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Original Research Article

Assessment of KaraShield™ properties in supporting the immune health of healthy subjects: a randomized, parallel, double-blind, placebo-controlled clinical study

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ABSTRACT

Background: This study aims to investigate whether a novel herbal extract blend, *KaraShield*™ could be used to help build a healthy immune system that could reduce the number of incidences or severity of common upper respiratory tract infections (URTIs).
Methods: A randomized, parallel, double-blind, placebo-controlled clinical study of 60 days was done on 120 healthy subjects allocated to a treatment arm (500 mg/day, *KaraShield*™) or placebo arm (500 mg/day).
Results: A 500 mg daily dosage of *KaraShield*™ significantly improved the subjects' immune health as measured by parameters such as the frequency and severity of upper respiratory tract conditions, the serum IgG level, mean ISQ raw score, WURSS scale score, CRP level in the serum and WHOQOL-BREF score at the end of the study period of sixty days from the baseline compared to that of the placebo. The investigated product was found to be safe and well tolerated by the subjects.
Conclusions: *KaraShield*™ may represent a promising safe and effective formulation for building a healthy immune system that could then counteract URTIs.

Keywords: Clinical trials, Upper respiratory tract infections, Immunostimulation, *KaraShield*™, Indian herb

INTRODUCTION

Upper respiratory tract infections (URTIs) can occur spontaneously but are more frequent in autumn and winter.[1] URTI is characterized by a wide array of acute illnesses affecting the upper airways, including tonsillitis, sinusitis, otitis media, pharyngitis, laryngitis, and the common cold. The symptoms of URTIs usually occur and reach a peak 24-72 hours after the subject becomes infected but can continue for as long as 7-14 days.[2] Viruses (mainly influenza virus, rhinovirus, coronavirus, parainfluenza virus, adenovirus, and respiratory syncytial virus) are the most important agents involved in URTIs occurrence, with rhinovirus representing the principal cause of the largest number of cases.[3] It was estimated that 2 or 3 URTIs occur in adults yearly, while children can have up to 5 on average.[2] An investigation in rural Delhi showed that acute URTI episodes had a prevalence of 12.1%.[4] The first-line treatment for cold includes the maintenance of hydration status, rest, and the prevention

of bacterial or viral spread. Antibiotics use in case of common cold, nasopharyngitis, and other non-specific URTIs does not determine an outcome improvement since they are ineffective against viruses, but analgesics, decongestants, and antipyretics can be effective in reducing pain and cold-like symptoms.[2,5] Based on this background, investigating URTIs prevention or treatment using plants and herbs extract may represent an important research area. Nowadays, herbal preparations are widely available to users and have gained popularity worldwide.[6,7] Herbal and active natural compounds represent, indeed, a growing industry today due to their role in preventing or treating numerous diseases and ailments, including viral infections.[8] Furthermore, another strategy for counteracting URTI is improving immune function; hence immunostimulant natural compounds may prevent or mitigate URTIs.[1]

KaraShield™ is a novel nutraceutical product formed by four well-known Indian herb extracts: *Andrographis paniculata* (Burm.f.) Wall. ex Nees, *Withania somnifera* (L.) Dunal., *Moringa oleifera* Lam., and *Ocimum sanctum* L. These plants are, known for their antiviral or immunostimulant activity. For instance, Andrographolide, the main bioactive compound extracted from *A. paniculata*, showed potential against Influenza A virus (H9N2, H5N1, and H1N1), Hepatitis B and C virus, Herpes simplex virus, Epstein-Barr virus, Human papillomavirus, Human immunodeficiency virus, and Chikungunya virus.[8,9] Similarly, quercetin, from *M. oleifera*, was reported to protect from influenza virus infection (H1N1, H5N1, and H3N2).[8] On the other hand, several investigations have ascribed *W. somnifera* and *O. sanctum* anti-inflammatory and immunomodulatory activity.[10,11] Thus, it was decided to investigate KaraShield's anti-viral and immunostimulatant properties with this randomized, parallel, double-blind, placebo-controlled clinical study.

METHODS

Study design

The proposed study was a multicentre, double-blind, placebo-controlled, two-arm, parallel study of 60 days, including 6 medical examinations divided into 1 screening visit (V1), 1 baseline visit (V2, day 0), and 4 follow-up visits at day 7±1 (V3), day 15±2 (V4), day 30±3 (V5), and day 60±3 (V6) (Figure 1).

Once the study was approved by the Sri Venkateshwara Hospital Ethics Committee (Registration Number: ECR/298/Inst/KA/2013/RR-19), the study was registered at the Clinical Trial Registry, India (CTRI) (Registration Number; CTRI/2022/06/043331; 17/06/2022). The prospective subjects were registered by assigning a unique identification code/subject ID. This subject ID was maintained throughout the study duration to serve the subjects' confidentiality. Each registered subject had undergone a formal informed consent process, which was documented on the approved version of the informed consent form before undergoing the inclusion and exclusion criteria screening procedures. The starting date of the study is 20 July 2022, while the study's end date is 16 October 2022.

