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The effects of oral supplements with *Sambucus nigra*, Zinc, *Tyndallized Lactobacillus acidophilus* (H122), Arabinogalactans, vitamin D, vitamin E and vitamin C in otitis media with effusion in children: a randomized controlled trial

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Abstract. – OBJECTIVE: To evaluate the ability of oral supplements with immune-stimulating molecules (*Sambucus nigra*, Zinc, *Tyndallized Lactobacillus acidophilus* (H122), *Arabinogalactans*, vitamin D, vitamin E and vitamin C) to reduce the inflammation of the upper airway tract and improve the outcome of otitis media with effusion (OME) in children.

PATIENTS AND METHODS: Randomized controlled trial. One-hundred ninety-eight children (CI 95%: 12-96 months) were divided into four groups. Group 1 (48 subjects) received 10 ml of oral supplements (OS) with immune-stimulating molecules for three months (20 days consecutively, then 10 days of suspension – the therapeutic scheme was repeated three times); Group 2 (54 children) underwent treatment with 10 ml of OS for 90 consecutive days; Group 3 (48 subjects) received 15 ml of OS for 45 consecutive days; a control group (48 children) underwent the standard treatment for rhinitis and OME. Outcome measures included otoscopy, tympanometry, fibroendoscopy, and the pure tone audiometry (PTA) at T0 (before treatment), T1 (45 days after treatment), and T2 (90 days after treatment).

RESULTS: All children treated with OS showed a reduction of Upper Airway Infection (UAI) episodes and OME compared to the control group independent of the administration method and posology. The three groups treated with OS showed statistically significant differences between T0 and T2 for otoscopy, tympanometry, fibroendoscopy, and PTA. In Group 2, the otoscopy and the tympanometry scores improved at T1. Group 2 and 3 had better PTA results than Group 1.

CONCLUSIONS: OS with immune-stimulating molecules should be considered as a supporting therapy in children affected by recurrent episodes of UAI associated with OME due to their capacity to improve the immune response and reduce the inflammatory phenomena. OS can improve the fibroendoscopic findings by restoring middle ear ventilation, in addition to their ability to reduce inflammation in the middle ear.

Key Words:
Otitis media, Pure tone auditory test, Rhinopharynx, Treatment supplementation.

Abbreviations

OM: Otitis Media; AOM: Acute Otitis Media; TM: tympanic membrane; OME: Otitis Media with Effusion; PTA: Pure Tone Audiometry; CHL: conductive hearing loss; UAI: upper airway infections; OS: Oral Supplements; CG: Control Group.

Introduction

Upper Airway Infections (UAIs) are common in children. Due to their viral origin (rhinovirus, adenovirus, coronavirus), these infections do not respond to antibiotic treatment[1] and are often treated with symptomatologic agents such as corticosteroids and mucolytic and anti-inflammatory drugs.

UAI in children under six years of age commonly include ear involvement due to the accumulation of fluid in the middle ear (Figure 1); this condition, called otitis media (OM), affects

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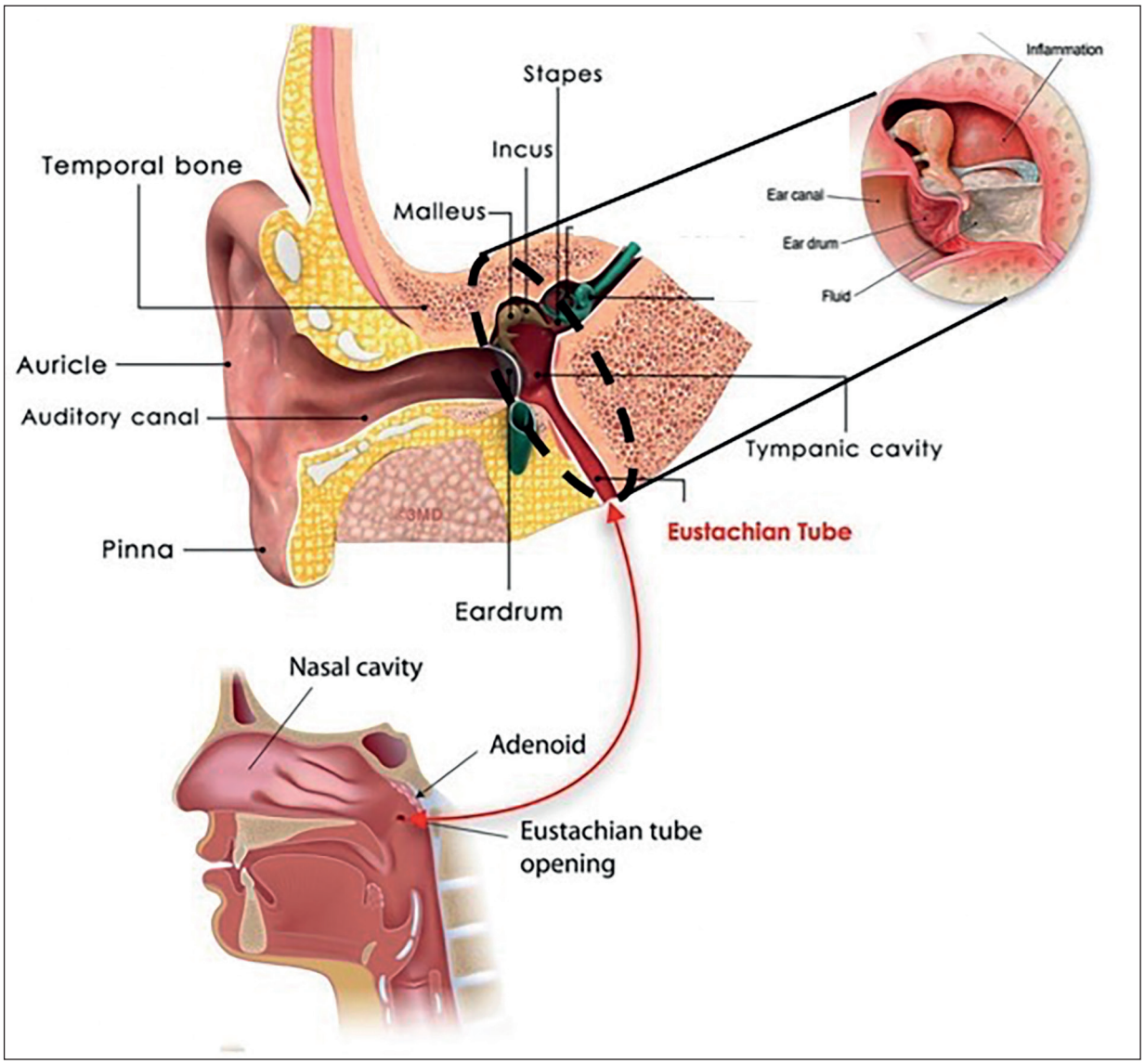

