

Research Article

Role of “NAFLAN” Immune (Nasal/ Oral) Spray in Prevention of Respiratory Virus Infections (Including COVID 19) in Indian Volunteers: The Preliminary Report of a Phase 1 Clinical Trial

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Abstract

Background & Objectives: The Immune-modulating Nutritional Supplements based on Grapeseeds rich in Sterols and Vitamins can be useful in Management of Respiratory Viral infections including COVID 19, but studies revealing their combined effects are lacking in literature. Therefore the German manufactured Oro-Nasal Immune spray (NAFLAN- A Nutritional Supplements Product) based on combination of Plant Sterols & Vitamin, B, D, E needs urgent exploration in context of Prevailing COVID 19 Pandemic across the world including India.

Methods: This clinical trial was done on 40 healthy Volunteers, as part of a Phase 1 trial Project of Pure Derm GmbH, Germany for its evaluation in Management of Respiratory Infections including COVID 19. The volunteers were selected from an NGO Hospital and this trial was enrolled at CTRI ICMR India and conducted according to WHO, PATH & CONSORT guidelines. Trial was done from 15th March 2021- 15th June 2021 in which this Immune spray was used for 1 month (1st May 2021- 31st May). This Clinical trial included all types of COVID 19 including Indian Mutation Delta Variant.

Results: Symptoms such as Cough and Diarrhoea were reduced significantly after use of Immune(Naflan Spray) [$\chi^2 = 9.25$ (Yates Correction), $P < 0.05$, RR Adjusted=1.8, 95% C.I=1.5-2.1], but no effect on high fever, headache, and Common cold were seen.

Interpretation & Conclusions: Nafalan Spray can be useful in Prevention of Cough in Respiratory Infections (possibly also COVID 19). However Large scale Multicentric trial is however advocated by authors before reaching a more definite conclusion of role of Nafalan Spray in Prevention of COVID 19 Infection.

Introduction

Currently uncontrolled Covid 19 Pandemic across the world has compelled a rapid exploration of Immune Modulatory foods available in Nature. One such compound found in Nature is Sterols found in Plants. Sterols are crucial lipid components that regulate

membrane permeability and fluidity and are the precursors of bioactive steroids, from which there is a high possibility of their potential role in Immune Modulation. Beta-sitosterol (BSS) and its glycoside (BSSG) are sterol molecules which are synthesized by plants. In animals, BSS and BSSG have been shown to exhibit anti-inflammatory, anti-neoplastic, anti-pyretic, and

immunomodulating activity [1]. It has also been evident from current literature that in Upper Respiratory Infections such as Chronic rhinitis and sinusitis: A small, placebo-controlled study on affected individuals indicated that this allergic condition was controlled by BSS: BSSG complex [1].

The BSS: BSSG complex is now considered a new, natural immune modulator which has demonstrated promising results in few studies [1, 2]. These important plant constituents that seem to specifically target T-helper cells and may help to restore balance between TH1 and TH2 cells. The result of this immune modulation is an increase in TH1-related cytokines, a decrease in TH2-related cytokines, increased lymphocyte proliferation, and greater NK cell activity [1]. Currently available literature also reveals that there is a reduced risk of numerous diseases with a diet high in fruits and vegetables, and indicate that specific molecules, including β -carotene, tocopherols, vitamin C, and flavonoids, confer some of this protective benefit [1]. In this regard, many Immune modulator foods are recently emerging and they are under research in COVID 19 era in many countries; such as Grape seed which is rich in phenolic compounds, fatty acids, and vitamins, with economic importance to pharmaceutical, cosmetic, and food industry.

