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Comparison of a Hypertonic Tannin-rich Solution vs 3% NaCl Solution as Treatment for Rhinosinusitis

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ABSTRACT

Rhinosinusitis (RS), chiefly viral in origin, is increasingly widespread, and puts heavy financial burdens on society. Treatments range from home remedies and alpha-adrenergic agonistic decongestants to antibiotics, corticosteroids, and even surgery. Much discomfort, pain, individual and societal cost, could be avoided through efficient, judicious and cost-effective treatments. In absence of a specific and effective treatment for RS, saline water nasal irrigation is considered safe and beneficial. In a simple scientific approach to find a novel remedy that would target not only the symptoms but also the causes of RS, a tannin-rich hypertonic solution for nasal spray was formulated and evaluated for efficacy against traditional saline. Tannin-rich plant extracts (procyanidins or PCDs) showing anti-bacterial, influenza virus-neutralizing activity were identified *in vitro*, and incorporated into a hypertonic glycerol solution. A pilot randomized, single blind trial was conducted on 113 RS patients not undergoing any other treatment: 51 patients received a 3% NaCl solution as placebo saline spray (PSS), while 62 patients received the PCD-containing, hypertonic solution. 3-4 sprays of the products were applied twice daily for 21 days (maximum) or until recovery. Rhinosinusitis symptom severity scores were recorded. Although PSS proved beneficial, the PCD-glycerol solution produced a much greater, statistically significant improvement with regard to speed and degree of symptom reduction, leading to a lesser need for antibiotherapy. Results show that the use of a non-irritant hypertonic solution containing specific tannins represents a new, efficient, safe and cost effective approach to treat RS.

Keywords: Biofilm, hypertonic, glycerol, procyanidins, rhinosinusitis, tannins.

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INTRODUCTION

Rhinosinusitis (RS) is an inflammation of the sinonasal passage, comprising the nasal cavity and the sinuses, which are mucosa-lined, air-filled cavities within the cranial bones.

Although it may sometimes be of bacterial, fungal or allergic origin[1,2], RS usually starts as a viral infection, following a common cold and involving the influenza, parainfluenza, rhino or corona viruses. The virus enters the nasal sinuses, infects mucosal cells and multiplies, liberating an important amount of free virus particles into the sinus cavity. These virions attack new healthy cells and spread the infection. The initial viral infection is then often followed by bacterial superinfection, formation of bacterial biofilm and blockage of sinus openings, which may transform Acute Rhinosinusitis (ARS) into Chronic Rhinosinusitis (CRS). The biofilm is described as sessile bacteria (15%) embedded in a polymeric substance or matrix (85%) containing polysaccharides, proteins and bacterial wall debris.[3] The nasal mucosa responds to infection through increased mucus production and recruitment of mediators of inflammation, which cause congestion and swelling of the sinuses and nasal passage.[4] The diameter of the sinus openings leading to the nasal cavity is further blocked by the bacterial biofilm, eventually resulting in complete sinus blockage and development of ARS or CRS symptoms.[5] As it blocks the sinus openings, the biofilm creates an infection-prone environment as well as a microbial reservoir, and should therefore be the key target when treating rhinosinusitis.[6]

Theoretically, treating Rhinosinusitis should consist in opening the sinonasal passage, accelerating nasal drainage and eliminating bacterial and viral contaminants from the sinuses. Unfortunately, the poor vascularization of sinuses renders systemic treatments ineffective, while their location hinders topical medication.[7] Currently, only symptomatic treatments, particularly saline nasal wash, and eventually antibiotherapy, are accepted as the most effective therapies.[8] Since there is a lack of effective topical antiviral drugs for RS, we focused our research in that direction, and sought novel means to remove the source of RS infections.

Tannins, widely available in the plant kingdom, are big molecules known to possess strong binding properties with several proteins and macromolecules, including bacterial lipopolysaccharides (LPS).[9,10] On the other hand, the surface of certain viruses presents several glycoprotein (GP) structures, such as hemagglutinin and neuraminidase (H and N) on the influenza virus, involved in the virus' entry into the cells.[11] As tannins are known to bind with such molecules and may therefore represent a very simple means of neutralizing viruses and bacteria, the aim of our research was to identify specific tannin-rich plant extracts (PCDs)

showing affinity for viral GPs and bacterial LPS. These PCDs were then incorporated into a hypertonic solution containing glycerol, as described by Shrivastava.[12] It was postulated that glycerol, being much more hypertonic than saline, yet non-irritant, should break open the biofilm and drain the contaminants, while tannins should bind to bacterial LPS and viral GPs to minimize intra-sinus infection. The hypertonic, PCD-containing solution, specifically formulated for the treatment of RS, was identified as NS-2, and a pilot clinical trial was conducted to evaluate its efficacy against a 3%NaCl solution (NS-1 or PSS) commonly employed as nasal wash.