Figure 1. Anatomy of the rhinopharynx, its relationship with the Eustachian tube and the normal anatomy of middle ear. In case of otitis media, the accumulation of mucus in the middle ear doesn't allow a correct function of the bone chain ear with consequent alteration of auditory apparatus.

18% of European children[2]. The pathophysiology of OM is related to mucous ascension from the rhinopharynx into the middle ear due to the short Eustachian tube[3]. When mucus in the middle ear is inflamed due to a viral-bacterial infection, the OM is classified as acute (AOM); in addition, AOM may cause a spontaneous perforation of the tympanic membrane (TM). In cases with uninfected mucus and the presence of a glue-fluid liquid, the OM is classified as Otitis Media with Effusion (OME)[4,5]. Both conditions may present a hearing deficit that is detected by pure tone audiometry (PTA). Hearing function is extremely important in children[6,7] as in adults[8-10]; thus, every form of OM should be promptly treated to restore the functionality of the middle ear.

The standard treatment for AOM is systemic antibiotic therapy, corticosteroids in the form of nasal spray or in a saline solution suspension[11,12]; OME is usually treated with a mucolytic therapy associated with corticosteroids[11]. In children affected by OME with a middle-severe form of conductive hearing loss (CHL), the elective treatment is a ventilation tube inserted into the TM to allow middle ear ventilation and mucus removal[4,11]. The application of a ventilation tube has to

be performed under general anesthesia[11,13], so less invasive forms of treatment should be exhausted prior to this option.

Currently, the success of OM treatment is highly variable; in fact, the resolution of this condition is strictly related to the state of the upper airways[3,4]. Furthermore, young children are often susceptible to UAI because their immune system is not completely mature[14]. Fever and lack of appetite – conditions commonly associated with viral infections – make the children's immune systems even weaker[15] by establishing a chronic infection condition that is sometimes difficult to resolve.

Some authors have proposed oral supplements (OS) to improve the immune response[16,17]. Vitaminic oral compounds have been successfully used in the adult population to improve the immune response in patients suffering from immune-deficit syndromes[18] and in patients affected by cancer[19]. However, to date, the impact of OS on the outcomes of OME in children following the reduction of UAI episodes has not been investigated. We speculate that, by stimulating the immune system, it is possible to improve the outcomes of OM.

The aim of this study is to test the efficacy of an OS that contains immune-stimulating molecules (*Sambucus nigra, Zinc, Tyndallized Lactobacillus acidophilus* (H122), *Arabinogalactans*, vitamin D, vitamin E and vitamin C) to improve the ventilation of middle ear and consequently the hearing abilities in children that suffering from OME in a randomized controlled trial.

Patients and Methods

This study was conducted in the Department of Otolaryngology of Santobono-Pausilipon, a tertiary pediatric referral center, from January to November 2018. All procedures were approved by the Local Institutional Review Board Committee and were conducted in accordance with the Ethical principles outlined in the Declaration of Helsinki. The participating children's parents signed a written informed consent document authorizing their enrollment in the study.

Inclusion criteria were children with OME of different severities (from light form to glue ear) aged < 8 years with no previous surgery of upper airways and/or ear.

Subjects were casually randomly assigned by the physician (single-blinded study) to one of the four groups: Group 1 (G1), Group 2 (G2), Group 3 (G3), or control group (CG). G1, G2, and G3 were treated with OS with immune-stimulating molecules (*Sambucus nigra,* Zinc*, Tyndallized Lactobacillus acidophilus* (H122)*, Arabinogalactans*, Vitamin D, Vitamin E and Vitamin C) (Humana Italia Spa) in combination with the standard treatment while CG underwent standard treatment only. The standard treatment was a nasal wash with Fluticasone, a mucolytic agent and hypertonic solution. All children started the treatment within two days after the first clinical evaluation.

Group 1 received 10 ml of OS with the following posology: 10 ml for 10 consecutive days, followed by a 20-day of treatment suspension; this therapeutic scheme was repeated for three months consecutively. Group 2 received 10 ml of OS every day for 90 consecutive days; G3 received 15 ml of OS for 45 consecutive days.

Outcome measures included otoscopy, tympanometry, fibroendoscopy, and the PTA. Three time points were identified: T0=before treatment (baseline), T1=45 days after treatment with DI, and T2=90 days after treatment and both clinical evaluations and auditory tests were performed at each time point.