The Grape seed in its oil form has beneficial properties for health that are mainly detected by *in vitro* studies, such as anti-inflammatory, cardioprotective, antimicrobial, and anticancer properties, and may interact with cellular and molecular pathways. These effects have been related to Grape seed oil constituents, mainly tocopherol, linolenic acid, resveratrol, quercetin, procyanidins, carotenoids, and phytosterols [3]. Few studies [4, 5] in literature also hint towards possible potential benefits of Grape seed oil in Management of Complications of Respiratory Infections such as COVID 19. In one study [4] the humans, the effects of grape seed oil consumption on inflammation and insulin resistance were evaluated in overweight/ obese women. and found that on homeostatic model assessment of insulin resistance scores, ultrasensitive C-reactive protein (us-CRP), and TNF- α decreased in the grape seed oil group. In another study Grape seed oil also showed a decrease of $8.4\% \pm 1\%$ in platelet aggregation when compared with the peanut oil (rich in oleic FA) consumption, which decreased aggregation by $10.4\% \pm 1\%$ [5].

Many studies [6-9] further reveal the potential benefits of Plant sterols from Grapes Seeds and Oil in Immune Modulation. In this regard, study [6] in literature reveal that Grape seed extract has also many properties as it contains proanthocyanin, which is a phenolic chemical belonging in the larger group of plant phytochemicals (flavonoids-a subgroup of tannins). Proanthocyanin may protect the blood vessels and cardiovascular system. It may also have an antioxidant effect. The tannins in grape seed extract are active antioxidants and antimutagenics. Because of these properties, grape seed extract may possibly also protect the lining of blood vessels and other tissues from damage from Respiratory Viral Infections Including COVID 19. One study [7] in this area also emphasize that Grapeseed oil which comes from the pressed seeds of grapes has Anti-inflammatory, Antimicrobial, Antioxidant properties. Another study has also found that eating a diet rich in black grapes may reduce the risk of

contracting certain cancers [8] One more study [9] in this regard has also emphasized on similar role of Plant-Sterols in Immune Modulation.

There are also other studies [10-12] in literature which emphasize on roles of various nutritional products such as Vitamins, Minerals in management of Respiratory Viral Infections such as COVID-19. Among them vitamins, A and D showed a potential benefit, especially in deficient populations. Among trace elements, selenium and zinc have also shown favorable immunomodulatory effects in viral respiratory infections. Micronutrients may be beneficial in nutritionally depleted elderly population [11]. A deficiency of these vitamins and minerals in the plasma concentration may lead to a reduction in the good performance of the immune system, which is one of the constituents that lead to a poor immune state [12].

Studies [13-14] from literature show promising roles of Vitamin E & D on Immune Functions. One study [13] who examined the role of vitamin E on the immune system, found that all forms of Vitamin E apart from α -tocopherol; other tocopherols as well as tocotrienols, have immunomodulatory functions. Another study [14] on Vitamin D have shown Promising results on Immune Functions. Vitamin D metabolizing enzymes and vitamin D receptors are present in many cell types including various immune cells such as antigen-presenting-cells, T cells, B cells and monocytes. *In vitro* data show that, in addition to modulating innate immune cells, vitamin D also promotes a more tolerogenic immunological status. *In vivo* data from animals and from human vitamin D supplementation studies have shown beneficial effects of vitamin D on immune function [14]. Few studies [13-14] also emphasize that future research should also characterize the effects of non-tocopherol vitamin E & D on immune cell function as well as their potential clinical application [14]. Several nutraceuticals and probiotics may also have some role in enhancing immune functions. One review study [15] on Nutraceuticals also led to the conclusion that the evidence of the efficacy of nutraceuticals is heterogeneous, and for some of these products, there is no information in the literature to recommend their use.

Moreover, Antiseptic gargling and nasal antiseptics are simple preventive measures that has probably been forgotten. Gargling was used to reduce upper respiratory tract infections and to treat bacterial/viral infections, but it has gone out of fashion [14]. Currently in India there are many Oral Sprays available in India Market such as that of Colgate Vedshakti Mouth Protect Spray and Oral Spray such as that of Zydus Cadilla (Viroshield) in Indian Market, is an Enzyme based Formula, which is claimed to reduce 99% viral load is available, but the long-term results of these sprays on LRTI are unknown [16]. Unlike in Europe, daily gargling has a long tradition in Japan for the prevention of respiratory infections and was strongly promoted by the Japanese Ministry of Health, Labour and Welfare during the H1N1 swine flu pandemic in 2009. It has now been explicitly recommended again to the population for daily use in the COVID-19 pandemic [17].