MATERIALS AND METHODS

1. Preparation of PCD-rich plant extracts: 136 tannin-rich plants were selected, and PCD-rich extracts were prepared, as described by Khanal et al.[13] The percentage of tannins in the plant extracts varied between 40% and 55% (w/w). Extracts were atomized by drying, and then diluted in the culture medium or in the test product base before use.
2. Pre-selection of PCDs: Extracts were initially screened for cytotoxicity, as described by Shrivastava et al,[14] and antibacterial activity,[15,16] as per the protocol published in the European Pharmacopeia's 7th Edition, using the *Staphylococcus aureus* strain commonly implicated in RS. Seventy-two (72) non-cytotoxic (at concentration up to 100 µg/ml), water-soluble plant extracts showing moderate to high antibacterial activity were then selected to evaluate their specific antiviral properties.
3. Virus cell Culture: Cell Culture models, where cells remain exposed to the external environment, were used to mimic topical viral infection. MDCK (Madine-Durby canine kidney, ATCC, USA), influenza virus-sensitive, cells were grown *in vitro*.
4. Virus Titer: To determine appropriate 50% or 100% virus tissue culture infective dose ($TCID_{50}$ or $TCID_{100}$), MDCK cell cultures were infected with different virus concentrations, as described by Shrivastava.[17]
5. Evaluation of anti-viral activity of the plant extracts: To evaluate viral GP-PCD binding, preselected PCDs were homogenized in Dulbecco's Modified Eagle's Medium (DMEM) (50 µg/ml) and pre-incubated in a test tube with $TCID_{100}$ virus concentration at 37°C for 1h to allow PCD-viral GP interaction. MDCK cells were then infected with this PCD-virus suspension and virus titer was measured. Four plant extracts, showing maximum virus-neutralizing effect (between 50-60%), were then associated with each other at half

concentration (25 µg/ml), and the PCD association (VB-PCDs) capable of neutralizing 100% of virus growth *in vitro* was finally selected.

6. Preparation of NS-2: According to a formula based on *in vitro* results, VB-PCDs (0.56%) were thoroughly mixed in a hypertonic solution of glycerol (32.49%) and water (66.95%), as described by Shrivastava et al.[18] The transparent, slightly viscous solution was filled into 15ml, low density, polyethylene containers fitted with a sprayer for nasal application, and labelled NS-2. Identical containers, labelled NS-1, were filled with 3% NaCl solution and used as PSS.
7. Safety evaluation: Conformingly to norm NF ISO 10993, toxicity, irritation and sensitization studies had been undertaken by BIOMATECH, Elevage Scientifique des Dombes and VITROBIO laboratories, *in vitro* and *in vivo* (all possible steps being taken to avoid animal suffering at each stage of the testing), prior to any human testing. Those tests demonstrated the safety of the specific NS-2 formulation.

Clinical trial

Objective of the study: Evaluate the efficacy of a hypertonic solution containing 4 selected antiviral and antibacterial PCDs for the treatment of the clinical RS manifestations in ARS or CRS patients. Effects on requirement for antibiotherapy were also observed.

Design

Single blind, randomized, pilot clinical trial, conducted at Bhavan Hospital & Research Centre, Manoramaganj, Indore (M.P.), India, between 12/2010 and 09/2011. All patients visiting the hospital and diagnosed for ARS or CRS were screened for inclusion in the study.

Ethical aspects

After approval of the Institutional Review Board/ Independent Ethical committee, and the Indian Council of Medical Research (ICMR), this pilot study was conducted according to GCP (Good Clinical Practice) and principles laid down in the 1964 declaration of Helsinki and its later amendments. The investigative institute is authorized to conduct clinical trials and is regularly inspected by the regulatory authorities. Only the subjects who gave informed consent were included in the study.