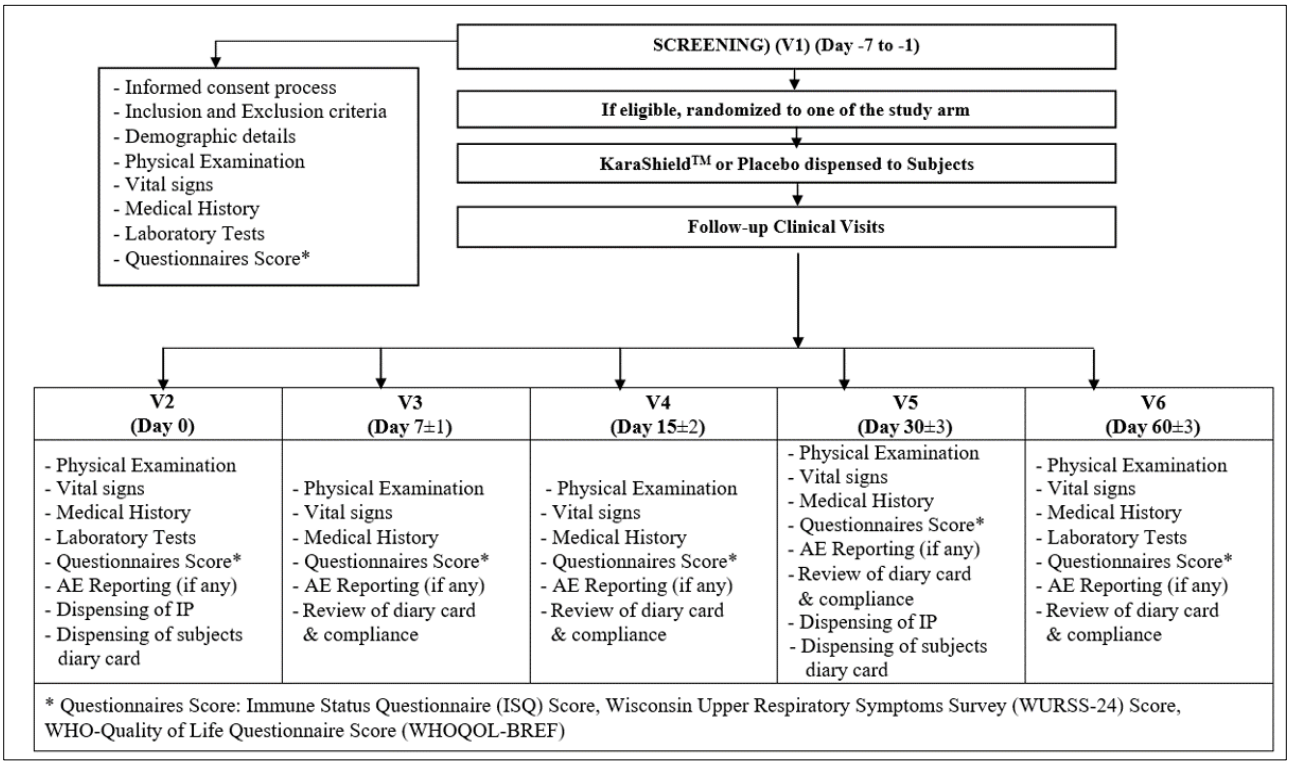

Figure 1: Schematic representation of study design.

Inclusion criteria

Healthy male and female subjects aged 18–60 years with a history of recurrent incidences (at least 2 episodes in the last 2 months) of clinically confirmed symptoms of the upper respiratory tract, such as common cold, cough, sore (scratchy) throat, nasal discharge (runny nose), nasal obstruction (plugged or congested), sneezing, headache, tiredness/body ache, chillness, etc. due to the common cold and/or seasonal change-related symptoms (except the allergic conditions). Subjects are willing to participate and comply with the protocol procedures by signing an informed consent form.

Exclusion criteria

Subjects with the following were excluded: current habit or history of cigarette smoking, current habit of alcohol consumption of more than 2 standard drinks/day; IgE level <700 KU/l (allergic patient); pneumonia or bronchitis; allergic rhinitis, sinusitis/pharyngitis, or any other oropharyngeal disorder; who underwent or needed tonsillectomy or adenoidectomy; any known significant systemic disease/ disorder, i.e., hepatic, renal, oesophageal, gastrointestinal, cardiovascular, psychological, or neurological; suffering from proteinuria (loss of protein in urine); who were on any seizure medication; who were on medication known to reduce IgG levels; known history of any malignant disease; known history of autoimmune disease and other systemic diseases related to the immune system; chronic immune diseases like HIV; suffering from HBsAg and HCV; who were treated with antibiotics less than one week before the study, or any vaccination less than 4 weeks before the study, or concomitant immunosuppressive or immune-stimulating therapy 3 months before the study starts; who were on concomitant treatment with corticosteroids; who participated in another clinical trial less than 3 months prior to this study; suffering from any communicable disease; female subjects who were pregnant, breastfeeding, or expecting pregnancy during the study period; history of consumption of any recreational drugs (such as cocaine, methamphetamine, or marijuana); who were scheduled for any surgery within 3-month period of completing the study; pre-diabetic/diabetic or hypertensive or hyperlipidemia; who were unable/unwilling to abide by the requirements of the protocol; who were incompetent to sign an informed consent form; and any criteria which in the opinion of the Investigator, suggested that the subject would not be compliant with the study protocol.