Hence it is desirable that there be further independent, rigorously randomized, and controlled studies that take into consideration the clinical effects, rather than the purely biological

ones, in vivo or vitro, of many molecules, both in therapy and in the prevention of respiratory infections. Therefore, the role of Oro- Nasal Immune sprays remains an unexplored area of research in context of Prevailing COVID 19 Pandemic across the world.

So, for a viral disease like COVID-19, where no pharmacological strategies for prevention or treatment are presently available and where the exact time of the ending of the alarming situation is unknown, nutritional strategies for enhancing immunity is something to be explored, this was the main reason for conducting this clinical trial on a Preparation of Plant Sterol with Vitamin B, E & Vitamin D in Germany, and hence this Clinical trial Phase 1 was conducted in India, where 2nd wave of COVID 19 caused huge mortality. Till this date no study has explored the Role of Combined effect of Plant sterols & Vitamin's combination as a Oro-nasal spray for Prevention of Respiratory Infections Including COVID 19 in Indian Population, Making it as a Unique in its own kind.

Material & Methods

Research Question

This study aimed to answer the following Research Question as per requirement of Manufacturer: Does use of NAFLAN-Immune Spray on Volunteers for 1 month reduces the Risk of Symptoms from Respiratory Infections (Including Covid 19)?

Aim of Trial

The trial finally aimed towards detection of highest dose of treatment can be given safely without side effects.

Material and equipment used

Nomenclature: NAFLAN Immune Spray **NAFLAN (THE IMMUNE CARE)** which is presumed to be able to fight with many respiratory viruses of oral cavity and nasal passages.

Manufacturers details: Pure Derm GmbH, Germany, which was given approval for marketing by State Certified Diploma Chemist in Germany (letter attached as Appendix).

Source: this nutritional Product is a food Supplement (T helper activator) In Naflan Spray the main content is Plant Sterols undertaken from Black Grape Seeds along with Vitamin E, D & B. The rationale of Synergy of Sterols and Vitamins was – it can provide best immune cure effect.

The composition of this product made for daily intake of 20 sprays as specified from company is as follows: a) Plant Sterols: 840mg, b) Vit E: 18mg α -TE (150% NRV) c) Vitamin- D: 5 μ g ((100% NRV) d) Vitamin-B: 122.5 μ g (100% NRV).

Product Safety and Quality Control Measures taken by Company

Naflan spray was developed after an official test report for sample no:20/112528-H dated 23.11.2020 and its free sales certificate of bilacon GmbH 13088(from 23.11.2020) is now considered to be marketable as a food supplement, within federal republic of germany.

The Procedures Adopted

Dosage of Spray: The recommended daily dose by manufacturer is 5x4 sprays with moutpiece for children above 5 years and Adults. This check was based on European and German Regulations.

For Prevention of Infections (Including COVID 19)

ORAL & NASAL spray (50 ml) was given to all participants for use for prevention of virus infections including covid 19. For Supporting Immune reaction and preventing the infection at least 5 Sprays (2 Nasal one per each passage & 3 Oral) 4 times per day were advised.

Assumption Criteria: Above spraying was considered enough for preventing infection migration to human body via Oral and Nasal pathways.

For treatment of any Viral Infected patients

Nasal spray frequency was used 2 times on each application (for preventing the virus immigration to Lower Respiratory Tract).

Ethical Research Quality Assurance Strategy adopted

This research was carried out in India on human volunteers, for which the ICMR's Ethical guidelines for biomedical and health research on human participants (2017) were strictly adhered.

IEC approval of Study

The permission from Ethics Committee/ Institutional Review Board (IRB) from NGO was taken for this phase 1 trial on human subjects (NGO Authorization letter attached).

Trial Registration

This Clinical Trial was registered at CTRI (ICMR- India) with registration no: REF/2021/01/040350 dated 24th Jan 2021 and it was authorized by Consent of NGO: Jain Medical Centre, Kanpur for Conduction of this study. The study protocol was approved by the institutional/local ethics committee of NGO and written consent was obtained from the participants by NGO.

