on a lemon pulp extract for the treatment of allergic rhinitis in autistic subjects, for whom there are legal limitations regarding the possible harmful side effects associated with the long-term use of traditional treatments, such as the use of cortisone, antihistamines, and leukotriene modifiers. Moreover, this nasal spray can be administered to very young children because it contains no alcohol.

Methodology: In this placebo-controlled study, the efficacy of the product was tested on 20 patients aged between three and eight years old (10 males and 10 females, mean age 5.5 years). Nasal cytology was performed using an exfoliative technique with a sterile swab soaked in sterile saline solution and then rubbed on the middle part of the inferior turbinate. The collected sample was May-Giemsa Grumwald stained to highlight the morphological changes in the nasal epithelium before and after the nasal spray therapy.

Results: The experimental data demonstrated that the lemon-based nasal spray has anti-inflammatory effects and is therapeutically safe as an aid in reducing the inflammatory cells observed in the nasal cytology.

Conclusion: The lemon pulp extract can be used as a nasal spray for allergic rhinitis in all autistic patients, even in children, because it contains no alcohol.

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21 **Keywords:** Autism; Allergic rhinitis; Nasal cytology; Nasal spray; Lemon pulp
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1. INTRODUCTION

Autism, originally called Kanner's syndrome, is a disorder that is considered to affect brain function by the international scientific community; individuals suffering from this disease show a marked decrease in social integration and communication. More precisely, given the variety of symptoms and the clinical complexity of the syndrome, the rubric of Autism Spectrum Disorders (ASDs) has recently been proposed. Currently, the aetiology of this disease remains unknown, but there are many theories that currently link autism to brain injury resulting in early neurocognitive damage. The exposure to toxic metals, environmental pollution and poor dietary habits may interact with genetic factors that predispose to the characteristic immune dysfunction and gastrointestinal abnormalities, that may then become aetiological factors.

Previous clinical and genetic studies have suggested that autism spectrum disorders (ASDs) are associated with immunological abnormalities involving cytokines, immunoglobulins, inflammation, and cellular immunity, but epidemiological reports are still limited [1]. In a recent epidemiological study, a total of 1596 patients with ASDs were identified and were found to have a significantly higher prevalence of allergic and autoimmune diseases than the control group. In particular, the patients with ASDs had an increased risk of allergic rhinitis (OR=1.70, 95% CI=1.51-1.91) [2]. These results support the association between ASDs and allergic diseases and autoimmune comorbidities (type 1 diabetes and Crohn's disease), although further studies are required to elucidate the possible underlying mechanisms and the roles of allergy immunity and autoimmunity in the aetiology of ASDs.

In some studies, a correlation has been shown between ASD mastocytosis and the pathological form of complex eosinophilia [3-5] that involves both the activation and proliferation of cutaneous mast cells, with the appearance of nettle-rash pigmentosa both observed in other organs and leading to skin reactions, food allergies, rhinitis, and asthma, often in the absence of a positive skin test [6,7]. Mast cells and eosinophils are not only determinants of allergic reactions but also of inflammation because they are involved in the permeability of the tissue membranes [8-10]. The search for therapeutic methods that can inhibit their proliferation is thus of paramount importance. Acute infections of the upper respiratory tract are common among autistic children as are rhinosinusitis, sinusitis, pharyngitis/tonsillitis, ear infections, laryngitis, rhinitis and allergic asthma. Asthma is one of the most common chronic diseases and is a major cause of morbidity in children worldwide. The symptoms of asthma in children include recurrent episodes of wheezing, dry cough, chest tightness with inflammation and airway obstruction; triggering factors for the disease are atopy, allergens, infections, obesity, and smoking.