Subjects' enrolment and randomization procedure

The final eligibility of the subject was ascertained through clinical assessments and blood and urine test reports. A subject was confirmed eligible for enrolling into the study only when all inclusion criteria questions were answered "Yes", and all exclusion criteria questions were answered "No". Fourteen subjects were disqualified for the inclusion criteria due to abnormal findings of biochemistry test results (e.g., abnormal serum glucose value, liver function test parameters, CRP values, and a high value of IgE). Eight subjects voluntarily withdrew themselves to participate before enrolment/randomization due to their respective personal reasons. Each eligible subject was dispensed capsules bottles in sequential order on a "*first come, first serve*" basis on the scheduled randomization day. The numeric code labelled on the capsules bottle served as the unique randomization number assigned to the particular subject. The investigators strictly followed the code sequence while dispensing the capsule bottles to maintain the integrity of the randomization and blinding. Eligible subjects were allocated to one of the two study arms (groups) following the randomization code mentioned on the label of the capsules bottles: study arm 1 (n=60): *KaraShield*™ (500 mg/day), and study arm 2 (n=60): placebo (500 mg/day).

KaraShield™ is a blend formed by standardized extracts of four well-known Indian herbs: *Andrographis paniculata* (Burm.f.) Wall. ex Nees, *Withania somnifera* (L.) Dunal., *Moringa oleifera* Lam., and *Ocimum sanctum* L.. *KaraShield*™ has been developed by Karallief Inc. Karallief Inc have applied for (or may apply for) trademarks and patents covering KaraShield™. Placebo is formed by only excipients.

Treatment duration and compliance

Subjects were advised to take one capsule daily after the first meal in the morning for 60±6 days. The intake of the capsules was recorded on the daily diary card issued to them on the randomization day. Missed dose, if any, was also recorded with the reason in the prescribed section of diary cards. The entries of capsules consumption, missed doses, and leftover capsules in the bottles were physically verified by the investigators and assigned site staff to ascertain the compliance of the subjects. A total of four subjects from the treatment group and five from the placebo group discontinued the study due to personal reasons (Figure 2).

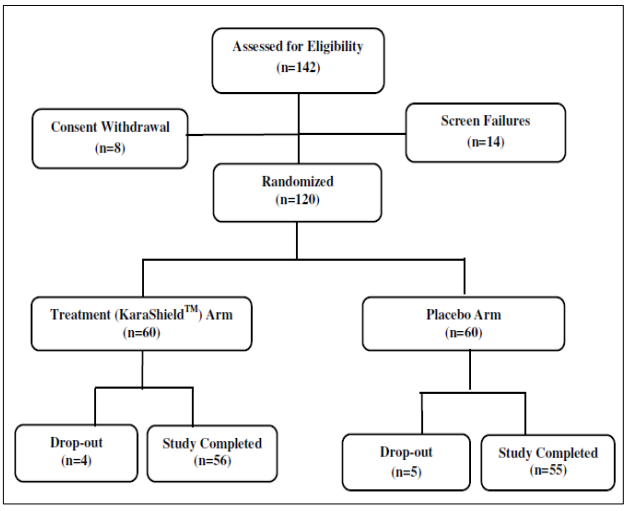

Figure 2: CONSORT diagram showing the study flow.

Primary efficacy parameters

Efficacy was based primarily on the change from baseline to end of the trial period in the episodes of clinically confirmed incidences of upper respiratory tract symptoms (URTIs) mentioned under the Wisconsin upper respiratory symptom survey (WURSS-24) questionnaire. The mean change in the number of episodes of clinically confirmed symptoms in the active group was compared with that of the placebo group.

Secondary efficacy parameters

Secondary parameter assessment was based on the change from the baseline to the end of the trial period in mean symptoms severity score (WURSS-24 score), functional impairments and abilities score (WURSS-24 score), global severity score (WURSS-24 score), immunity status questionnaire (ISQ) score, immunoglobulin G (IgG) level, CD3, CD4, and CD8 count, C-reactive protein (CRP), and WHO-quality of life (WHOQOL-BREF).

Safety parameters

A complete physical examination was conducted at all visits including head, eyes, ears, nose, throat, neck (including thyroid), heart, lungs, abdomen (including liver and spleen), lymph nodes, extremities, nervous system, and skin, blood pressure and body weight. A complete medical history was recorded during the screening period and a review of concomitant medication throughout the study period. Possible adverse events were registered at each visit. Each subject had undergone the clinical laboratory tests listed in Table 11, while urine and blood samples were collected at the screening visit and final visit (V6). The investigator reviewed any abnormal findings for clinical relevance. The adverse events (AE) were recorded in the respective AE forms.

Statistical analysis

The missing observations were imputed using the last observation carried forward (LOCF) approach. Further results were analyzed using the per-protocol (PP) analysis set, a subset of the Intention-to-treat (ITT) population, consisting of subjects with no major protocol deviations affecting the primary efficacy variables. Statistical significant differences were evaluated using the p value for ANCOVA or ranked ANCOVA and the p value for a paired t test or Wilcoxon signed-rank test. Unless otherwise stated, all hypotheses were tested at a significance level of 0.05.

RESULTS

Subject characteristics

Subjects analyzed ranged in age from 18 to 60 years. The 120 subjects were randomized by forming two arms of 60 patients each - *KaraShield*™ arm and placebo arm. All participants were closely monitored regarding medication and visit compliance. All completers had undergone all the assessments on their respective scheduled clinical visits at day 0 (V2), 7±1 (V3), 15±2 (V4), 30±3 (V5), and 60±3 (V6) days. A slight but significant reduction in weight and BMI was seen at V5 and V6 for the treatment group, and at V6 for the placebo group (Table 1).