Setting and participants

The main inclusion criteria required for the patients to:

- give informed consent to participate in the trial;
- belong to the 18-70 age group;

- complain of clinical manifestations of RS, with a minimum RS severity (RSS) score of 10 out of 20. RSS was calculated as sum of the scores assessing the severity, on a 0 (not present) to 4 (very severe) scale, of the following symptoms: 1) purulent nasal discharge; 2) nasal obstruction or congestion; 3) maxillary pain; 4) headache and swelling around the eyes; 5) sore throat or pharyngitis.

Patients were excluded if one or more of the following criteria applied:

- pregnant or breast-feeding women, and women planning on becoming pregnant at the time of the study;
- already under treatment for respiratory infection;
- having taken antibiotic, anti-histaminic or steroid drugs in the previous 14 days;
- suffering from asthma;
- RS of known fungal or allergic origin;
- abnormal structural narrowing of the sinus passages, such as a deviated septum or any other kind of anatomical obstruction, or CRS with nasal polyps. When patient was diagnosed with CRS during screening, endoscopic examination (of middle and anterior ethmoid sinuses and middle turbinate) was performed, and CRS patients presenting those signs were excluded.

Randomization Process

During the screening visit, enrolled patients were randomly assigned by the investigator to 2 groups, using a randomization sequence based on a random-number table (1 to 200), with a predetermined reading direction. Even numbers were allocated to the first group (NS-1: PSS) and odd numbers to the 2nd group (NS-2). Product identity was known to the investigator but not to the patients. Population distribution was homogenous between the groups with respect to age, sex, body mass index, symptoms and type of rhinosinusitis (ARS/CRS) (Table 1). Medical history and endoscopic examinations were used to classify the patients as ARS or CRS. PSS group comprised 17 ARS and 28 CRS patients; NS-2 group comprised 22 ARS and 36 CRS.

For all intents and purposes the patients were randomized fairly evenly according to initial clinical symptoms.

Number of patients

After initial eligibility assessment of 127 patients, 113 patients were randomized, and 45 participants were finally analyzed as per the protocol in the NS-1 (PSS) group, and 58 in the NS-2, active treatment, group (Figure.1 consort flow chart).