In particular, allergic rhinitis is the most common disease of the upper airways, and it is believed that approximately 20% of the population is affected by this disease. Although it can occur at any age, onset is common in puberty and before age 20. Over the years, changes are possible with regard to the severity of the disorder and the number of substances to which one is allergic. In particular, the present study was conducted on autistic subjects because previous clinical and genetic studies have suggested autism spectrum disorders (ASDs) are associated with immunological abnormalities involving cytokines, immunoglobulins, inflammation and cellular immunity, although epidemiological reports are still limited. In this context, the aim of this study was to examine the behaviour of the nasal membrane in autistic subjects before and after treatment with a nasal spray made of lemon pulp extract. The nasal spray tested in this work was chosen for the treatment of allergic rhinitis in autistic subjects in order to avoid possible harmful side effects related to traditional long-term therapy; however, such a therapy could also be used by non-autistic subjects. Moreover, the advantage of this spray is that it could be administered to very young children because it contains no alcohol.

2. FLAVONOID PROPERTIES

The flavonoids are a group of plant pigments responsible for much of the colour of many fruits, vegetables and flowers with antioxidant activity. Under this name are 5000 compounds classified into subclasses that depend on their chemical structure. A flavonoid skeleton is composed of two aromatic rings (commonly designated as A and B), which are connected through a pyrone ring (C) in the case of flavones, or a dihydropyrone ring in the case of flavanones. Flavonoids are mainly present in citrus fruits as their glycosyl derivatives. Aglycones (the forms lacking the sugar moieties) occur less frequently in juices, owing to their lipophilic nature and hence their low solubility in water. The presence of a relatively large number of flavonoids in citrus juices is a result of the many different combinations that are possible between polyhydroxylated aglycones and a limited number of mono- and disaccharides. The health effects of flavonoids have been analysed in recent years and relate to their antioxidant, anti-inflammatory, anti-allergy, antiviral and anti-tumour activities. The effect of flavonoids seems to be mainly preventive. In particular, some natural flavonoids, such as quercetin and luteolin, seem to reduce the release of inflammatory molecules such as histamine and kinins from mast cells. Quercetin can reduce oxidative stress in autistic subjects with a concomitant decrease in the level of lipid hydro-peroxides and antioxidant enzymes [11]. Luteolin inhibits the production of microglia, reducing the inflammatory action of glial cells [12,13]; inhibits the release of cytokines; and has neuroprotective action that may be useful in the treatment of neuroinflammatory diseases alone or as an adjuvant for other therapeutic approaches. Flavonoids, however, and in particular luteolin, are lipophilic substances and are poorly absorbed after oral administration, being metabolised by the liver.

3. NASAL CYTOLOGY

Nasal cytology is of remarkable importance in the study of rhino-sinus diseases, especially Vasomotor Rhinitis (VMR), as it represents a valuable means of differential diagnosis between allergic/non-allergic diseases and bacterial/viral infections. It is a popular and proven method that dates back to 1889, when Gollash [14] first identified the numerous eosinophils in the nasal secretion from an asthmatic patient and attributed the presence of eosinophils as a key role in the pathogenesis of asthma. The use of nasal cytodiagnosis was recently encouraged by the study of Charles Eyermann [15], who identified eosinophils in the nasal exudate of allergic patients and highlighted their diagnostic importance. Since then, many researchers have focused their attention on cytology and particularly on the presence of different types of inflammatory cells in nasal diseases [16,17]. Different factors have contributed to the increased interest in the cytological study of the nasal mucosa, making this procedure more widespread; the sampling is easy to perform and minimally invasive, allowing the examination to be repeated as often as is required in the follow-up visits in the case of vasomotor disorders and for monitoring the effectiveness of some treatments. Some studies have shown that the rhinocytograms of patients with allergies varies according to the topical nasal steroid treatment. Some authors have shown that fluticasone dipropionate and beclomethasone dipropionate are able to effectively control perennial and seasonal allergic symptoms and vasomotor rhinopathy as well as to induce cytological changes with a significant reduction in the number of eosinophils and basophils in the nasal mucosa [18-21]; similarly, it has been shown that the anti-inflammatory effects of topical corticosteroids are doubtlessly proven by the reduction in the immune-inflammatory components observed on the rhinocytogram. Cortisone therapy, despite being effective in most cases, has disadvantages related to side effects after prolonged use; it is not tolerated by allergic individuals; it may not be used during pregnancy and lactation; and finally, it may not be used by children under the age of twelve.