Episodes of clinically confirmed incidences of URT and related condition

Incidences referred to symptoms associated with upper respiratory tract conditions mentioned under the Wisconsin upper respiratory symptom survey (WURSS-24) score. Episodes were the number of times the subjects showed any clinically confirmed upper respiratory tract symptoms during the last sixty days before the study initiation and during the treatment duration of sixty days. The following symptoms of upper respiratory tract conditions were considered for the selection of participants in this study: runny nose, plugged nose, cough, sore throat, sneezing, scratchy throat, head congestion, hoarseness, feeling tired, chest congestion, body aches, headache, and fever.

A reduction of 81.47% in the episodes of incidences was observed in the *KaraShield*™ group at the end of the study period. The placebo group experienced a relatively smaller reduction of 39% in incidence episodes (Table 2).

Symptoms score of URT and related condition

The WURSS-24 includes 10 items assessing symptoms of upper respiratory tract conditions. For each parameter of symptoms, the individual score ranges from 0 (=no symptom) to 7 (=severe condition) (Table 3).

The reduction in the total symptoms score is referred to as the improvement in the participants' general health and vice versa. An improvement through a statistically significant reduction (82.5%; p value <0.0001) in the mean symptoms score of the upper respiratory tract and related conditions was prominently observed throughout the study duration in the *KaraShield*™ group. The placebo arm also observed a mild reduction (34.5%; p value <0.0001) in the severity of symptoms. However, after adjusting the baseline score, there was a statistically significant difference between the groups at the end of the study (ANCOVA p value <0.0001), indicating that *KaraShield*™ treatment is more effective in reducing the symptoms' severity (Table 5).

Functional impairments and abilities score

The WURSS-24 includes 9 items assessing functional impairments and abilities of participants. For each parameter of symptoms, the individual score ranges from 0 (=no symptom) to 7 (=severe condition) (Table 4).

The reduction in the total functional impairments and abilities score is referred to as the participant's functional ability improvement and vice versa. The treatment group

showed better results, with a reduction of 10.91 units (60.8%; p value <0.0001) when compared to that of the placebo group, with a reduction of 5.22 units (33.4%; p value 0.0002) at the end of the study (Table 5).

Table 1: Statistical analysis of demography; weight and body mass index (BMI) (per protocol population).

Variable	Treatment group (n=56)	Placebo group (n=55)	P value[a]
Weight (kg) at day 0 (V2)	61.30±4.74	62.25±5.11	0.6149
Weight (kg) at day 7 (V3)	61.31±4.75	62.25±5.11	
P value[b]	0.6588	-	
Weight (kg) at day 0 (V2)	61.30±4.74	62.25±5.11	0.0505
Weight (kg) at day 15 (V4)	61.22±4.61	62.31±5.08	
P value[b]	0.1618	0.3218	
Weight (kg) at day 0 (V2)	61.30±4.74	62.25±5.11	0.0057
Weight (kg) at day 30 (V5)	60.93±4.46	62.20±5.08	
P value[b]	0.0031	0.2606	
Weight (kg) at day 0 (V2)	61.30±4.74	62.25±5.11	0.0744
Weight (kg) at day 60 (V6)	60.52±4.11	61.76±4.88	
P value[b]	0.0002	0.0020	
BMI (kg/m^2) at day 0 (V2)	23.62±2.91	23.58±3.15	0.6365
BMI (kg/m^2) at day 7 (V3)	23.63±2.92	23.58±3.15	
P value[b]	0.6298	-	
BMI (kg/m^2) at day 0 (V2)	23.62±2.91	23.58±3.15	0.0979
BMI (kg/m^2) at day 15 (V4)	23.59±2.88	23.61±3.14	
P value[b]	0.1733	0.3369	
BMI (kg/m^2) at day 0 (V2)	23.62±2.91	23.58±3.15	0.0201
BMI (kg/m^2) at day 30 (V5)	23.48±2.85	23.56±3.12	
P value[b]	0.0032	0.2569	
BMI (kg/m^2) at day 0 (V2)	23.62±2.91	23.58±3.15	0.2365
BMI (kg/m^2) at day 60 (V6)	23.32±2.75	23.40±3.08	
P value[b]	0.0003	0.0020	

[a]P value compared between groups; p value for ANCOVA, [b]p value compared within groups; p value for paired t-test.

Table 2: Summary of episodes of incidences (per protocol population).

Variable	Statistics	Treatment group (n=56)	Placebo group (n=55)
Incidence episodes at day 0 (V2)	Mean±standard deviation	2.59±0.87	2.18±0.84
	Median	3.0	2.0
	(Q1, Q3)	(2.00, 3.00)	(2.00, 3.00)
	(Min, max)	(1.00, 4.00)	(1.00, 4.00)
Incidence episodes at day 60 (V6)	Mean±standard deviation	0.48±0.66	1.33±1.23
	Median	0.0	1.0
	(Q1, Q3)	(0.00, 1.00)	(0.00, 2.00)
	(Min, max)	(0.00, 2.00)	(0.00, 4.00)

Table 3: Items of upper respiratory symptoms of WURSS-24.

	Do not have this symptom	Very Mild		Mild		Moderate		Severe
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Runny nose								
Plugged nose								
Sneezing								
Sore throat								
Scratchy throat								
Cough								
Hoarseness								
Head congestion								
Chest congestion								
Feeling tired								

Table 4: Items assessing functional impairments and abilities of WURSS-24.