Trial Conduction Method

This trial took help of **CONSORT (Consolidated Standards of Reporting Trials) 2010 guideline** in which 25 items Check list was followed for bringing transparency in conduction of Phase 1 trial.

Inclusion Criteria

Audience of Volunteer was randomly sampled in such a way that: they were healthy people but had risk of any Respiratory Infection. All participants who were at least 18 years of age (being eligible for consent), So audience of volunteer was already exposed to high risk of getting COVID 19, as COVID positivity rate of Kanpur district where this study was conducted was ranging between 12% to 16% in Month of May 2021.

Working Definition of Symptoms

Volunteer was considered symptomatic, if he/she had experienced any episode of any symptom as verified by attending

Physician at NGO in this study i.e. Sudden High fever, Cough, Diarrhoea, Headache, Cough & Cold, Muscle and Joint Pain and any skin problem.

Study Design

Study Participants: The present study was double blinded, single arm clinical trial on 40 healthy volunteers, selected randomly but those who gave consent for enrolment, thereafter, were taken in this study.

Sample Size: As per requirement of Phase 1 trial- at least 40 Volunteers were enrolled.

Place of Conduction of study

The volunteers were selected from an NGO Hospital of a Jain Medical Centre, Mahaveer Shiksha Sansthan, Gandhi Gram, 108 B Vinobha Nagar, Kanpur, Kanpur Nagar, Uttar Pradesh (208007) India and this trial was enrolled and conducted as per CTRI Guidelines of ICMR India. This NGO gave authorization and consent for Clinical Trial on Immunity Spray and registering trial at appropriate authority in India before start of this study.

Duration of Conduction of study

Study was done from 15th March 2021- 15th June 2021 in which this Immune spray was used for 1 month (1st May 2021- 31st May) by healthy Volunteers. This Phase 1 trial on Volunteers was done as per the following Gantt Chart Method.

Clinical Trial Registration at CTRI(ICMR) India	24th Jan 2021
Recruiting of subjects,	Started from 15th March 2021*
No of Subjects	40 Volunteers
Place of Recruitment of Subjects:	Jain Medical Centre, KANPUR (INDIA) NGO
EXPECTED DATE OF COMPLETION OF DATA	31st May 2021
STATISTICAL ANALYSIS Completion	15th June 2021
JOURNAL ARTICLE COMPLETION	30th June 2021

*Current Phase 1 clinical trial was conducted during 2nd wave of COVID 19 pandemic in India,

Statistical Analysis

This was done by statistical software Epi-Info ver 7.2.2.2. The Proportions were calculated, and Chi-square test of Significance was applied to know P values.

Results

Majority of Volunteers were in age group 20-30 years (52.5%), Female (70%), Class 12th Passed (52.5%), Housewife (70%) and had total family income between 3-4 lakhs per annum (40%) (Table no :01). Out of 40 Volunteers participated in study, 10% had history of covid19 Infection,15% had recent COVID +ve antigen. After use of Naflan spray for 1 month, No Covid +VE Volunteer was found. Symptoms such as Cough and Diarrhoea were Reduced (improved) by 120% after use of Immune (Naflan Spray), but no effect on high fever, headache, and Common cold

Table 1: Socio-demographic Profile of Volunteers (N=40).

Age group	No & %
<than 20	1(2.5)
20-30	21(52.5)
30-40	12(30)
40-50	2(5)
50-60	2(5)
>than 60	2(5)
Sex	No & %
Male	12(30)
Female	28(70)
Education Level	No & %
10th passed	7(17.5)
12th Passed	21(52.5)
Graduate	12(30)
Occupation	No & %
Teacher	2(5)
Shopkeeper	2(5)
Agriculture	1(2.5)
Clerk	1(2.5)
Student	2(5)
Not Employed	1(2.5)
Salesman	1(2.5)
Lic agent	1(2.5)
Housewife	28(70)
Private worker	1(2.5)
Family Income	No & %
up to 1 Lakh PA	Nil
1-2 Lakh PA	7(17.5)
2-3 Lakh PA	5(12.5)
3-4 Lakh PA	16(40)
4-5 Lakh PA	12(30)

were seen, after use of Naflan Immune Spray. So in the Immune spray user group maximum reduction was seen in Cough & Diarrhoea (45% each respectively). The use of Spray gave significant reduction (p<0.05) in few symptoms such as Cough. (Table no: 02).