4. LEMON

The lemon belongs to the family Rutaceae, which also includes oranges, mandarins, bergamot, cedar, and grapefruit. The main cultivars of lemons are as follows: Femminello, Monachello and Interdonato. In particular, the femminello Sorrentino, also known as the "Oval of Sorrento" lemon and the "lemon of Massa" and is medium-large in size (each lemon weighs no less than 85 grams), has a pulp that is straw yellow in colour with a highly acidic juice and is rich in vitamin C. The lemon has been known for its therapeutic properties for several generations; it helps to strengthen the immune system and cleanses the digestive tract. Moreover, it is not only a blood purifier but also helps the body to fight disease. Lemon juice in particular is very useful in the treatment of kidney stones, in the treatment of heart attacks and in the reduction of body temperature [22]. The health benefits are due to the many compounds that are contained in the lemon, such as vitamin C, vitamin B, phosphorus, proteins and carbohydrates. The lemon contains flavonoids, which contains the antioxidant compounds, have anti-inflammatory effects, act as a natural anti histamine and have anti cancer properties. Additionally, the lemon helps to prevent diabetes, constipation, and hypertension as well as is helpful in skin care, in the treatment of fever, in hair care, in occasional dental therapies, in cases of indigestion and many other health problems [23]. Some studies have shown that lemon juice or lemonade is able to cure kidney stones by forming urinary citrate, which prevents the formation of crystals [24]. The aim of this study was to examine the behavior of the nasal membrane in autistic subjects before and after treatment with nasal spray made of pulp extract of lemon.

5. MATERIAL AND METHODS

Instrumentation and chemicals. The following were used: Naviglio Extractor 500 mL model (Atlas Filtri Engineering, Padua, Italy); a microscope (Nikon Instruments S.p.a., Florence, Italy); and the May-Grünwald- Giemsa Reagent (Carlo Erba, Milano, Italy).

A 1% Lemon extract titrated with 6% citric acid, pure aloe juice, essential oil of ravsara, soluble Propoli WSEP-70®, and essential oil of Niaouly (Intermedia Synergie s.r.l., Cernobbio, Como, Italy) were used in the preparation of the nasal spray.

Procedure. For the preparation of the lemon pulp extract, the Naviglio extractor, demineralised water and 2 kg of lemons (12 lemons) were used following a previously reported procedure [25]. The lemon extract had a pH of between 3 and 3.5 and a citric acid content ranging between 6% and 7% (w/w); the juice of Aloe barbadensis Miller, Propoli WSEP-70® and small quantities of Ravensara Niaouly essential oil were added to obtain a nasal spray with no preservatives and no alcohol and that is suitable even for children.

Recruitment of patients and therapy. Twenty patients associated with the AIAS (Italian Association to Spastic Assistance) structure of Afragola (Naples, Italy) were selected; both males and females, with a mean age of 5.5 years (min. 3, max 8 years), showing the autistic spectrum and not on drug treatment were chosen. All the parents administered the spray and performed the sampling protocol.

For all the subjects, after the completion of the formalities, an amnestic and objective examination was performed in the presence of the parents who helped to define the clinical picture of the subject, which has been designated as a number from 0 (no symptoms) to 3 (presence of any allergic symptoms). In particular, the scale of the symptoms ranged from 0 to 3, where the value 0 corresponded to the absence of symptoms, the value 1 corresponded to nasal secretion or nasal obstruction more or less important, the value 2 to bouts of sneezing, itching of the nose and/or palate, the value 3 corresponded to the presence of numerous allergic symptoms, such as frequent conjunctivitis with watery eyes, redness and tingling sensation. During the examination, in addition to the presence of the parents, it was necessary that qualified personnel be present to prevent panic attacks or bouts of hyperactivity, which are very difficult to manage.