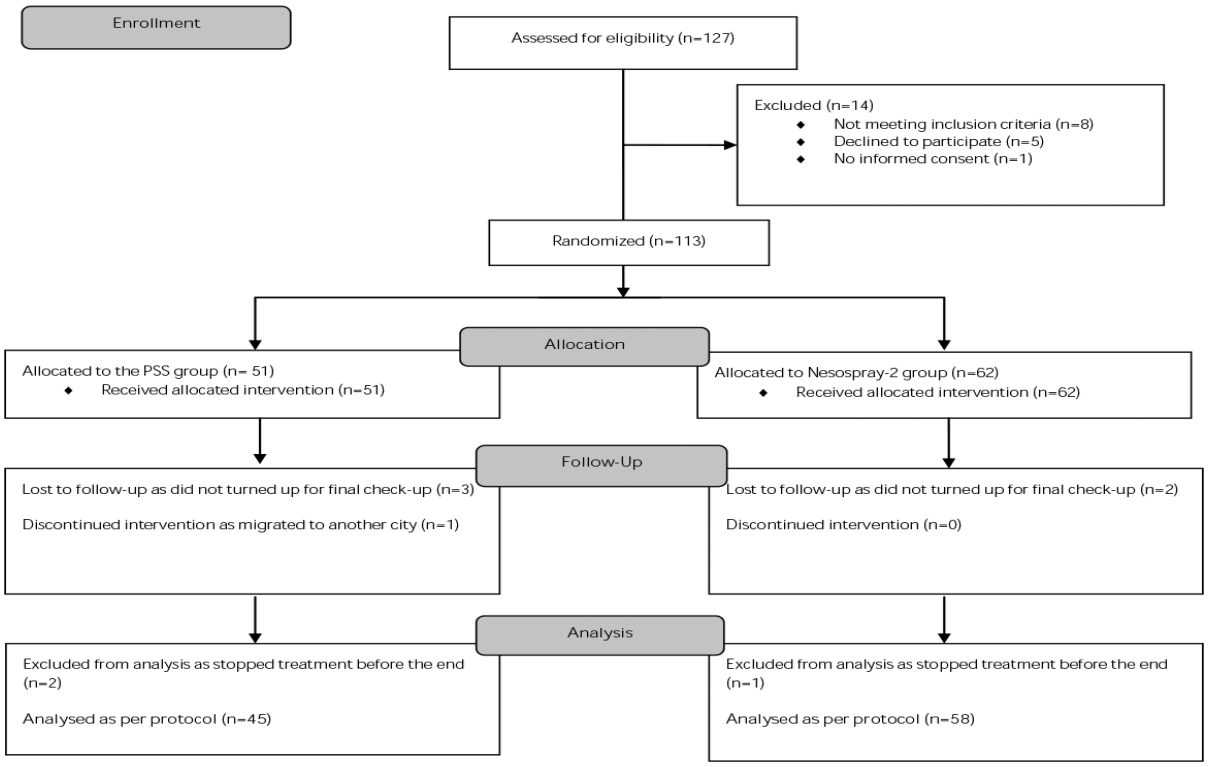

Figure.1 : CONSORT flow of patients and dataset for analysis

As this was a pilot trial, it was decided to enroll a minimum of 40 patients in each group. Since the efficacy of 3%NaCl is thoroughly documented in scientific literature, the investigator deliberately allotted more participants to the NS-2 test group (56.3%) than to the PSS control group (43.7%), so as to have more data for the new treatment. Every 10th patient (in chronological order) was therefore moved from the PSS group (n=51) to the NS-2 group (n=62).

Product application

Patients were asked to spray the product into the nasal cavity (3-4 sprays per application), twice a day, for a maximum period of 21 days or until complete recovery. Each spray delivering approximately 0.1ml of product, and the pressure exerted on the sprayer being subject to slight fluctuation, the dose per application varied between 0.3 and 0.4 ml. As the process was identical in both groups, eventual variations were considered acceptable. Assessment of compliance: During the study, patients were monitored by the chief investigator and three patient-assigned doctors.

Main Outcomes Measured

After recruitment, patient's medical history was recorded in the patient's observation table. Each patient was then asked to assess intensity of: a) nasal congestion, b) runny nose, c) sinus pain upon pressure on the face, and d) overall RS condition, on a 0 to 4 scoring scale, where 0 indicated no symptoms, 1-mild, 2-moderate, 3–severe and 4–very severe symptoms.

Table 1: Demographic and clinical characteristics of participants at study outset

Characteristics	Placebo Saline Solution (PSS) group (n=45)	NS-2 group (n=58)	Study Mean (n=103)
Age: Mean (SD)	33.5 ± 13.4	36.9 ± 12.8	35.2 ± 12.9
Sex (men/women): n (%)	26 (58%) / 19 (42%)	36 (62%) / 22 (38%)	62 (60%) / 41 (40%)
Body Mass Index in kg/m^2; mean (SD)	21.1 ± 4.4	23.5 ± 6.2	22.7 ± 5.9
ARS/CRS: n (%)	17 (37. 8%) / 28 (62.2%)	22 (37.9%) / 36 (62.1%)	39 (37.9%) / 64 (62.1%)
Nasal congestion: n (%)	36 (80%)	51 (88%)	87 (84%)
Rhinorrhea: n (%)	35 (78%)	49 (84%)	84 (82%)
Sinus and maxillary pain: n (%)	38 (84%)	44 (76%)	82 (80%)
Rhinosinusitis Severity Score (SD)	12.81 ± 2.1	12.46 ± 3.3	12.6 ± 2.7

Table 2: Mean scores (maximum 4) obtained on day 0 (before the start of treatment), 30 minutes after the first product application and from day 1 up to day 21, for nasal congestion, runny nose, sinus pain and overall rhinosinusitis grade, in the placebo saline solution (PSS, n=45) and NS-2 (n=58) groups ± *SD* of the mean. % change indicates mean difference within the same group (NS-2 or PSS) compared to day 0 (pre-treatment) values or between the NS-2 and the PSS group at a given time-point. Values in bold indicate statistically significant ($p<0.05$) difference.