	Not at all	Very Mild		Mild		Moderate		Severe
	0	1	2	3	4	5	6	7
Think clearly								
Sleep well								
Breathe easily								
Walk, climb stairs, exercise								
Accomplish daily activities								
Work outside the home								
Work inside the home								
Interact with others								
Live your personal life								

Table 5: Statistical analysis of WURSS-24 scores (per protocol population).

Variable	Treatment group (n=56)	Placebo group (n=55)	P value[a]
Symptoms score at day 0 (V2)	25.27±4.85	24.80±5.22	0.0078
Symptoms score at day 7 (V3)	19.55±6.86	23.22±5.29	
P value[b]	<0.0001	0.1196	
Symptoms score at day 0 (V2)	25.27±4.85	24.80±5.22	<0.0001
Symptoms score at day 15 (V4)	15.43±8.36	22.82±6.22	
P value[b]	<0.0001	0.0487	
Symptoms score at day 0 (V2)	25.27±4.85	24.80±5.22	<0.0001
Symptoms score day 30 (V5)	10.63±8.71	20.47±6.86	
P value[b]	<0.0001	0.0006	
Symptoms score day at 0 (V2)	25.27±4.85	24.80±5.22	<0.0001
Symptoms score at day 60 (V6)	4.41±6.52	16.24±6.43	
P value[b]	<0.0001	<0.0001	
Functional impairments and ability score at day 0 (V2)	17.93±7.03	15.64±8.80	0.5914
Functional impairments and ability score day 7 (V3)	14.54±7.52	15.00±8.74	
P value[b]	0.0007	0.6141	
Functional impairments and ability score at day 0 (V2)	17.93±7.03	15.64±8.80	0.5873
Functional impairments and ability score at day 15 (V4)	13.70±7.37	14.05±8.69	
P value[b]	0.0002	0.1884	
Functional impairments and ability score at day 0 (V2)	17.93±7.03	15.64±8.80	0.6244
Functional impairments and ability score at day 30 (V5)	11.59±6.91	12.27±8.41	
P value[b]	<0.0001	0.0202	
Functional impairments and ability score at day 0 (V2)	17.93±7.03	15.64±8.80	0.0091
Functional impairments and ability score at day 60 (V6)	7.02±5.36	10.42±7.64	
P value[b]	<0.0001	0.0002	
Global severity score at day 0 (V2)	3.39±1.63	3.42±1.55	0.6621
Global severity score at day 7 (V3)	3.13±1.24	3.31±1.43	
P value[b]	0.1856	0.6759	
Global severity score at day 0 (V2)	3.39±1.63	3.42±1.55	0.0319
Global severity score at day 15 (V4)	2.93±1.39	3.47±1.29	
P value[b]	0.0518	0.7756	
Global severity score at day 0 (V2)	3.39±1.63	3.42±1.55	0.0016
Global severity score at day 30 (V5)	2.41±1.41	3.42±1.52	
P value[b]	0.0028	0.9641	
Global severity score at day 0 (V2)	3.39±1.63	3.42±1.55	<0.0001
Global severity score at day 60 (V6)	2.20±1.34	3.44±1.40	
P value[b]	<0.0001	0.9955	

[a]P value compared between groups; p value for ANCOVA or ranked ANCOVA; [b]p value compared within groups; p-value for paired t-test or Wilcoxon signed-rank test

Global severity and global change score

The scoring refers to the subjects' own assessments and reporting of health status. The score ranges from 1 (=very much better) to 7 (=very much worse). The reduction in the score is referred to as the participants' general health improvement and vice versa. The treatment group showed better results (35% reduction; p value <0.0001) in maintaining the general health conditions of the participants when compared to that of the placebo group (0.6%; p value 0.9955). After adjusting the baseline score, there was a statistically significant difference between the groups at the end of the study (ANCOVA p value <0.0001). This implies that the treatment (*KaraShield*™) has performed better than the placebo in maintaining the general health conditions (Table 5).

Summary of ISQ score

The ISQ is a validated scoring form useful for clinical practice and research requiring a quick screening of the immune status of the subjects. It consists of seven items related to the immune status: headache, sudden high fever, skin problems (e.g., eczema and acne), diarrhea, common cold, muscle and joint pain, and coughing. Each of the ISQ items can be scored as follows: never=0 points; sometimes=1 point; regularly=2 points; often=3 points; (almost) always=4 points. The ISQ raw scores interpretation considered that the lower the total score, the higher the subject's immune fitness, and vice versa.

A significant reduction (4.89 units=53.5%; p value ≤0.0001) in the mean score of the Immune Status Questionnaire (ISQ) from the baseline to the end of the treatment was observed in the *KaraShield*™ treatment group, whereas a very minimal reduction (0.82 units=9.1%; p value 0.0433) was observed in the placebo group. After adjusting the baseline score (ANCOVA p value≤0.0001), the treatment group showed a statistically significant outperformance compared to the placebo group (Table 6).

Immunoglobulin-G (IgG) level in the serum

An IgG serum level increment of 12.3% (p value<0.0001) in the treatment group and a reduction of 4.7% (p value 0.0471) in the placebo group were observed. This implies that the treatment of *KaraShield*™ supported a better increase of the protective immunoglobulins G (IgG) in the serum and provided better defensive immunity to the participants when compared to that of the placebo (Table 7).

CD3, CD4, and CD8 count

There was no statistically significant change in the CD3, CD4, and CD8 values for either the treatment or placebo group (Table 8). This finding is significant because it implies that no major infections or autoimmune phenomena were observed in any of the study participants during the trial.