In the study, particular attention was paid to the sampling times and cytological processing. The cytological sampling was performed by an exfoliative technique, with a sterile swab soaked in sterile saline that was rubbed on the middle part of the inferior turbinate. The collected material was then transferred onto a glass slide by spreading it thoroughly into a thin layer to stratify the cells. The cells were subsequently stained following the method of May-Grunwald-Giemsa to aid in the identification of all the cellular components normally present in normal cells or in immune inflammation. Observation was performed with an optical microscope (the Nikon Eclipse 200) using oil immersion at 1000 x. Fifty fields were read, examining the entire surface of the slide to find the cell phenotype with a primary focus on diagnosis (neutrophils, eosinophils, lymphocytes, mast cells). At the same time, we observed the cells of the nasal epithelium.

The study used a randomised placebo-controlled, double-blind procedure. Randomised controlled trials (RCT) are experimental studies that allow the evaluation of the effectiveness of a specific treatment in a given population. The assignment of the subjects to a treatment group was performed using a randomisation method. The randomisation increased the likelihood that other variables, not considered in the study design, were distributed uniformly in the experimental group and in the controls. In this way, the possible differences observed between the two groups could be attributed to the treatment. This was a double-blind procedure because both the parents of the subjects and the experimenters were unaware of any information that could have affected the results of the experiment.

Cytology was first performed based on the inclusion of the patients in the study (Figures 1-6). The twenty autistic subjects were divided into two groups of ten: Group A received the lemon-based nasal spray: 1% Lemon Extract with citric acid titrated to 6%, pure aloe vera juice, essential oil Ravensara, Propolis-soluble WSEP 70®, essential Oil Niaouli (two puffs - equal to 0.14 ml - 3 times daily); Group B was administered an equal amount of saline. (two puffs - equal to 0.14 ml - 3 times daily).

The study was conducted according to the guidelines outlined in the Declaration of Helsinki, and all the procedures were according to those approved by the Federico II University Ethics Committee. Moreover, after describing the design of the study, all of the children's parents signed the informed consent form about the study including the possibility of receiving a placebo.

Figures 1, 2, and 3. Rhinocytograms of the patients in group A before treatment with the nasal spray with scores of 2, 2, and 3 in Figure 1; Figure 3 shows the eosinophils highlighted by a blue arrow; Figure 2 shows mast cells indicated by a red arrow.

Cytology was first carried out based on the inclusion of patients in the study, and the Rhinocytograms are shown (Figures 1-6). Figures relating to the rhinocytograms of the patients in group A, with scores of 2, 2, and 3 before the treatment with the nasal spray are shown in Figures 1 and 3 in which eosinophils are highlighted by the blue arrows. Mast cells are highlighted by a red arrow in Figure 3, along with large areas of eosinophil degranulation.

Figures 4, 5, and 6. Rhinocytograms of the patients in group B, before the treatment with the saline solution and with scores of 2, 2, and 3 are shown in Figure 4. Figure 5 shows mast cells and eosinophils. Figure 6 shows that, in addition to mast cells, neutrophils are also present and are highlighted by the black arrow, and there is a large area of eosinophil degranulation.

In the rhinocytogram figures from the patients in group B with scores of 2, 2, and 3 before the saline solution treatment, it is possible to observe mast cells and eosinophils (Figures 4 and 5). In Figure 6, in addition to mast cells, neutrophils indicated by the black arrow, are present as is a large area of eosinophil degranulation. After 10 days of therapy with the nasal spray, we made nasal cytology observations. Similarly, we made rhinocytological observations in all cases after 30 days of therapy (Figures 7-12). A Wilcoxon-Two-Sample Test (t approximation) was used for the statistical evaluation of the experimental results, with statistical significance set at the $p < 0.05$ level.

Figures 7, 8, and 9 Rhinocytograms of the patients in group A after the topical administration of the lemon spray. The nearly complete disappearance of eosinophils and mast cells can be observed, with the persistence of some neutrophil and eosinophil granules.

Figures 10, 11, and 12 Rhinocytograms of the patients in group B after the treatment with saline solution: no evidence of significant changes was observed.