Discussion

In recent years, there has been a growth in scientific interest in many nutritional supplements such as Nutraceuticals, which can be extracted, used for food supplements, or added to foods. There has long been interest in the antiviral properties of Nutraceuticals, which are especially topical in the context of the ongoing COVID-19 pandemic [15].

It is also evident from literature that Balanced nutrition can help in maintaining immunity for prevention and management of viral infections [18] Clinical evidence has shown that numerous therapies based on natural products and /or food supplements with immune-stimulatory and antiviral actions capable of

Table 2: Symptom’s status of Volunteers after use of NAFLAN Immune Spray (N=40).

Sr. No.	Symptoms as Identified by Physician at NGO	Symptom found before use (In No & %) (n=40)	Symptoms Reduced after 1 Month Use (In No & %) (n=40)
	Asymptomatic	10	0
	Pre-Symptomatic	30	40
Chi square test: $\chi^2 = 9.25$ (Yates Correction), $p = 0.002$ (< 0.05), [RR Adjusted=1.8, 95% C. I=1.5-2.1]			
1	High Fever (Never/Sometimes/ Regularly/Always)	0	1(2.5)
2	Headache (Never/Sometimes/ Regularly/Always)	0	1(2.5)
3	Common Cold (Never/Sometimes/ Regularly/Always)	0	1(2.5)
4	Cough (Never/Sometimes/ Regularly/Always)	15 (37.5)	18(45)
5	Diarrhoea (Never/Sometimes/ Regularly/Always)	15 (37.5)	18(45)
6	Another Specific symptom (N/S/ R/A)	0	1(2.5)
7	Past H/o covid19 Infection	4 (10%)	0
8	Test of Covid19 RTPCR/Antigen	6 (15%)	0

supporting and increasing the body’s immune defenses might be able to reduce the duration and severity of symptoms related to colds, flu, and respiratory viruses in general, as well as to prevent the onset of serious complications [19]. In the context of emerging role of such food supplements prevention and management of viral infections including COVID 19, in our present study the Volunteers used Immune spray for 1 month and this revealed mixed issues from this phase 1 clinical trial.

The present study reveals that majority of Volunteers were in younger age group females, who were basically Class 12th passed housewife and had total family income between 3-4 lakhs/yr, which shows that socioeconomic background was of lower middle-class category. As our present study found that only 10% had history of covid19 Infection, 15% had recent COVID + ve antigen, this could be due to variable exposure and the personal interpretation of Volunteers during 2nd wave of COVID 19 Pandemic in India in month of April- May 2021. Finding of 0 Covid +ve Volunteer after use of Naflan spray for 1 month (based on Combination Plant Sterols with Vitamins B, D & E), may be due to the possible positive effect of Immune (Naflan Spray) on Local Respiratory Immunity. This finding is like many research studies [20-23] across the world on possible role of Nasal Sprays & Nasal Vaccines in Prevention of Respiratory Infections including Covid -19 Infection.

Our findings of Reduction in Cough and Diarrhoea, suggests that NAFLAN SPRAY is possibly having positive effect on Local Upper and Lower Respiratory and GI Tract Immunity without any side effects. This may be due to Vitamins B, D & E in Combination with Plant Sterols. This finding is similar to many Immune studies [13, 14], [18, 19], [20-34] on their role in Respiratory Immunity. Our study is therefore very similar to a recent study, which

reported that nasal vaccines in the form of nasal sprays are being developed by a US company called *Altimmune*, who claim that it can be more effective in preventing COVID-19 transmissions, especially in children. A biotech startup called *Altimmune* based in Maryland, USA, is in a bid to develop a nasal spray to fight COVID-19.

