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Research Article

Immune-Modulatory Effect of a Poly-Herbal Blend in Individuals Frequently Susceptible to Cold and Flu: A Randomized, Double-Blind, Placebo-Controlled Study

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Abstract

Introduction: Optimal functioning of the immune system is critical to fight infections. OmniActive Health Technologies (OAHT) has developed a Poly-Herbal Blend (PHB) consisting standardized extracts of Ashwagandha, Boswellia, Neem, Star Anise and a formulated turmeric extract (Ultrasol curcumin®) with known ability to modulate immune system. The current study evaluated the efficacy and safety of PHB in healthy human subjects frequently susceptible to cold and flu.

Methods: Thirty subjects were randomized to receive one tablet of either PHB or placebo every morning after breakfast for 60 days. Hematological parameters such as WBC count, WBC differentials, cell counts for Absolute Lymphocyte, Platelet, IgG, IgM, CD4 and CD8, CD4/CD8 ratio, CD45, CD 3, Natural killer (CD16/56), and CRP along with parameters of stress, sleep and common cold symptoms using Perceived Stress Score (PSS), Pittsburg Sleep Quality Index (PSQI), and Common Cold Questionnaire (CCQ), respectively were evaluated at baseline, day 30 and day 60 post supplementation. Adverse events and blood measures were monitored to assess safety.

Results: PHB showed significant improvement ($p<0.05$) in markers of innate immunity like WBC, natural killer cell, and platelet counts and markers of adaptive immunity with increased absolute lymphocyte count, lymphocytes %, CD4, CD8, CD4/CD8 ratio, CD3, CD45 counts and IgG at several time points compared to placebo. A significant decrease ($p<0.05$) was noted in CRP levels, decreased common cold symptoms, increasing trend for IgM plasma levels ($p=0.0514$) and trend of improvement in sleep quality ($p=0.0976$) was seen in PHB as compared to placebo. There were no adverse events reported.

Conclusion: The study findings indicate significant improvement in the overall immune status of subjects in PHB group. Further, PHB was well tolerated without any adverse events related to supplementation. PHB could be effective for individuals susceptible to cold and flu looking for herbal alternative to boost their immune system.

Clinical Trial Registration: CTRI/2021/07/035206 (Clinical Trials Registry - India).

Keywords: Adaptive immunity; Herbal formulation; Immunity; Innate immunity; Viral infections

Introduction

Immunity is a biological response of host against a foreign body that include infectious agents such as bacteria and virus [1]. The outcome of an immune response may lead to protection against infectious agent or an exaggerated response resulting in tissue damage and disease. Immunity is mediated through innate and adaptive immunity and include complex integrated network of cells, tissues, organs and soluble mediators. Innate immunity is non-specific in nature and provide immediate host defense whereas adaptive immune response is precise and antigen-specific and takes several days to develop [2]. Innate immunity is highly conserved and consist of cells that include Natural Killer (NK) lymphocytes, neutrophils, monocytes, macrophages, complement, cytokines, and acute phase proteins whereas adaptive immunity is mediated through T lymphocytes and B lymphocytes [3]. Both innate and adaptive immunity work synergistically to eliminate foreign bodies or infection. Immune system is further assisted by network of cytokines that regulate immune cells and play an important role in maintaining homeostasis of the immune system [4-8]. Immune response generally remains in a homeostatic balance in healthy condition, however is modulated during infection or injury leading to excessive response causing allergy and autoimmunity whereas suppression of immune response may lead to opportunistic infection [9]. Further, immunity is influenced by sleep and stress and sleep deprivation affects both adaptive and innate immune system, increases the risk of infection and induces psychological stress. Several studies confirm that poor sleep is associated with increased susceptibility to viral infections leading to upper respiratory tract infection [10].