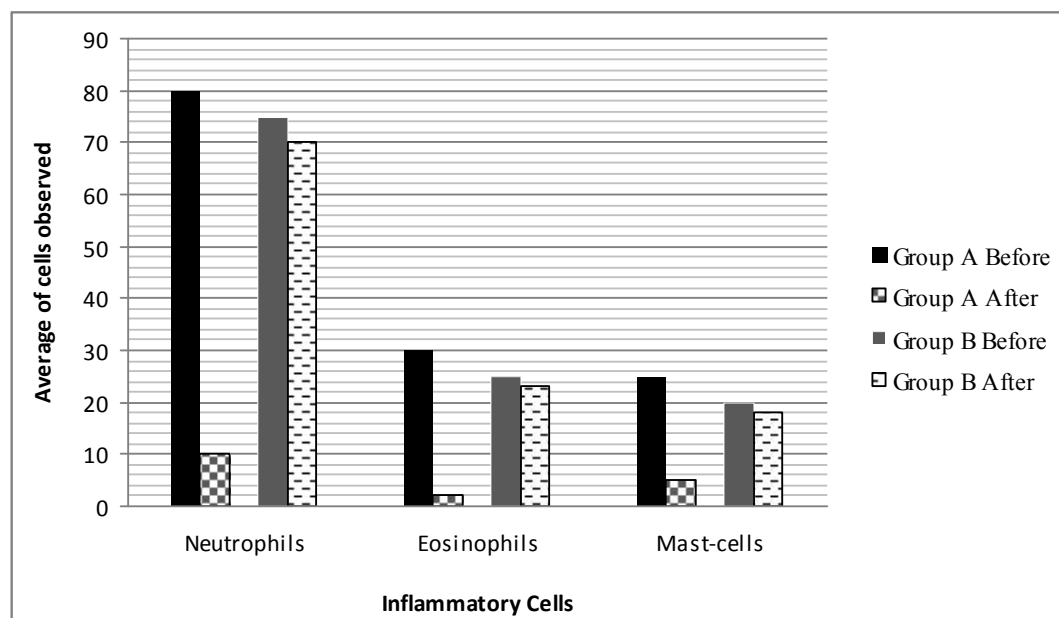
6. RESULTS

The evaluation of the rhinocytograms in the autistic subjects showed a significant neutrophilic component, with a discrete but constant representation of eosinophils and mast cells in both groups before the therapy (See Figures 1-3). In all of the subjects in group A, the examination revealed an improvement in the first ten days.

After ten days of topical administration of the lemon spray, the rhinocytograms of the patients in group A showed the almost complete disappearance of eosinophils and mast cells, with the persistence of some of neutrophilic and eosinophilic granules, while the patients in group B did not show any significant changes after the saline treatment (Table 1). Moreover, during the treatment, it was important to perform constant hygienic nasal washes or showers with saline solution to remove the favourable conditions for the

development of an allergic reaction. Sometimes, the severity and subjective symptoms required an increase in the dose of the lemon-based spray, without any observable side effects. Two patients in group A used a higher dose (two puffs - 0.14 ml 5 times a day instead of 3). Similarly, two patients in group B were given an equal amount of saline. Both groups showed no side effects, such as redness or irritation of the nose (data not shown). The symptoms for all of the subjects in group A improved within the first ten days. Side effects such as irritation and redness of the nasal cavities were not detected during the entire period of the treatment.

TAB. 1 Total progress of inflammatory cells for both group before and after therapy. (total slide surface 2,5 x 6 cm)