Clinical Evaluation

Children were evaluated by a pediatric otolaryngologist with over 10 years of experience. The physician evaluated their TM with a Sensera microscope (Zeiss, Oberkochen, Germany). The state of the TM was photo-recorded and then scored from 1 to 3 as follow: 1 = TM opaque and retracted, 2 = TM opaque, and 3 = healthy TM (Image 2a). Then, the same physician investigated the state of the nose and the rhinopharynx using flexible fibroendoscopy (Stortz, Tuttilingen, Germany) and Olympus CV-170 camera (Olympus, Shinjuku, Tokyo, Japan) to determine the presence and volume of adenoid tissue. The findings were classified using the Cassano assessment[20] with scores ranging from 1 to 4.

Audiological Evaluation

Children's hearing ability was evaluated with two tests: PTA and tympanometry. All tests were performed by a technician with over 20 years of experience. PTA was performed to determine the auditory hearing threshold and the type of hearing loss (i.e., sensorineural or conductive) using a clinical audiometer (Madsen Astera, Otometrics, Taastrup, Denmark). Tym-

panometry was performed to evaluate the sound transmission capacity of the middle ear using a Clarinet Middle Ear Analyzer (Inventis, Padua, Italy) (Figure 2).

The auditory threshold was scored following the American Speech-Language-Hearing Association guidelines for hearing loss with "1" referring to mild hearing loss (26-40 dB), "2" indicative of slight hearing loss (16-25 dB), and "3" for a typical auditory threshold (10-15 dB). Children that showed a PTA indicative of sensorineural hearing loss were excluded from the study.

Tympanometry test produced three different function curves for the motility of the middle ear structure: tympanogram type A represented typical function; tympanogram type B indicated the presence of fluid/infection in the middle ear that prevented auditory transmission; and tympanogram type C indicated negative pressure in the middle ear, as in the case of a poorly functioning Eustachian tube or obstruction of the rhinopharynx. We scored the results of the immittance test as follows: 1=tympanogram type B, 2=tympanogram type C, 3=tympanogram type A (Image 2b).

Statistical Analysis

The statistical analysis was performed by using STATA®. One-way ANOVA was used to evaluate the score variation within each group (G1, G2, G3, and CG) at the tree time points (T0, T1, T2) for the otoscopy findings. The same test was repeated to evaluate the variance of the tympanometry results, fibroendoscopy and PTA findings.

Differences in the otoscopy findings, tympanometry, fibroendoscopy, and PTA results between (and within) the four groups (G1, G2, G3, and CG) at T1 and at T2 were analyzed with one-way ANOVA. A Bonferroni-Holms *ad hoc* test was performed for each one-way ANOVA. A p-value < 0.05 was considered to be statistically significant.

Results

Each variable's severity in each group was equally distributed to guarantee the homogeneity of samples. Specifically, all groups included patients that presented PTA scores from 1 to 3 and same for tympanometry test. In addition, the

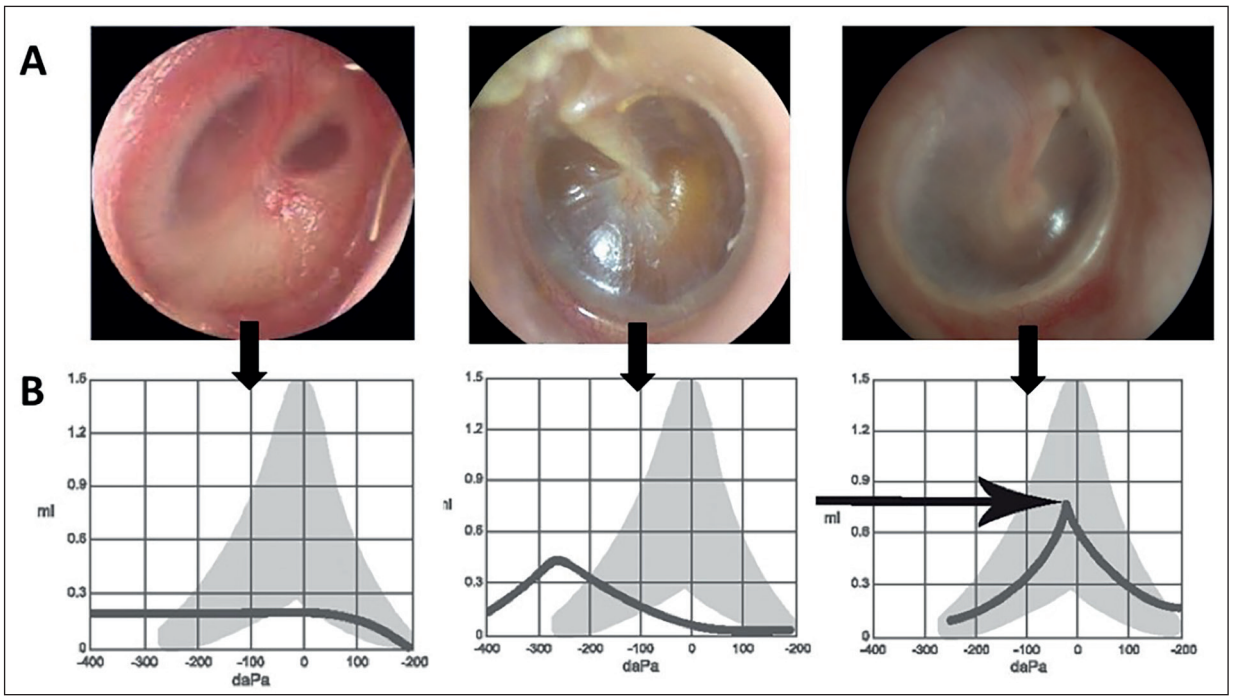

Figure 2. ***A,*** From left to the right the image shows the three aspect of the tympanic membrane (TM) that we considered in our study, respectively AOM, OME and normal aspect. ***B,*** The different shapes of the tympanometry test correlated to the different TM aspects. From left to right: tympanogram type B, tympanogram type C and tympanogram type A. The back arrow shows the maximum peak reached during the test.