Table 6: Statistical analysis of IQS score (per protocol population).

Variable	Treatment group (n=56)	Placebo group (n=55)	P value[a]
ISQ at day 0 (V2)	9.14 ± 2.32	9.00 ± 2.07	0.1869
ISQ at day 7 (V3)	8.91 ± 2.20	9.16 ± 2.13	
P value[b]	0.0625	0.4670	
ISQ at day 0 (V2)	9.14 ± 2.32	9.00 ± 2.07	0.0081
ISQ at day 15 (V4)	7.43 ± 2.05	8.58 ± 2.30	
P value[b]	<0.0001	0.3952	
ISQ at day 0 (V2)	9.14 ± 2.32	9.00 ± 2.07	0.0051
ISQ at day 30 (V5)	6.88 ± 2.07	8.29 ± 2.28	
P value[b]	<0.0001	0.1144	
ISQ at day at 0 (V2)	9.14 ± 2.32	9.00 ± 2.07	<0.0001
ISQ at day 60 (V6)	4.25 ± 2.51	8.18 ± 2.50	
P value[b]	<0.0001	0.0433	

[a]P value compared between groups; p value for ANCOVA or ranked ANCOVA; [b]p value compared within groups; p-value for paired t-test or Wilcoxon signed-rank test

Table 7: Statistical analysis of IgG level in the serum (per protocol population).

Variable	Treatment group (n=56)	Placebo group (n=55)	P value[a]
IgG (g/l) at day 0 (V2)	12.33±1.69	12.90±2.16	<0.0001
IgG (g/l) at day 60 (V6)	13.85±1.84	12.30±2.33	
P value[b]	<0.0001	0.0471	

[a]P value compared between groups; p value for ANCOVA or ranked ANCOVA; [b]p value compared within groups; p-value for paired t-test or Wilcoxon signed-rank test

Table 8: Statistical analysis of CD3, CD4, and CD8 count in the serum (per protocol population).

Variable	Treatment group (n=56)	Placebo group (n=55)	P value[a]
CD3			
CD3 (/ul) at day 0 (V2)	1816±433.5	1874±586.2	0.5261
CD3 (/ul) at day 60 (V6)	1863±427.1	1829±488.5	
P value[b]	0.5060	0.5730	
CD4			
CD4 (/ul) at day 0 (V2)	1158±485.1	1162±433.0	0.8418
CD4 (/ul) at day 60 (V6)	1128±398.2	1142±359.1	
P value[b]	0.6032	0.6927	
CD8			
CD8 (/ul) at day 0 (V2)	711.6±340.7	744.8±325.9	0.9178
CD8 (/ul) at day 60 (V6)	674.3±256.2	704.7±275.8	
P value[b]	0.9263	0.4737	

[a]P value compared between groups; p value for ANCOVA or ranked ANCOVA; [b]p value compared within groups; p-value for paired t-test or Wilcoxon signed-rank test

Table 9: Statistical analysis of C-reactive protein (CRP) level in the serum (per protocol population).

Variable	Treatment group (n=56)	Placebo group (n=55)	P value[a]
CRP (mg/l) at day 0 (V2)	3.43±1.82	2.92±1.85	<0.0001
CRP (mg/l) at day 60 (V6)	1.81±1.10	3.20±1.76	
P value[b]	<0.0001	0.2526	

[a]P value compared between groups; p value for ANCOVA or ranked ANCOVA; [b]p value compared within groups; p-value for paired t-test or Wilcoxon signed-rank test

Table 10: Adverse events.

Subject ID	Visit no.	Adverse effects description	Concomitant medication	Severity	Relationship	Outcome	Group
RAPA008	4	Constipation	Lactulose	Moderate	Not related	Completely recovered	*KaraShield*™
MSBA030	6	Headache	-	Mild	Not related	Completely recovered	*KaraShield*™
KARA072	6	Body heat	-	Mild	Not related	Completely recovered	*KaraShield*™
CHEA076	4	Diarrhoea	Loperamide 2 mg	Mild	Not related	Completely recovered	*KaraShield*™
NANB033	4	Fever	Paracetamol-500 mg	Mild	Not related	Completely recovered	*KaraShield*™
RBSA050	5	Constipation	-	Mild	Not related	Completely recovered	Placebo
TRPA084	5	Acidity	Gelusil	Moderate	Not related	Completely recovered	Placebo
SUNB002	5	Headache	-	Mild	Not related	Completely recovered	Placebo
ROHB026	5	Dryness of mouth	-	Mild	Not related	Completely recovered	Placebo

Table 11: Clinical laboratory tests for safety assessment.

Parameters	V1 screening visit (day -7 to -1)	V6 final visit (day 60±3)
Complete blood profile (CBP)	√	√
Liver function test (LFT)-SGOT, SGPT, GGT, ALP, serum albumin, serum bilirubin, total protein	√	√
Renal function test (RFT)-serum urea, serum creatinine	√	√

Continued.

Parameters	V1 screening visit (day -7 to -1)	V6 final visit (day 60±3)
Uric acid	√	√
Immunoglobulin G (IgG)	√	√
Immunoglobulin E (IgE)	√	X
CD3, CD4 and CD8 count	√	√
C-reactive protein (CRP)	√	√
HIV, HBsAg and HCV tests	√	X
Urine analysis (Routine)	√	√
**** Urine pregnancy test (UPT)**	√	√

Table 12: Statistical analysis of WHOQOL-BREF-scores (per protocol population).