Scale Bar: 38,05 x 13,85 cm

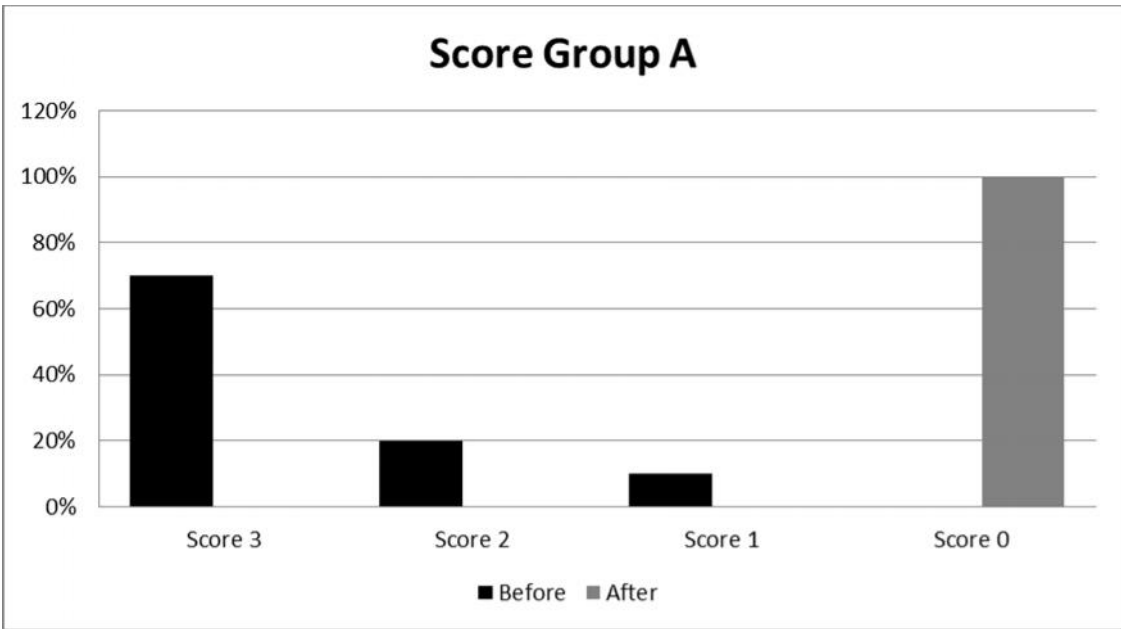
The analysis of the rhinocytograms was in perfect agreement with the clinical improvement in the autistic patients as reported by the parents. In fact, an improvement in the overall symptoms has always corresponded with a concomitant reduction in the inflammatory cells in the nasal secretions. The improvement of the rhinocytological framework may be explained by mechanisms related to the synergistic action of the natural substances present in the spray; however, further study will be required to confirm this. Although there are few cases to draw conclusions from, the positive results speak to the likelihood that the spray has anti-inflammatory activity.

Upon the first observation, the rhinopathic subjects had typical allergic symptoms: nasal obstruction, rhinorrhea, and sneezing that were more or less acute. Hypertrophic turbinates of a bruised-pale colouring were apparent in the rhinoscopy. In Tables 2 and 3, we report the values of the scores before and after the treatment for groups A and B. The patients, from a subjective-overall symptomatology point of view, before the therapy had the following scores: in group A, 70% had a score of 3, 20% had a score of 2, and 10% had a score of 1. The scores in group B were as follows: 70% had a score of 3, 20% had a score of 2, and 10% had a score of 1. After the therapy, the scores in group A were as follows: 100% had a score of 0; while the scores in group B were as follows: 65% had a score of 3, 20% had a score of 2, and 15% had a score of 1 (Tables 2 and 3). The evaluation of the initial group A rhinocytograms revealed a rich neutrophilic component, with a discrete but constant presence of eosinophils and mast cells and there were infrequent lymphocytes in nine cases. In all the subjects, after the first ten days, a clear regression of the individual symptoms was objectively detected; it was only

359 necessary to increase the number of daily doses in two cases because of persistent
360 allergic symptoms. Microscopically, we observed an absence of neutrophils and
361 lymphocytes, with a reduction by more than 50% of eosinophils and mast cells. In some
362 rare cases, eosinophilic and mast cell degranulations were observed.
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365 **Tab. 2.** Group A scores before and after treatment