TM and the fibroendoscopy scores were equally distributed in the four groups by meaning that in all groups were present children with TM scores from 1 to 3, and fibroendoscopy scores from 1 to 4.

All children attended correctly to the check-up after 45 (T1) and 90 (T2) days.

Treatment Results "within" Group Comparison

Figure 3 summarizes the results of the three groups treated with OS at the two follow-up time-points (T1 and T2) (Figure 3).

Group 1

This group included 48 children (33 males, 68.7%; 15 females, 31.3%) with a mean age of 5.3 years (SD: 1.65; CI 95%: 2-8).

We observed a statistically significant improvement when comparing T0, T1, and T2 in otoscopy (CI 95%: 1-3; ANOVA: $p < 0.0001$), tympanometry (CI 95%: 1-3; ANOVA: $p < 0.0001$), fibroendoscopy (CI 95%: 1-4; ANOVA: $p < 0.0001$), and the PTA (CI 95%: 1-3; ANOVA: $p = 0.0024$).

We did not observe statistically significant variations for otoscopy (T1= mean: 1.8; SD: 0.6; CI 95%: 1-3), tympanometry (T1= mean: 1.7; SD: 0.7; CI 95%: 1-3), fibroendoscopy (T1= mean: 2.6; SD: 0.8; CI 95%: 1-4), and PTA (T1= mean: 1.7; SD: 0.6; CI 95%: 1-3) between T0 and T1.

At T2, we identified statistically significant variances for the otoscopy findings (mean: 2.6; SD: 0.4; CI 95%: 2-3) relative to both T0 (BH: $p < 0.01$) and T1 (BH: $p < 0.01$). Tympanometry values improved (mean: 2.7; SD: 0.4; CI 95%: 2-3) with statistically significant scores both for T0 *vs.* T2 (BH: $p < 0.01$) and T1 *vs.* T2 (BH: $p < 0.01$). The fibroendoscopy findings ameliorated (mean: 3.5; SD: 0,5; CI 95%: 2-4) with statistically significant p values compared to T0 (BH: $p < 0.01$) and