Immunomodulators that modify immune system by inducing, amplifying, attenuating immune responses have been extensively used in clinics to achieve specific therapeutic goals [9]. Modulation of immune functions using traditional medicinal plants that target immune system at multiple steps is considered more beneficial to attain desirable health benefits. Several medicinal plants and phytochemicals are known since ancient times for their ability to modulate the immune function [11]. The current study describes a poly herbal combination that includes standardized extracts of Ashwagandha, Boswellia, Neem, Star anise and a formulated turmeric extract (Ultrasol curcumin®), widely known to modulate immune system along with anti-bacterial and anti-viral properties [12-22] and are well-tolerated with no known adverse events in humans when consumed at safe doses [18].

Ashwagandha (*Withania somnifera*) is extensively used as an adaptogen in traditional Indian medicine and is an established immunomodulatory agent due to their withanolide glycosides content [23]. Curcumin from turmeric extract is widely established immune-modulating agent and has antimicrobial and anti-viral properties [24]. 3-acetyl-11-keto boswellic acid from Boswellia serrata extract also has potent anti-inflammatory as well as immune stimulatory effect [25]. Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antifungal, antibacterial, antiviral, antioxidant properties [26]. Star anise (*Illicium verum*), a traditional medicinal herb contains Shikimic acid which is being used as a precursor for the production of Tamiflu with potent anti-viral properties [27].

Methods

Study Design and Procedures

This was a prospective, randomized, double-blind, multiple doses, parallel, placebo-controlled, clinical interventional study to evaluate the efficacy and safety of PHB on the immunity of healthy human subjects. The subjects consumed one tablet a day for 60 days. The study was started after due approval from an institutional ethics committee, Ethics Committee of Telerad Rxdx Healthcare Private Limited (EC registration number ECR/1494/Inst/KA/2021). The study was conducted as per the requirements of the Indian Council of Medical Research (ICMR) ethical guidelines, International Council for Harmonization (ICH) 'Guidance on Good Clinical Practice' (E6R2), and 'Declaration of Helsinki'. The study was registered with the Clinical Trials Registry of India (CTRI/2021/07/035206). Informed consent was voluntarily obtained from every participant before enrolment for the study. Subjects were randomly assigned in a 1:1 ratio to receive either PHB or placebo. The randomization schedule was generated by a non-study assigned, independent expert ensuring the treatment balance by using SAS® statistical software, version 9.4. Staff who were involved in the investigational product related activities were not involved in the study related activities to ensure double blindness of the study. The total study duration was a maximum of 63 days which included the screening period of 3 days, followed by the treatment period of 60 days. Information about gender, age, body weight, height, BMI, medical history, concomitant medication history, and oral contraceptives were obtained during the screening visit. Blood samples for evaluation of laboratory parameters and validated questionnaires for stress, sleep and common cold symptoms were collected at baseline, day 30, and day 60.

Inclusion/Exclusion Criteria

The participants were enrolled in the study as per the inclusion and exclusion criteria outlined in the Table 1.

Study Material: Details on study products have been provided in Table 2.