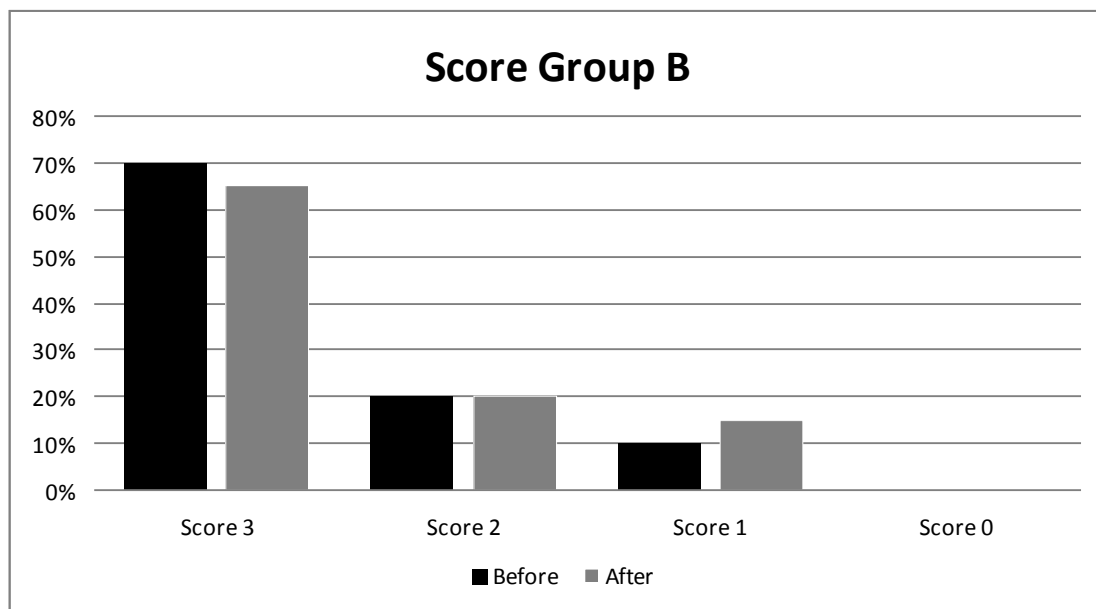
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Scale Bar: 3,96 x 6.64 cm

370 **Tab. 3.** Group B scores before and after treatment



Scale Bar: 3,96 x 6.64 cm

At the end of the therapy, all the subjects in group A reported individual symptomatology equal to 0 (no symptoms). This finding was confirmed by a local objective examination, which documented a clear improvement of the mucosa by rhinocytograms that detected a reduction in the numbers of mast cells (average 20.26 to 2.18; standard dev. 19.56 to 3.17), eosinophils (average 13.6 to 2.5; standard dev. 16.4-6.38), neutrophils (average 37 to 1.74; standard dev. from 55.21 to 5.15) and lymphocytes (average from 5.32. to 0.88, standard dev. 11.45 to 2.95) ($p < 0.01$). The subjects in Group B did not show substantial improvements in either symptoms or cytology ($p < 0.01$).

Our study was an analysis of individuals with autism, in whom there is a greater susceptibility to colds; nasal cytology, the method we used in this study, is undoubtedly valid and is well tolerated and easy to perform [14-18].

The cell morphologies studied have revealed a cytologic pattern that shows a significant proportion of neutrophils, eosinophils and mast cells that are pathognomonic of cellular forms [26-30]. Lymphocytes are most likely related to previous viral infections.

The main aim of our study was to assess the variation in the nasal cellularity, and in particular, of those cells known to be involved in allergic complications using a lemon-based nasal spray treatment. A placebo effect was verified by means of the control group.

The topical administration of the lemon-based spray showed the almost complete disappearance of eosinophils and mast cells, with the persistence of some neutrophils or lymphocyte and rare eosinophils and metachromatic granules. Therefore, frequent hygienic nasal washes with saline solutions are essential during the treatment to remove the conditions conducive to an allergic reaction. In two cases, the severity and subjective symptoms rendered it necessary to increase the dose of the lemon-based spray, without any observable side effects.

7. DISCUSSION AND CONCLUSION

Different studies have reported the efficacy of daily lemon extract on the nasal mucociliary clearance, and the properties of water-soluble flavonoids on venous micro-circulation [31,32]. In this study, the nasal spray showed immediate effects such as the clearing of the nasal cavity that lead to improved breathing; the cytological findings were consistent with this result. From the first uses of the product, there was a significant elimination of fluid that helped to remove mucus and irritant deposits, promoting better

nasal hygiene. Furthermore, the essential oils present have beneficial effects that contribute to a sanitising activity [33]. Natural substances present in the spray undoubtedly exhibited anti-inflammatory properties; these substances are applicable for all such diseases in which the use of corticosteroids may be contraindicated.