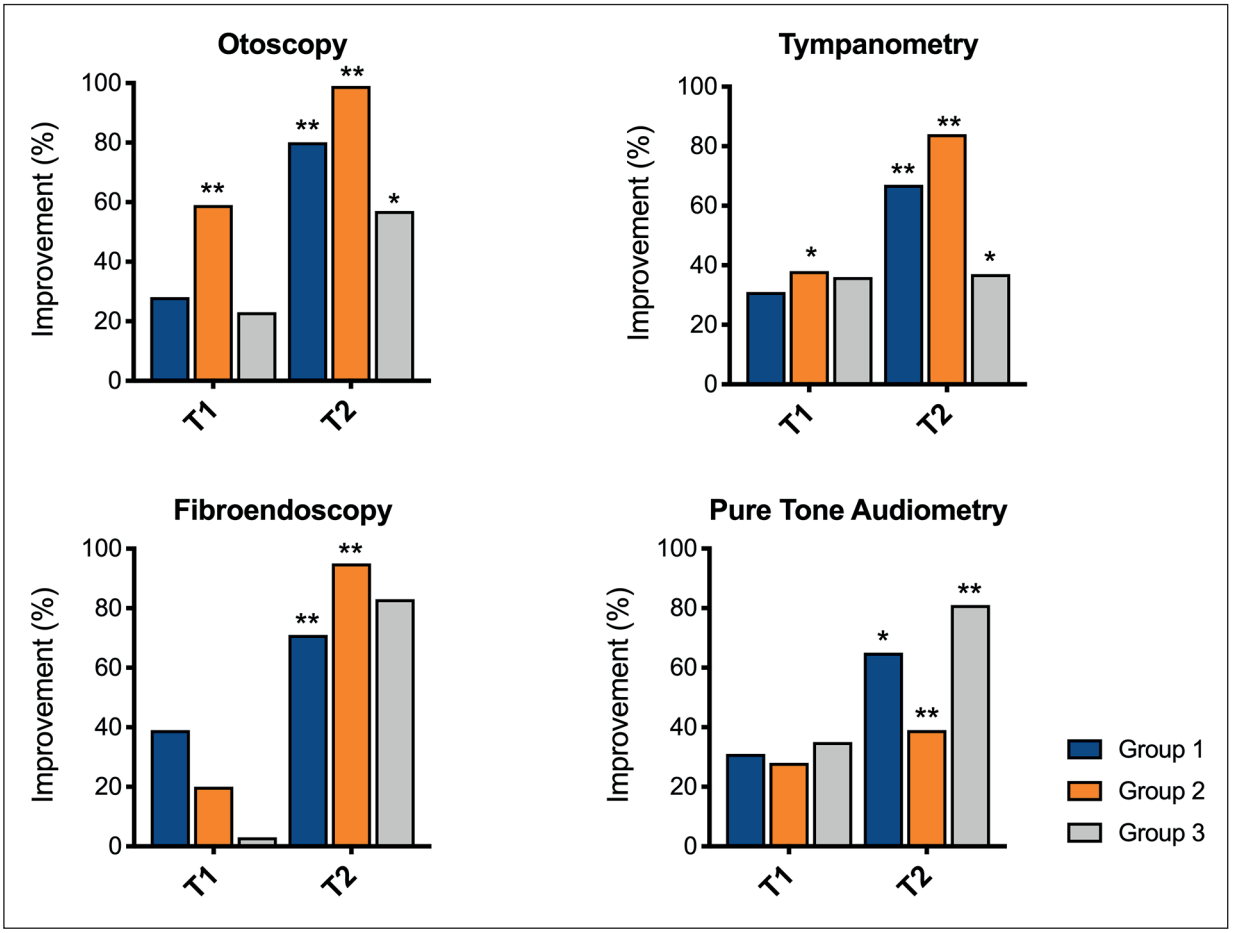

Figure 3. The results of "within group" comparison. Two asterisks (**) represent a p value < 0.01, while one asterisk (*) indicates a $p < 0.05$. p-values are considered in function of the starting conditions (T0).

T1 (BH: $p < 0.01$). Finally, PTA also significantly improved compared to T0 (BH: $p < 0.01$) and T1 (BH: $p < 0.05$) (mean: 2.4; SD: 0.7; CI 95%: 2-3).

Group 2

This group included 54 subjects (39 males, 72.2%; 15 females, 27.8%) with a mean age of 5.1 years (SD: 1.84; CI 95%: 3-8).

We observed statistically significant improvements when comparing T0, T1, and T2 for otoscopy (CI 95%: 1-3; ANOVA: $p < 0.0001$), tympanometry (CI 95%: 1-3; ANOVA: $p < 0.0001$), fibroendoscopy (CI 95%: 1-4; ANOVA: $p < 0.0001$), and PTA (CI 95%: 1-3; ANOVA: $p < 0.0001$).

The improvement between T0 and T1 was statistically significant for the otoscopy findings (mean: 1.8; SD: 0.4; CI 95%: 1-3; BH: $p < 0.01$) and tympanometry (mean: 1.7; SD: 0.5; CI 95%: 1-3; BH: $p < 0.05$), while fibroendoscopy (mean: 2.7; SD: 0.7; CI 95%: 1-4) and PTA (mean: 1.8; SD: 0.6; CI 95%: 1-3) variances did not reach scores of statistical significances.

At T2, we identified statistically significant variations for the otoscopy findings (mean: 2.9; SD: 0.2; CI 95%: 2-3) between T0 (BH: $p < 0.01$) and T1 (BH: $p < 0.01$).

Tympanometry scores (mean: 2.9; SD: 0.3; CI 95%: 2-3) improved with statistically significant scores both for T0 (BH: $p < 0.01$) and T1 (BH: $p < 0.01$). The fibroendoscopy findings (mean: 3.7; SD: 0.4; CI 95%: 2-4) at T2 also generated statistically significant p-values compared to T0 (BH: $p < 0.01$) and T1 (BH: $p < 0.01$). Finally, PTA (mean: 2.8; SD: 0.3; CI 95% : 2-3) also improved significantly over T0 (BH: $p < 0.01$) and T1 (BH: $p < 0.01$).

Group 3

Forty-eight children were included in this group (27 males, 56.2%; 21 females, 43.8%) with a mean age of 4.6 years (SD: 1.47; CI 95%: 3-8).

We observed a statistically significant improvement in this group when comparing T0, T1, and T2 for otoscopy (CI 95%: 1-3; ANOVA: $p = 0.0032$), tympanometry (CI 95%: 1-3; ANOVA: $p = 0.0006$), fibroendoscopy (CI 95%: 1-4; ANOVA: $p < 0.0001$), and PTA (CI 95%: 1-3; ANOVA: $p = 0.0002$).

We did not observe statistically significant variations in otoscopy (mean: 1.7; SD: 0.6 CI 95%: 1-3), tympanometry (mean: 1.6; SD: 0.6; CI 95%: 1-3), fibroendoscopy (mean: 2.4; SD: 0.5; CI 95%: 1-3) and PTA (mean 1.8: SD: 0.6, CI 95%: 1-3) between T0 and T1.

At T2, we identified statistically significant differences for the otoscopy findings (mean: 2.2; SD: 0.6; CI 95%: 1-3) compared to T0 (BH: $p < 0.01$) and T1 (BH: $p < 0.05$). Tympanometry scores (mean: 2.2; SD:0.6; CI 95%: 1-3) improved with statistically significant scores relative to T0 (BH: $p < 0.01$) and T1 (BH: $p < 0.05$). The fibroendoscopy (mean: 3.3; SD: 0.5; CI 95%: 2-4) findings ameliorated with statistically significant p-values compared to T0 (BH: $p < 0.01$) and T1 (BH: $p < 0.01$). Finally, PTA (mean: 2.5; SD: 0.5; CI 95%: 2-3) improved with statistically significant values both for T0 *vs.* T2 (BH: $p < 0.01$) and T1 *vs.* T2 (BH: $p < 0.01$).

Control Group

This group included 48 patients (36 males, 75%; 12 females, 25%) with an average age of 5.3 years (SD: 1.66; CI 95%: 2-8).