		Day 0	30min	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 14	Day 21
Nasal congestion	**PSS**	3,378	2,689	2,756	2,778	2,733	2,711	2,400	2,244	2,356	2,289	1,867
	± *SD*	*0,535*	*0,557*	*0,802*	*0,517*	*0,751*	*0,626*	*0,657*	*0,83*	*0,484*	*0,626*	*0,786*
	NS-2	3,224	2,224	1,914	1,707	1,379	1,293	1,172	1,086	0,845	0,569	0,241
	± *SD*	0,893	0,773	0,904	0,918	0,952	0,817	0,861	0,884	0,834	0,652	0,432
	% change NS-2 vs day 0	**-**	**-31,02%**	**-40,63%**	**-47,05%**	**-57,23%**	**-59,89%**	**-63,65%**	**-66,32%**	**-73,79%**	**-82,35%**	**-92,52%**
	% change PSS vs day 0	**-**	**-20,40%**	**-18,41%**	**-17,76%**	**-19,09%**	**-19,75%**	**-28,95%**	**-33,57%**	**-30,25%**	**-32,24%**	**-44,73%**
	% change NS-2 vs PSS	**-4,56%**	**-17,29%**	**-30,55%**	**-38,55%**	**-49,54%**	**-52,31%**	**-51,17%**	**-51,60%**	**-64,13%**	**-75,14%**	**-87,09%**
	p-value	**0.1198**	**0.0004**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**

Runny nose	**PSS**	1,400	1,844	1,667	1,222	1,289	1,400	1,644	1,289	1,644	1,111	0,867
	± *SD*	*0,72*	*0,737*	*1,168*	*0,85*	*0,661*	*0,751*	*0,883*	*0,727*	*0,957*	*0,714*	*0,726*
	NS-2	1,224	3,241	2,638	1,845	1,621	1,466	1,397	1,241	0,569	0,569	0,414
	± *SD*	0,974	0,683	0,873	0,854	0,97	0,754	0,954	0,924	0,624	0,652	0,563
	% change NS-2 vs day 0	**-**	**164,79%**	**115,52%**	**50,74%**	**32,43%**	**19,77%**	**14,13%**	**1,39%**	**-53,51%**	**-53,51%**	**-66,18%**
	% change PSS vs day 0	**-**	**31,71%**	**19,07%**	**-12,71%**	**-7,93%**	**0,00%**	**17,43%**	**-7,93%**	**17,43%**	**-20,64%**	**-38,07%**
	% change NS-2 vs PSS	**-12,57%**	**75,76%**	**58,25%**	**50,98%**	**25,76%**	**4,71%**	**-15,02%**	**-3,72%**	**-65,39%**	**-48,78%**	**-52,25%**
	p-value	**0.0364**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**0.0005**	**0.3397**	**0.0010**	**0.4644**	**P<0.0001**	**P<0.0001**	**P<0.0001**
Sinus pain	**PSS**	2,667	2,622	2,600	2,600	2,444	2,222	1,800	1,444	1,244	0,778	0,311
	± *SD*	*0,674*	*0,65*	*0,889*	*0,863*	*0,624*	*1,106*	*0,842*	*0,967*	*0,857*	*0,902*	*0,468*
	NS-2	2,793	2,6	1,155	0,966	0,586	0,379	0,259	0,345	0,414	0,397	0,138
	± *SD*	0,614	0,598	1,04	0,878	0,531	0,489	0,442	0,479	0,563	0,591	0,348
	% change NS-2 vs day 0	**-**	**-6,91%**	**-58,65%**	**-65,41%**	**-79,02%**	**-86,43%**	**-90,73%**	**-87,65%**	**-85,18%**	**-85,79%**	**-95,06%**
	% change PSS vs day 0	**-**	**-1,69%**	**-2,51%**	**-2,51%**	**-8,36%**	**-16,69%**	**-32,51%**	**-45,86%**	**-53,36%**	**-70,83%**	**-88,34%**
	% change NS-2 vs PSS	**4,72%**	**-0,84%**	**-55,58%**	**-62,85%**	**-76,02%**	**-82,94%**	**-85,61%**	**-76,11%**	**-66,72%**	**-48,97%**	**-55,63%**
	p-value	**0.5276**	**0.7538**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**
Overall rhinosinusitis	**PSS**	3,156	2,444	3,200	3,378	3,267	3,067	2,956	2,800	2,600	1,556	0,822
	± *SD*	*0,638*	*0,624*	*0,588*	*0,49*	*0,688*	*0,688*	*0,796*	*0,815*	*0,751*	*0,867*	*0,777*
	NS-2	2,914	2,793	1,19	1,138	0,879	0,655	0,759	0,741	0,586	0,466	0,138
	± *SD*	0,904	0,744	0,712	0,868	0,818	0,608	0,802	0,664	0,726	0,503	0,348
	% change NS-2 vs day 0	**-**	**-4,15%**	**-59,16%**	**-60,95%**	**-69,84%**	**-77,52%**	**-73,95%**	**-74,57%**	**-79,89%**	**-84,01%**	**-95,26%**
	% change PSS vs day 0	**-**	**-22,56%**	**1,39%**	**7,03%**	**3,52%**	**-2,82%**	**-6,34%**	**-11,28%**	**-17,62%**	**-50,70%**	**-73,95%**
	% change NS-2 vs PSS	**-7,67%**	**14,28%**	**-62,81%**	**-66,31%**	**-73,09%**	**-78,64%**	**-74,32%**	**-73,54%**	**-77,46%**	**-70,05%**	**-83,21%**
	p-value	**0.0365**	**0.0205**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**