There are published accounts that lemon juice is among the most powerful natural antiseptic and bactericides; it is beneficial in ear infections and in colds as well as has a certain efficacy in treating inflammation of the throat, mouth ulcers, gingivitis and inflammation of the tongue.

Lemon juice contains hesperedin, eriodictyol, and diosmin [31,32]. The pharmacological effects of flavonoids were first observed in 1935 by Szent-Gyorgyi [34], who separated a substance capable of decreasing capillary permeability and of increasing vascular resistance from the lemon peel; this substance is called citrina, which was later discovered to be made of two flavonoids, hesperidin and eriodictyol.

Aloe juice has an anti-allergenic and anti-allergic effect that has proven to be highly efficient in most cases; moreover, the effect of a tincture of aloe has long been known in nasal-oropharyngeal infections [35]. The complex acemanane sugar together with the bradykinin present in the aloe have a reinforcing and modulating effect on the defensive and anti-inflammatory system; this finding has been confirmed by clinical studies that show its antioxidant, immuno-stimulant, anti-inflammatory, anti-allergic and purifying effects [36]. The carboxypeptidase present in the aloe acts on tissue inflammation and indirectly alleviates pain.

The essential oil of Niaoulj represents an effective protective agent in the treatment of infections of the respiratory tract because its vapours have bactericidal, immuno-stimulant, hyperemising, mucolytic, and balsamic properties [37]. Hence, the Niaoulj can be used as a valid remedy to sustain the functions of the respiratory apparatus in the event of rhinitis, pharyngitis, laryngotracheitis, bronchitis, and pneumonia. Moreover, due to its anti-spasmodic properties, the vegetable extract of Niaoulj can be used as a remedy to sustain organic functions in the presence of spasmodic coughs [38].

Because of its anti-bacterial, anti-viral, and expectorant properties, the essential oil of Ravensara represents an excellent remedy for infections of the respiratory tract. It can be used as a valid remedy to sustain the functions of the respiratory system in the event of sinusitis, rhino-pharyngitis, bronchitis, coughs, and whooping cough [39].

The propolis WSEP-70® standardised at 10% (w/w) in quercetin and 75% (w/w) in total polyphenols expressed in galangin is an extract of water-soluble propolis that enhances the natural defences of the organism against inflammatory disturbances to the nasal and oropharyngeal cavities [40]. It displays a high anti-oxidising and protective effect on the mucosa, due to the high bio-availability of the active components at the biological fluid level and to the high absorption of active components by the oropharyngeal mucosa and the first gastro-oesophageal tract, compared with those of traditional propolis [41].

The analysis of the rhinocytograms was consistent with the clinical improvements. In fact, the improvement of the subjective symptoms has always coincided with a reduction in the numbers of inflammatory cells in the nasal secretions. The improvement of the rhinocytologic framework was likely due to the synergistic action of the natural substances present in the spray and is still under botanical study. Citrus fruits and citrus juices stand out among the most common phenolic rich dietary sources [22]. Epidemiological studies have shown an inverse association between risk and the intake level of some specific flavonoids, but further clinical trials are needed to assess a more precise correlation between the level of flavonoid intake and human health benefits.

Our survey, however, is too small to draw general conclusions. We can affirm that the spray can be used in autistic children for whom the traditional therapy is contraindicated. Moreover, we argue that the nasal cytology may be a useful and objective method to assess not only the phases of clinical rhinitis but also the effects of therapy on the inflammatory cell component, the reduction of which is a guarantee of effective therapy.

The lemon pulp extract based nasal spray tested in this work has been used for the treatment of allergic rhinitis in autistic subjects to avoid the possible harmful side effects related to traditional long-term therapy. However, this therapy could also be used in non-autistic subjects. Moreover, this spray may be administered to very young children because the spray is devoid of alcohol.

COMPETING INTERESTS

All authors can confirm that there is no conflict of interest, financially or otherwise.

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