We only observed statistically significant variations in the otoscopy (CI 95%: 1-3) (ANOVA: $p = 0.0032$) when comparing T0 to T2 (BH: $p > 0.01$). The other outcomes [tympanometry (CI 95%: 1-3), fibroendoscopy (CI 95%: 1-4), and PTA (CI 95%: 1-3)] did not improve enough to reach a statistically significant level.

Treatment Results Between Group Comparison at T1

Otoscopy: OS with immune-stimulating molecules improved patient outcomes compared to the CG (ANOVA: $p < 0.0001$) independently of the administration method; specifically, we observed a statistically significant variation at T1 between G1 (mean: 1.8; SD: 0.6) and CG (mean: 0.6; SD: 0.8) (BH: $p < 0.01$), between G2 (mean: 1.9; SD: 0.5) and CG (BH: $p < 0.01$), and G3 (mean: 1.7; SD: 0.6) and CG (BH: $p < 0.01$). No statistically significant differences were observed when comparing G1 and G2, G1 and G3, and G2 and G3.

Tympanometry: OS improved patient outcome compared to the CG (mean: 0.5; SD: 0.9) (ANOVA: $p < 0.0001$) independent of the administration method used; specifically, we observed a statistically significant variation at T1 between G1 (mean: 1.7; SD: 0.8) and CG (BH: $p < 0.01$), between G2 (mean: 1.7; SD: 0.5) and CG (BH: $p < 0.01$), and G3 (mean: 1.7; SD: 0.6) and CG (BH: $p < 0.01$). No statistically significant differences were revealed by comparing G1 and G2, G1 and G3, and G2 and G3.

Fibroendoscopy: We did not observe any statistically significant variations between the four

groups (G1 (mean: 2.6; SD: 0.8), G2 (mean: 2.8; SD: 0.6), G3 (mean: 2.4; SD: 0.5), and CG (mean: 2.4; SD: 1.1)) at T1 for this outcome.

PTA: OS treatment improved patient outcome compared to the CG (mean: 1.7; SD: 0.7) (ANOVA: $p = 0.0040$) but only for G3 (BH: $p < 0.05$). Furthermore, for this specific finding, the outcomes in G3 (mean: 2.5; SD: 0.5) were statistically different for G3 with G1 (mean: 1.9; SD: 0.6) (BH: $p < 0.05$) and G3 with G2 (mean: 1.8; SD: 0.6) (BH: $p < 0.05$). No statistically significant variations were observed between G1 and G2, G1 and CG, G2 and CG. Figure 4 summarizes the results of the outcomes between the three groups (G1, G2, and G3) and the CG at T1 (Figure 4).

Group Comparison at T2

Outcome summary at T2 is shown in Figure 5 (Figure 5).

Otoscopy: treatment with OS improved patient outcome compared to the CG (ANOVA: $p < 0.0001$) independent of the administration method; specifically, we observed statistically significant variations at T2 between G1 (mean: 2.7; SD: 0.5) and CG (mean: 0.8; SD: 1.1) (BH: $p < 0.01$), between G2 (mean: 2.9; SD: 0.2) and CG (BH: $p < 0.01$), and G3 (mean: 2.2; SD: 0.6) and CG (BH: $p < 0.01$). Statistically significant variances were also revealed by comparing G1 and G3 (BH: $p < 0.05$) and G2 and G3 (BH: $p < 0.01$). No statistically significant differences were observed between G1 and G2.

Tympanometry: OS improved patient outcome compared to the CG (mean: 0.7; SD: 1.3) (ANOVA: $p < 0.0001$) independent of the administration method; specifically, we observed a statistically significant variation at T2 between G1 (mean: 2.7; SD: 0.5) and CG (BH: $p < 0.01$), between G2 (mean: 2.9; SD: 0.3) and CG (BH: $p < 0.01$), and G3 (mean: 2.2; SD: 0.6) and CG (BH: $p < 0.01$). A statistically significant variance was found when comparing G1 and G3 (BH: $p < 0.05$) and G2 and G3 (BH: $p < 0.01$). No statistically significant differences were observed between G1 and G2.

Fibroendoscopy: OS treatment improved patient outcome compared to the CG (mean: 2.4;