Initial observations were made by the patient in collaboration with the investigator to explain the modalities of treatment administration and ensure that the RS symptom severity (RSS) scoring system was understood. Thereafter, observations were made by the patient alone (days 1, 2, 3, 4, 5, 6, 7, 14 and 21). Any adverse side effect was also recorded by the patient, and monitored. Antibiotherapy during the study was authorized when judged necessary by the doctor assigned to the patient; the starting date and duration were then recorded in the patient's table. The mean RSS for the study was 12.6 (out of 20), indicating that the participants suffered from severe RS. Rhinosinusitis parameter scores for each time-point are summarized in Table 2.

Statistical analysis

Clinical data were analyzed using SAS 9.1.3. statistical software. Descriptive statistics, i.e. mean standard deviation (SD), minimum and maximum frequency distribution, were used for analysis of demographic details, clinical evaluation, and medical history parameters. If the p-value was greater than 0.05, the results were considered not significant.

RESULTS AND DISCUSSION

Viral and bacterial Rhinosinusitis, affecting millions of people, causes huge economic losses.[19] Although this infection is not initially life-threatening, ARS may lead to CRS and serious health consequences.[20,21] Symptomatic treatments provide limited relief,[22] while antibiotherapy presents the risk of increased bacterial resistance.[23] Saline nasal irrigation is a widely accepted symptomatic treatment[24,25] but the efficacy of a 3%NaCl solution remains limited.[26]

Bacterial biofilm, blocking the sinus openings and preventing medical treatment from reaching the sinuses, thus maintaining sinus infection, is the key hindrance to treating RS. The poor vascular supply and the anatomical structure of the sinus cavities equally hamper any approach to remove sinus contaminants. Consequently, breaking the biofilm to open the sinuses and to relieve intrasinusal pressure constitutes a fundamental requirement for the treatment of RS. Unfortunately nasal mucosa is not keratinized and is extremely sensitive to irritation, limiting the use of any mechanical or chemical device to break the biomembrane open. In absence of an effective drug, currently only 3.4% NaCl solutions or sea water are employed as nasal washes to alleviate nasal congestion. However, the osmotic pressure exerted at this NaCl concentration is insufficient to rupture the biofilm, while higher concentrations of NaCl or other, similar, solutes are irritating to the nasal mucosa. Therefore, we used the maximum non-irritating concentration of glycerol, with a total solute concentration of 3.47 compared to 0.58 for sea water (3.4% NaCl), and exerting nearly 5.9 times higher osmotic pressure on the biofilm than PSS. In effect,

the osmolality of 3.4% NaCl (molecular weight: 58.4) is 5 to 6 times lower compared to NS-2 which contains 32.49% Glycerol (molecular weight: 92.1).

We then proceeded to enhance this hypertonic glycerol solution's properties by adding PCD-rich plant extracts. Although a few tannin-rich plant drugs have shown capacity to reduce symptoms of RS,[27,28] recourse to phytotherapy as RS treatment is very limited, mostly because, at non-irritant concentrations, solutions with plant preparations are not sufficiently hypertonic to break the biofilm, and the observed beneficial effects are solely due to the presence of tannins and their capacity to bind with macromolecules. Tannins are very big molecules with a complex chemical structure, and present the advantage of being inert, non-toxic, non-irritant, and without any biological or pharmacological interaction with the nasal mucosa.[29] Since *in vitro* experiments conducted for tannin selection show that tannins are much more specific in their binding to viral glycoproteins than to bacterial polysaccharide membranes, we first selected those PCDs known to have antibacterial effect, and then focused on these PCDs' virus binding properties to restrict final selection to PCDs presenting optimal dual activity. We then added the selected, specific, tannin-rich plant extracts to the hypertonic glycerol solution to conjugate their respective properties in order to effectively clear the sinuses from infectious agents.