Inclusion criteria
a. Healthy male or non-pregnant, non- lactating female human subject between age of ≥ 30 and ≤ 70 years who frequently suffered from cold, flu, and fever b. Subjects who were willing to abstain from smoking and consuming tobacco throughout the study duration c. Subjects who were willing to abstain from consuming alcohol throughout the study duration d. Subjects without any significant disease or clinically significant abnormal laboratory values on laboratory evaluation at baseline measurement e. Subjects willing to avoid anti-inflammatory/ immunostimulant/ immunosuppressant medications during the study period f. Female subjects of childbearing potential practicing an approved method of contraception and willing to continue its use throughout the study duration or female subjects of non-childbearing potential g. Subjects willing to provide voluntary written consent h. Subjects willing and able to understand and comply with the requirements of the study, consume the investigational product as instructed, return at the appropriate scheduled visits, comply with therapy prohibitions, and be able to complete the study
Exclusion criteria
a. Subjects with history or evidence of hypersensitivity to any ingredient from the formula or its metabolites b. Subjects with clinically significant disease(s) or disorder(s) in the opinion of the investigator may (i) put the subject at risk because of participation in the study (ii) interfere with the study evaluations or (iii) cause concern regarding subject's ability to participate in the study c. Subjects with a history of hypo and hyperthyroidism d. Subjects with alarm signs or symptoms, including fever, gastrointestinal bleeding, unintentional weight loss, anemia, dysphagia, or abdominal mass e. Subjects with a history of milk, gluten allergies, or other known food intolerances and or any food allergies f. Subjects with a history of significant systemic diseases, seizures, psychiatric disorders, neurological disorders, depression, or mental illness and allergic rash g. Subjects taking ace-inhibitors, cholesterol-lowering medications h. Subjects with a history of difficulty with donating blood or difficulty in the accessibility of veins i. Subjects who were taking any prescription drugs or over-the-counter drugs (e.g.: cough and cold preparations, antacid preparations and natural products used for therapeutic benefits) within 30 days before screening j. Subjects who were taking any immunostimulant/ immunosuppressant supplement k. Subjects who were pregnant, nursing, or planning a pregnancy within the study participation period l. Female subjects with positive urine pregnancy test at the screening m. Subjects who were treated with any investigational drug or investigational device within 3 months before study entry

Table 1: Inclusion and exclusion criteria for subject selection into the study.

PHB			**Placebo**		
Ingredient name	**Dose**	**Active content**	**Ingredient name**	**Dose**	**Active content**
Withania somnifera (Ashwagandha)	100 mg	2.5 mg Total Withanolides	Microcrystalline Cellulose	475 mg	-
Boswellia serrata (Boswellia)	100 mg	2.5 mg AKBA, 2.5 mg KBA			
Azadirachta indica (Neem)	50 mg	0.25 mg Nimbolides			
Ultrasol Curcumin®	125 mg	25 mg Total curcuminoids			
Illicium verum (Star anise)	100 mg	5 mg Shikimic acid			
Total	475 mg	-	Total	475 mg	-

Table 2: Details of study products.

Safety and Efficacy Parameters

Safety assessments included monitoring of adverse events, physical examination, vital signs measurements, laboratory assessments, and urine pregnancy tests for females of childbearing potential monitored at baseline, day 30, and day 60. The efficacy parameters were evaluated at baseline, day 30, and day 60 and included WBC count, WBC differentials, Absolute Lymphocyte Count, platelet count, Immunoglobulins (IgG and IgM), CD3, CD4, CD8, CD4/CD8 ratio, CD45, and NK (CD16/56) cell counts, and CRP. Also, stress, sleep and common cold symptoms were analyzed using Perceived Stress Score (PSS), Pittsburgh Sleep Quality Index (PSQI), and Common Cold Questionnaire (CCQ), respectively.

Sample Size

Thirty healthy male or non-pregnant, non-lactating female subjects age between ≥ 30 and ≤ 70 years who frequently suffered from cold and flu were enrolled in the study with 15 subjects in PHB group and 15 subjects in placebo group.

Efficacy Analysis

Data were summarized for demographic and baseline characteristics, efficacy variables, and safety variables. For categorical variables, the number and percentage of each category within a parameter were calculated for non-missing data. For continuous variables with non-missing values, statistics included the number of observations, mean, standard deviation/standard error, median, minimum and maximum values. Percent change was calculated for each visit from baseline to present the standardized data. All statistical analyses were performed using SAS®, version 9.4. Subjects with missing data were excluded only from analyses for which data were not available. Mean change from baseline to Visit 2 - Interim visit (Day 30 ± 2 days) and baseline to Visit 3 - End of Treatment Visit (Day 60 + 2 days) were calculated using Paired T-test for within group analysis. Between groups analysis were conducted using ANOVA (Analysis of Variance). The criterion for the significant test by treatment was set at a p-value < 0.05.