Variable	Treatment group (n=56)	Placebo group (n=55)	P value[a]
Physical health domain score at day 0 (V2)	23.20±3.57	22.85±3.81	0.6094
Physical health domain score at day 7 (V3)	23.23±3.79	22.95±3.99	
P value[b]	0.6197	0.4540	
Physical health domain score at day 0 (V2)	23.20±3.57	22.85±3.81	0.0475
Physical health domain score at day 15 (V4)	23.50±3.58	24.53±3.81	
P value[b]	0.3711	0.0160	
Physical health domain score at day 0 (V2)	23.20±3.57	22.85±3.81	0.8618
Physical health domain score at day 30 (V5)	24.25±3.93	24.18±3.71	
P value[b]	0.0027	0.0643	
Physical health domain score at day 0 (V2)	23.20±3.57	22.85±3.81	0.0322
Physical health domain score at day 60 (V6)	25.00±3.89	23.55±3.60	
P value[b]	<0.0001	0.3897	
Psychological health domain score at day 0 (V2)	20.73±4.31	20.71±4.24	0.5452
Psychological health domain score at day 7 (V3)	21.16±3.94	21.09±3.43	
P value[b]	0.0187	0.3619	
Psychological health domain score at day 0 (V2)	20.73±4.31	20.71±4.24	0.7036
Psychological health domain score at day 15 (V4)	22.34±3.74	22.20±3.23	
P value[b]	0.0025	0.0136	
Psychological health domain score at day 0 (V2)	20.73±4.31	20.71±4.24	0.7680
Psychological health domain score at day 30 (V5)	22.54±2.79	22.29±2.28	
P value[b]	0.0037	0.0536	
Psychological health domain score at day 0 (V2)	20.73±4.31	20.71±4.24	0.2751
Psychological health domain score at day 60 (V6)	23.20±2.60	22.60±2.71	
P value[b]	0.0002	0.0107	
Environmental health domain score at day 0 (V2)	17.20±3.75	17.76±3.83	0.0552
Environmental health domain score at day 7 (V3)	17.25±3.54	18.40±3.37	
P value[b]	0.6313	0.1223	
Environmental health domain score at day 0 (V2)	17.20±3.75	17.76±3.83	0.2548
Environmental health domain score at day 15 (V4)	19.70±4.13	19.18±3.91	
P value[b]	<0.0001	0.0250	
Environmental health domain score at day 0 (V2)	17.20±3.75	17.76±3.83	0.0714
Environmental health domain score at day 30 (V5)	20.66±4.08	19.47±3.96	
P value[b]	<0.0001	0.0143	
Environmental health domain score at day 0 (V2)	17.20±3.75	17.76±3.83	0.0102
Environmental health domain score at day 60(V6)	21.89±3.89	19.96±4.24	
P value[b]	<0.0001	0.0035	
Social relationship domain score at day 0 (V2)	8.89±2.09	9.05±2.02	0.8036
Social relationship domain score at day 7 (V3)	9.39±1.85	9.36±1.88	
P value[b]	0.0216	0.2909	
Social relationship domain score at day 0 (V2)	8.89±2.09	9.05±2.02	0.8731
Social relationship domain score at day 15 (V4)	9.63±1.78	9.67±1.84	

Continued.

Variable	Treatment group (n=56)	Placebo group (n=55)	P value[a]
P value[b]	0.0165	0.0700	
Social relationship domain score at day 0 (V2)	8.89±2.09	9.05±2.02	0.9976
Social relationship domain score at day 30 (V5)	9.63±1.85	9.65±1.68	
P value[b]	0.0203	0.1020	
Social relationship domain score at day 0 (V2)	8.89±2.09	9.05±2.02	0.2962
Social relationship domain score at day 60 (V6)	10.32±1.66	9.89±1.96	
P value[b]	<0.0001	0.0279	

[a]P value compared between groups; p value for ANCOVA or ranked ANCOVA; [b]p value compared within groups; p-value for paired t-test or Wilcoxon signed-rank test

C-reactive protein determination

C-reactive protein (CRP) value was statistically significantly reduced in the treatment group (47.2%, p value <0.0001), whereas it slightly increased in the placebo group. The reduction of CRP value was also found to be statistically significant between the groups over the period (ANCOVA p value<0.0001). Thus the treatment of *KaraShield*™ showed better results in managing inflammatory biomarker-CRP levels during general health conditions of upper respiratory tract symptoms (Table 9).

WHO-quality of life questionnaire score

The WHO-quality of life questionnaire (WHOQOL-BREF) is an instrument formed of 26 items divided into four domains: physical health, psychological health, social relationships, and environmental health. Each domain of the WHOQOL-BREF is formed by a scale score from 1 to 5. An increase in the resultant score (sum of the positive scores - sum of negative scores) for the individual domains was considered the improvement in that domain and vice versa.[12] A higher score increase was observed in the treatment group compared to the placebo group at the sixty-day follow-up in all items, indicating an amelioration of quality of life in the treated arm compared to the placebo arm (Table 12).

Safety assessment

All the safety parameters, whole blood tests, biochemistry, and clinical observations did not exhibit any statistically significant change from the baseline to the end of the study in both groups. The study medication was well tolerated by the subjects. There were no serious adverse side effects. There were mild/moderate adverse events (AE) observed in 9 patients, evenly distributed between the treatment arm (5 patients) and placebo arm (4 patients). The main detected AE are constipation, headache, body heat, diarrhoea, fever, acidity, and dryness of the mouth (Table 10).