Based on the results obtained for all symptoms observed in this study, NS-2 showed statistically significant differences in efficacy compared to PSS ($p<0.005$), often right from day 1.

PSS sprays did relieve nasal congestion, with nearly 20% reduction on days 1 to 4, around 30% on days 5 to 14, and up to 45% on day 21, compared to day 0. However, despite a marked, progressive symptomatic improvement, PSS' decongestant effects were limited, and for a majority of patients (89.3%), complete relief of the symptom was not observed during the treatment period. NS-2 was only slightly more effective than PSS in the first 30 minutes ($p=0.004$), but showed much accrued and significant effect thereafter ($p<0.005$), as the mean reduction in nasal congestion was nearly 41% on day 1, and nearly 74%, 82% and 93% on days 7, 14 and 21, respectively, compared to pre-treatment. From day 7, the mean score goes below the 1-mark, and for CRS and ARS patients alike, the tendency towards symptom resolution is confirmed in the following two weeks, with over 90% of ARS and 80% of CRS patients reporting absence of symptom. Results indicate that NS-2 induces very early and progressive nasal decongestion, proving significantly more active than PSS, this outcome being consistent with changes observed in rhinorrhea.

In the PSS group, a strong increase in nasal discharge was recorded 30 minutes after first application (+32%) and on day 1 (+20%). However, this briefly increased nasal mucus fluidness

following each PSS application did not lead to any significant effect on nasal mucus flow in-between applications, and only in the third week of treatment did nasal discharge diminish to a final mean value 38% lower than pre-treatment. In the NS-2 group, a striking increase in nasal discharge was observed right after first product application (+165%) and on day 1 (+115%), compared to pre-treatment values ($p<0.005$). This extremely strong evacuation of mucus started about 20 minutes after application and lasted for 5 to 6 hours. Early discharge was also notably thicker, thinning progressively over the next days. The extreme nasal discharge observed within 30 minutes of NS application in the first 2-3 days, with subsequent reduction in RS symptoms, followed by return to normal discharge in the second and third weeks of treatment and concurrent relief in overall condition, suggest that the sinonasal passage has probably been opened, sinuses have been emptied, and intrasinusal pressure has been relieved, due the rupture of the sinus-blocking biomembrane. Endoscopy was performed on some CRS patients at the end of the trial, confirming our hypothesis, but this examination was not conducted systematically in every completely recovered patient.

As a consequence of relief in intrasinusal pressure, the associated pain was also alleviated.

In the PSS group, a gradual lessening of sinus pain was noticed from day 1 up to end of the study. The pain reduction was 53%, 71% and 88% on days 7, 14 and 21 respectively, compared to pre-treatment, indicating that saline wash is significantly effective in reducing sinus pain, but that the effects are slow and spread over time. In the NS-2 group, a statistically insignificant ($p=0.75$) change occurred in the first 30 minutes (-7%). Afterwards, however, pain scores showed statistically significant differences comparatively to PSS group, as the reduction was nearly 59%, 65%, and 79% on days 1, 2 and 3, respectively, compared to day 0. Most patients had nearly no residual pain from day 5, as mean reduction in severity then remains 85 to 95% compared to pre-treatment. Globally, PSS took more than twice the time, compared to NS-2, to bring sinus pain to comparably low levels (0.3/4).

From the reduction in the prominent symptoms associated with RS should ensue amelioration in general condition. In PSS patients, the alleviation of symptoms noticed immediately after treatment application does not appear to concretize into lasting effects since mean value remains the same on days 0 and 1, and a slight worsening in overall condition is registered on days 2 and 3. After day 7, however, overall RS severity progressively subsides, reduced by 51% and 74% on days 14 and 21, respectively. In contrast, after a statistically insignificant ($p=0.02$) initial reduction in overall RS, NS-2 strongly decreased overall RS severity from day 1 (-59%), with further incremental improvement up to the end of treatment, for both CRS and ARS patients.

Remarkably, it took 4 to 5 days for NS-2 to achieve considerable global amelioration (by around 75%) versus 21 days for similar results with PSS.