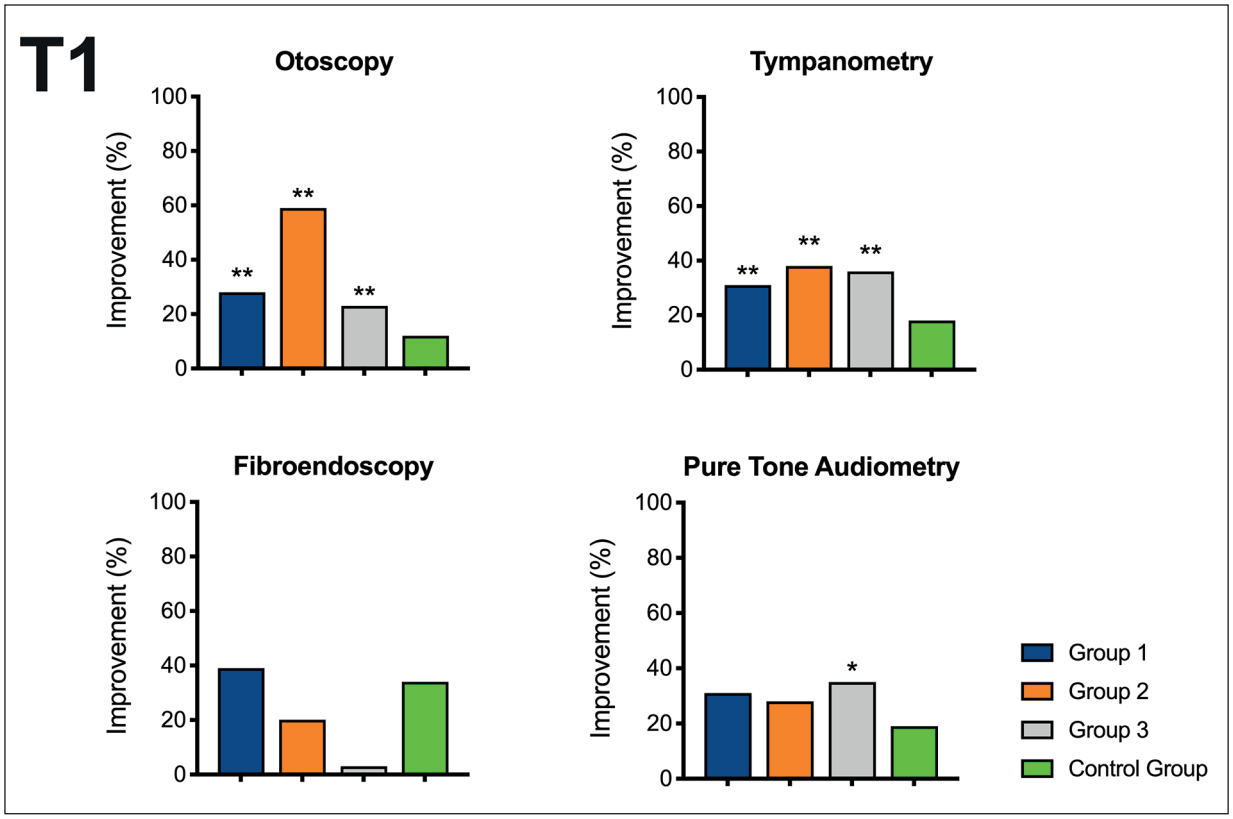

Figure 4. The changes observed for the four outcomes by comparing the groups of the study between T0 and after 45 days of treatment with oral supplements with immune-stimulating molecules (T1). Two asterisks (**) represent a p-value < 0.01, while one asterisk (*) indicates a $p < 0.05$.

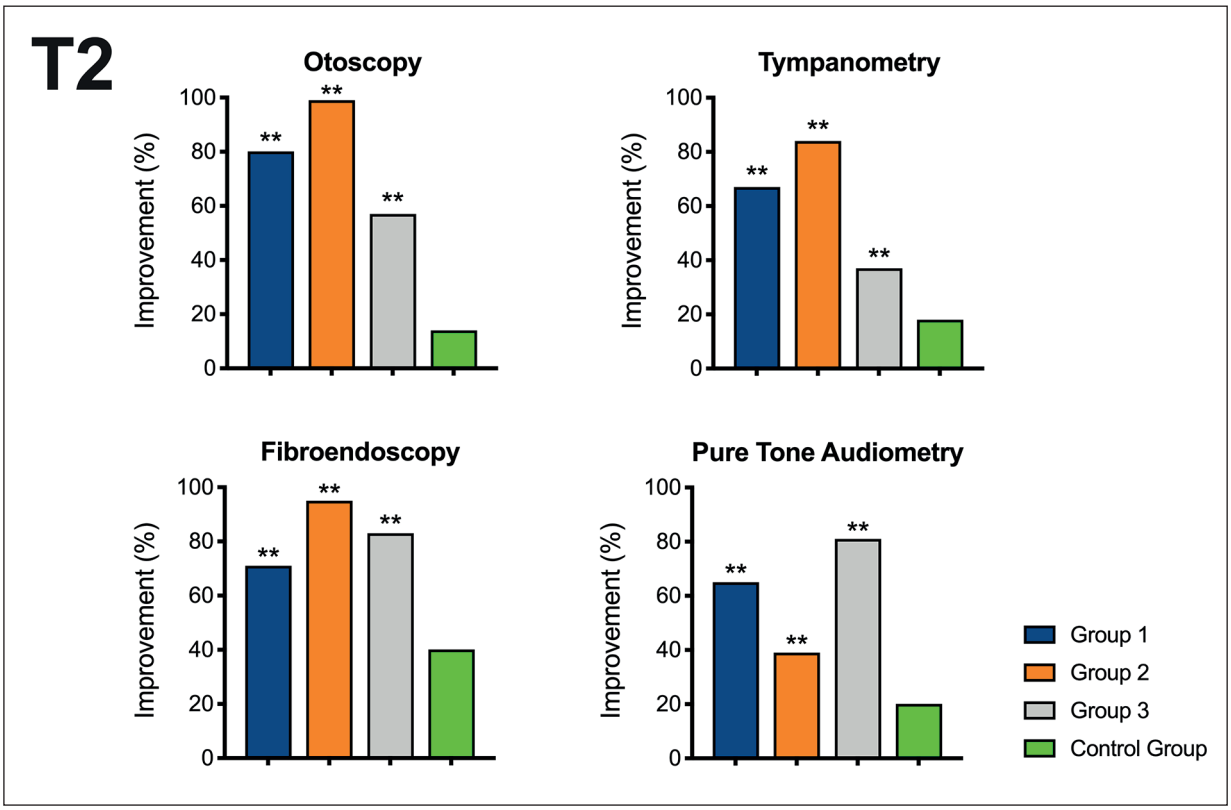

Figure 5. The changes observed for the four outcomes between the groups of the study after 90 days of treatment (T2) with oral supplements with immune-stimulating molecules or in case of G3 only after 90 days from the beginning of the study by comparing T0 *vs.* T2. Two asterisks (**) represent a p-value < 0.01, while one asterisk (*) indicates a $p < 0.05$.