DISCUSSION

The concept of "Reverse Pharmacognosy" is shifting the paradigm of herbal drug design through robust technology to understand the mechanisms of action of herbal remedies at multiple levels. As knowledge about herbal medicines expands, new drugs for new targets may evolve with the involvement of innovative techniques. Phytocompounds or phytochemicals with a long history of medicinal use are believed to interact with multiple targets to confer pharmacological or physiological effects at the cellular, tissue, or organ levels.[13,14] Based on these considerations, it was decided to test a new formulation *KaraShield*™ capable of counteracting UTRIs and inducing immune system stimulation by exploiting the synergistic effect of 4 natural species known for their antiviral, immunostimulating, and anti-inflammatory activities. Specifically, the current investigation is a multicentre, double-blind, placebo-controlled, two-arm, parallel study to explore the efficacy and safety of *KaraShield*™ (500 mg/day) compared to a placebo (500 mg/day) in the management of URTI conditions in healthy subjects. A total of 111 subjects completed the study comprising 56 subjects from the *KaraShield*™ group and 55 from the placebo group. Overall, it was seen that *KaraShield*™ administration reduced the incidence of URI episodes, symptoms severity, and functional impairments, evaluated with the WURSS-24, significantly more than the placebo. These improvements were also confirmed by a significant reduction in CRP levels in the treatment arm compared to the placebo group. It is known, indeed, that high CRP values are frequently found in viral or bacterial respiratory infections.[15] The obtained results should be attributable to active metabolites from herbal extracts in the analyzed formulation. It was indeed seen that Moringa A, from *M. oleifera*, can counteract influenza virus infection by inhibiting the expression and the nucleus transfection of cellular protein transcription factor EB (TFEB) and then the virus autophagy in infected cells.[16] Similarly, andrographolide from *A. paniculata* and its derivative 14-alpha-lipoyl andrographolide could reduce influenza virus infection probably by interfering with viral hemagglutinin, thereby blocking viral binding to cellular receptors.[17] Furthermore, a randomized double blind-placebo study demonstrated that *A. paniculata* extract administration reduced uncomplicated common cold intensity and symptoms compared to placebo.[18]

Another important issue is the induction of the immunity system since it plays an important role in counteracting viral infection.[19] Hence, the immunity status was also evaluated in the present clinical trial, evidencing an improvement in the ISQ score of the *KaraShield*™ group. In line with the latter result, an increase in IgG levels was seen in the treatment arm compared to the placebo group.

The important observation of decreased inflammation (CRP) but increased immunoglobulin G (IgG) suggests a better balance or "more efficient" defensive clearance of common pathogens. CRP is a measurement of how hard the immune system is currently working, e.g., "inflammatory marker." While the elevation of CRP can occur from bacterial infection, it can also be affected by diet, exercise, and stress. IgG is a defensive protein specifically produced following an infection, and low levels correlate with recurrent or potentially invasive infections. In addition, specific IgG for gut bacterial components, e.g. LPS, reduce fatigue and other systemic symptoms caused by "leaky gut." IgG plays a pivotal role in the immune system against viral infections by binding the viral surface epitope, inhibiting viral entry and, thus, infection.[20] Thus, IgG can defend the body from infection without triggering an inflammatory response. The evidenced immunostimulation in treated subjects can be attributable to *W. Somnifera* and *O. sanctum*. A previous randomized placebo-controlled double-blinded study demonstrated that *W. somnifera* extract significantly improves healthy subjects' immune profiles by modulating the innate and adaptive immune systems. In particular, a significant increase in immunoglobulins, cytokines, and quantitative lymphocyte subsets was evidenced.[10] Similarly, a double-blinded randomized controlled cross-over trial on healthy volunteers demonstrated the immunomodulatory effect of *O. sanctum*, as after four weeks of treatment, increased levels of IFN-γ, IL-4, and percentage of T-helper cells and NK-cells were observed.[21] Immunostimulation is not a single-direction metric, as over-stimulation can cause inflammation that can harm the host. Observing an improving CRP, with the improvement in the quality of life scores, and increasing IgG, demonstrates improved efficiency in clearing infections. This efficiency is responsible for clearing infections without the participants""feeling sick". Altogether, the reduction of URTI episodes and symptomatology severity, and the amelioration of the immunity system by the nutraceutical product, *KaraShield*™, results in improved life quality evaluated with the WHOQOL-BREF questionnaire. Hence this clinical trial demonstrated the effectiveness and synergistic effect of *KaraShield*™ in reducing upper respiratory tract infections in healthy subjects during 60 days of treatment. Moreover, the potential utilization of *KaraShield*™ in preventing URTIs is further enhanced by its safety profile demonstrating that the investigated nutraceutical product is safe and well-tolerated. The study's main strength is represented by the evidence that outputs obtained by self-reported questionnaires (WURSS-24, ISQ, and WHOQOL-BREF) were corroborated by laboratory results (evaluation of IgG and CRP levels). However, the limitations must also be considered since the current clinical trial was done on a small group of subjects and for a short time interval.

CONCLUSION

This multicentre, double-blind, placebo-controlled, two-arm, parallel study demonstrated that *KaraShield*™ (500 mg/day) could reduce the incidence and severity of URTIs in healthy subjects and thus may represent a promising safe and effective formulation of Indian herbs known for their antiviral and immunostimulant activity.

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