The novel coronavirus SARS-CoV-2 enters into the human body mainly through the ACE2 + TMPRSS2+ nasal epithelial cells. The initial host response to this pathogen occurs in a peculiar immune microenvironment that, starting from the Nasopharynx-Associated Lymphoid Tissue (NALT) system, is the product of a long evolutionary process that is aimed to first recognize exogenous airborne agents. Promoting the accumulation of SARS-CoV-2 specific T cells thanks to a nasal vaccination that may also guarantee a fast cellular adaptive response in case of exposure, thereby allowing to control viral spreading and preventing its dissemination to the lungs and other organs [39].

Therefore, this possible positive effect of Immune Care Naflan Spray can be well explained by studies [13, 14, 19], [35-38] available in literature on Roles of Vitamin D, B & E as well Plant Sterols. As Literature reveals that Vitamin E modulates T cell function through directly impacting T cell membrane integrity, signal transduction, and cell division, and also indirectly by affecting inflammatory mediators generated from other immune cells. Modulation of immune function by vitamin E has clinical relevance as it affects host susceptibility to infectious diseases such as respiratory infections, in addition to allergic diseases such as asthma [13]. A recent review that included 11,321 people from 14 countries demonstrated that supplementing with vitamin D decreased the risk of acute respiratory infections (ARI) in both those who had deficient and adequate levels of vitamin D. Overall, the study [39] showed that vitamin D supplements reduced the risk of developing at least one ARI by 12%. The protective effect was strongest in those with low vitamin D levels. Finally in our present study, symptoms such as Cough and Diarrhoea which were reduced significantly ($p < 0.05$) after use of Immune (Naflan Spray); but as no effect on high fever, headache, and Common cold were seen, after use of Naflan Immune Spray, this can be well explained by possible local Respiratory & Gut Immunity augmentation instead of Systemic Immunity by NAFLAN (Immune Care) Spray.

So, it is very logical that in COVID 19 Pandemic, as large proportion of those infected release the virus before initial symptoms appears, protective measures that reduce the viral load at the entry points by using Immune Care (Naflan Spray), since the probability of infection increases with exposure, and the initial viral load influences the severity of the infection as advocated in studies [27, 28] like finding of our present study.

Article Highlights

- There is no side effect of Volunteers after use of Naflan Spray for 1 month
- There is a positive effect on Respiratory & Gut Immunity after use of Naflan spray.
- Nafalan Spray can be useful in Prevention of Cough in Respiratory Infections (including COVID 19).

- Use of Naflan Spray for 1 month had no effect on high fever, headache, and Common cold.
- Naflan Spray in Oro- Nasal form can be a potential Food Supplement for Prevention of COVID 19.

Strengths of this Clinical Trial: Having healthy vitamin, B, D, E levels can enhance immune health and may be helpful in people with COVID-19.

Limitations of this Clinical trial: The challenges for in this phase 1 trials were: finding methods to assess the potency and effectiveness of these new Spray; sharing of safety information; calculation of the starting dose; design of dose-escalation protocols; and, in the interests of the well-being and safety of the subjects, Exact dose effect was also not seen.

Conclusions

Current Phase 1 clinical trial conducted during 2nd wave of COVID 19 pandemic in India, reveal possible positive effect on Respiratory & Gut Immunity use of Naflan spray for 1 month on Volunteers with no side effects reported by any Volunteer. Nafalan Spray can be useful in Prevention of Cough in Respiratory Infections (possibly also COVID 19). However large scale Multicentric Phase 2, 3, & 4 trials are however advocated by authors before reaching a more definite conclusion of role of Nafalan Spray in Prevention of COVID 19 Infection for Indian Population.

List of Abbreviations

SARS-CoV-2: severe acute respiratory syndrome

COVID -19: Corona Virus disease

BSS- Beta-sitosterol

BSSG- Beta-sitosterol Glycoside

NGO: Non- Governmental Organization

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Registration number in case of Clinical Trials: This Clinical Trial was registered at CTRI (ICMR- India) with registration no: REF/2021/01/040350 dated 24th Jan 2021.

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