SD: 1.1) (ANOVA: $p < 0.0001$) independent of the administration method; specifically, we observed a statistically significant variation at T2 between G1 (mean: 3.6; SD: 0.5) and CG (BH: $p < 0.01$), between G2 (mean: 3.8; SD: 0.4) and CG (BH: $p < 0.01$), and G3 (mean: 3.3; SD: 0.5) and CG (BH: $p < 0.01$). No statistically significant differences were found when comparing G1 and G2, G1 and G3, and G2 and G3.

PTA: OS improved patient outcome compared to the CG (mean: 1.7; SD: 0.7) (ANOVA: $p < 0.0001$) independent of the administration method; specifically, we observed a statistically significant variation at T2 between G1 (mean: 2.4; SD: 0.7) and CG (BH: $p < 0.05$), between G2 (mean: 2.8; SD: 0.4) and CG (BH: $p < 0.01$), and G3 (mean: 2.5; SD: 0.5) and CG (BH: $p < 0.01$). No statistically significant differences were revealed by comparing G1 and G2, G1 and G3, and G2 and G3.

Discussion

Our results show that children with OME treated with OS with immune-stimulating molecules presented better outcomes compared to those receiving the standard treatment alone regardless of the administration method and posology of the oral supplement. The standard treatment improved the fibroendoscopy findings but was not able to ameliorate the MT aspect, i.e., to improve the tympanometry results and to restore the PTA threshold.

Children treated with OS showed an improvement in all of the four parameters investigated. This improvement was quite homogeneous and independent of the administration method and posology of the OS when comparing T2 to T0 but some statistically significant differences between G1, G2, and G3 were present at T1 relative to T0. Furthermore, we noticed that some differences in the improvements when compar-

ing the study groups at T0 to T1 and at T1 to T2. When we singularly analyzed the variation (within comparison) in each group, we observed that children in G2 were the only ones that improved in terms of otoscopy and tympanometry at T1, while in G1 and G3 the variation did not reach a statistically significant level at the same set-point. By comparing all four groups at T1 (between comparison), we found that children in G3 were the only ones with improved PTA. In addition, we observed that in Group 3 at T1, the improvement to the MT was better than G1 and G2. All patients treated with OS presented good outcomes at T2 in all of the four outcome measures. Subjects in Group 3 showed the best results of the three groups, in fact, they presented with a prevalence of score 3 (highest result) in all four outcomes investigated. At T2, G3 children completely resolved their OME with recovery in MT, tympanogram, and hearing thresholds with complete closure of the air-bone gap (indicative of CHL). Furthermore, the fibroendoscopy results from these patients showed good patency of the rhinopharynx in terms of the reduction of adenoid tissue hypertrophy and consequent improvement of middle ear ventilation. When we analyzed the variation within the same group, G2 seemed to be the optimal treatment regimen for obtaining a quick resolution of OME as supported by the improvement of otoscopy, MT, and tympanometry. However, when we looked at the absolute best treatment between the three different doses of OS and administration methods, the highest dosage (15 ml) (G3) gave the best outcome. Furthermore, children in G3 continued to improve their outcome by remaining the best of the three study groups at T2 after treatment suspension. Overall, our results suggest that treatment with 15 ml of OS fortified with phytotherapeutic extracts with immune-stimulating action for 45 consecutive days could be considered for children with severe forms of OME and likely even as a supporting treatment for AOM. Treatment with 10 ml for 90 consecutive days may be the best option in cases of light forms of OME or for preventing inflammation of the superior airways and consequent OME. The immune-stimulating molecules in the OS administered in the study groups stimulate the immune system function [21-24] by improving the white cells functions and by increasing the IgA in the mucus. *S. nigra* inhibits viral replication in the early stages of infection [25] by reducing the aggressive capacity of the virus. The vitamins A, C, D, and E improve the response of the immune system by increasing mucosal IgA (A and E)[26,27] and by actively stimulating macrophage and lymphocyte activity[19,28]. As with *S. nigra*, increased IgA improves the resistance of the superior upper airway tract to the virus[25], while the improved activity of white blood cells (macrophages and lymphocytes) increases the immune response when the infection reaches the blood[29].

Lactobacillus acidophilus presents several benefits on health. At first, it reduces the level of systemic inflammation and the concentration of reactive oxygen species (ROS) that depress the answer of the immune-system[30] by inducing an indirect immune-stimulation; secondly, *lactobacillus* inhibits the adhesion and the growth of gram-negative bacteria[31] and inhibits the viral growth and the penetrance of virus inside the cells[32] by protecting the subject from viral and bacterial aggression.

The results of immune-stimulating OS treatment in children reported in this study are consistent with results observed in adults[15,16,18]. The improvement in the immune response was showed by a reduction in the volume of the adenoid tissue indicative of the resolution of the infective/inflammatory process[33]. The decrease in the inflammation of the adenoid tissue acted on the resolution of OME by reducing the production of mucus and by restoring a healthy space in the rhinopharynx. The combination of these conditions allowed for the restoration of middle ear ventilation[4,33] and the recovery of healthy hearing function, as indicated by the PTA results.

Conclusions

The use of OS fortified with phytotherapeutic extracts with immune-stimulating action should be considered as a supporting therapy both for UAI and OME. In the present work, OS reduced the viral aggressiveness, improved the immune response, and helped the recovery process. Further studies to evaluate the effect of OS with immune-stimulating molecules directly on specific subsets of immune cells are necessary.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding Declaration
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