A secondary endpoint of our study was to observe whether the use of the PCD-containing NS-2 solution had any effect on requirements for antibiotherapy, often resorted to in the course of treating RS. Among the 136 tannin-rich plant extracts we initially screened, 72 extracts showed at least 2 log reduction in *S. aureus* growth while having no cytotoxic potential at the concentration of 100µg/ml in the culture medium. Among those extracts, only 8 were found to induce between 45% and 56% inhibition of virus growth. When 4 of these extracts were associated with each other (25 µg/ml), 100% virus growth inhibition was observed. This association, called VB-PCDs, was used to prepare NS-2. Addition of tannins to the hypertonic solution appears to reduce intrasinusal microbial activity as our results prove that this approach cuts by half the requirement for antibiotherapy, and shortens its duration. Antibiotherapy (oral) was prescribed when the patient's assigned doctor suspected risks of subsequent pulmonary infection. Choice of antibiotics included amoxicillin and amoxicillin/clavulanic acid combination (500mg t.i.d). Recommended duration was typically 5 to 10 days, with prolonged treatment for refractory infections. As shown in Figure. 2, 18/45 patients (40%) in PSS group and 12/58 (21%) in NS-2 group necessitated antibiotherapy during the study period, with average duration of 10.5 days in PSS group versus 7.41 days in NS-2 group. Some patients, (n=5 in PSS group, n=6 in NS-2 group) required antibiotics very early in the trial (between days 0 and 2). As the trial progressed, there were only 6 (10.35%) instances of later antibiotherapy initiation in NS-2 group (days 5 to 12), compared to 13 (28.9%) in PSS group (days 4 to 14). Among those were 6 PSS patients initially diagnosed with ARS (35.3%), versus only 2 (9%) for NS-2. In NS-2 group, later antibiotics recipients were mostly CRS patients. These findings denote a correlation between the early and consistent symptomatic improvement of RS obtained with NS-2 treatment and an apparent lesser need (by nearly 50%) for antibiotherapy, compared to PSS group.

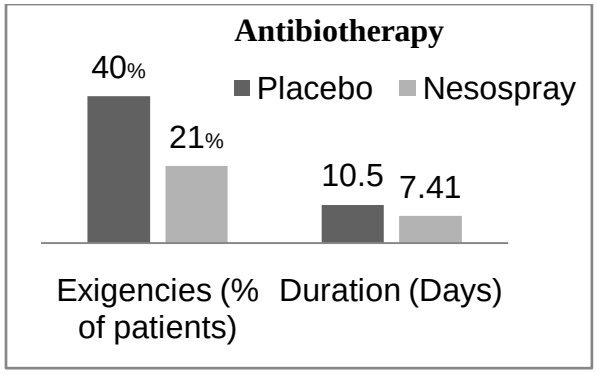

Figure.2: Antibiotherapy requirement

Percentage of population requiring antibiotic therapy (1a) and mean duration of treatment (1b) in PSS group compared to NS-2 group during the study period. n = 45 in the PSS group and 58 in the NS-2 group. P<0.005 compared to the PSS group.

It has already been demonstrated that specific tannin-rich plant extracts possess antiviral [30,31] properties and neutralize viral infectivity *in vitro*,[32,33] while certain tannins have strong affinity for bacterial LPS [34] and also for *Staphylococcus aureus*,[35] the two main pathogens involved in RS. We therefore strongly believe that the anti-microbial activity of NS-2 involves strong PCD-macromolecule binding.

Additionally, as tannins also conjugate with sugar molecules, they should help retain glycerol film over the nasal mucosa.

CONCLUSION

Results obtained from this comparative study demonstrate the efficacy of the PCD-containing, hypertonic solution NS-2 as treatment for Rhinosinusitis. Improvement brought forth by NS-2 varied according to symptoms: sinus pain dropped to very low scores between days 2 and 3, while nasal congestion and discharge took up to a week. Overall condition sharply improved from day 3, and comprehensive results point out to optimal treatment duration between 3 and 5 days. No side effects or allergic reaction were noticed in any of the patients, except for a slight nasal irritation reported by most NS-2 patients and lasting for 20-30 seconds following product application, which was not observed in the PSS group. Our study's results show that the novel association of glycerol and specific tannins is highly effective as topical treatment for RS. Future clinical studies might also evaluate this treatment on CRS with nasal polyps, since *S. aureus* is increasingly prevalent in that subgroup.[36] Although this study presents the limitations of involving a limited number of patients, without radiological imaging of every sinus, and concerns the use of tannin-rich plant extracts but not purified fractions of tannins, we believe that the results obtained fulfill our objectives, as the aim of the study was to evaluate the potential of a new, logical and safe approach for the treatment of RS.

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