Safety Analysis

Safety analyses were performed using hematology, biochemistry, and urine analysis, the incidence of adverse events, physical examination, and vital sign measurements for all the randomized subjects who received at least one dose of the study supplement. Descriptive statistics included number of subjects, mean and standard error for continuous safety variables and frequency, percentage for categorical safety variables such as adverse events were summarized for supplementation.

Results

Subject Disposition

Of the 31 screened subjects, a total of 30 subjects were randomized as one subject did not fulfill the eligibility criteria and was considered as screen failure (Figure 1). The demographic characteristics are shown in Table 3. In the PHB group, a total of 15 subjects were enrolled out of which 12 subjects were males and 3 were females having mean age 41.27±6.6 yrs, mean body weight 75.05±13.94 kg, mean height 165.1±11.22 cm and mean BMI 27.56±4.51 kg/m^2. On the other hand, in the placebo group, a total of 15 subjects were enrolled out of which 10 subjects were males and 5 were females with a mean age 36.67±5.15 yrs, mean body weight 71.48±15.63 kg, mean height 161.2±8.50 cm and mean BMI 27.52±5.63 kg/m^2.

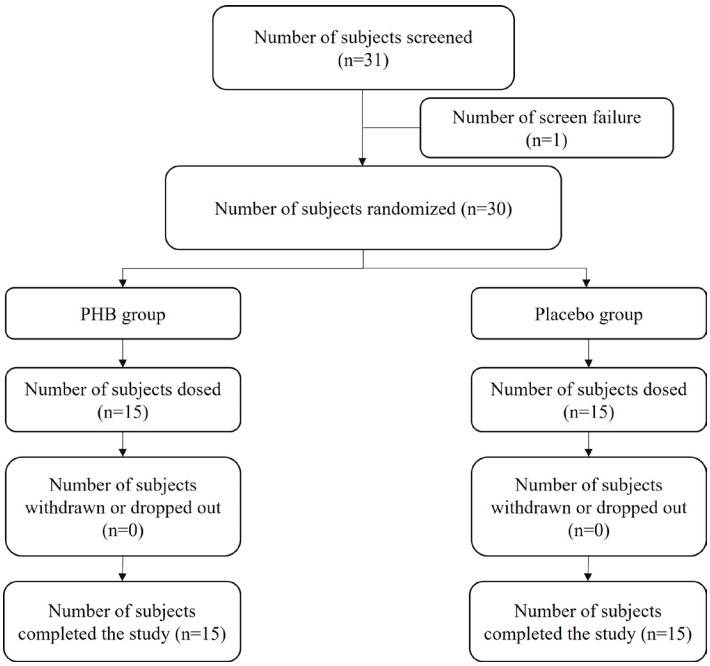

Figure 1: CONSORT.

Demographics Characteristics	PHB (N=15)	Placebo (N=15)
Age (Years)		
N	15.00	15.00
Mean ± SD	41.27±6.66	36.67±5.15
Median	41.00	36.00
Min, Max	33,52	30,50
p-value	0.0434	-
Sex, n (%)		
Male	12.00(80.00)	10.00(66.67)
Female	3.00(20.00)	5.00(33.33)
Height(cm)		
N	15.00	15.00
Mean ± SD	165.1±11.22	161.2±8.50
Median	167.0	163.0
Min, Max	145,183	146,175
p-value	0.2886	-
Weight(kg)		
N	15.00	15.00
Mean ± SD	75.05±13.94	71.48±15.63
Median	74.00	67.70
Min, Max	55,107	51,104
p-value	0.5149	-
BMI (kg/m^2)		
N	15.00	15.00
Mean ± SD	27.56±4.51	27.52±5.63
Median	26.40	26.30
Min, Max	20,36	19,39
p-value	0.9830	-

Abbreviations: N = number of subjects in specified treatment
n = number of subjects in specified category; PHB - Poly-Herbal Blend.

Note 1: Percentages are based on the number of subjects in the specified treatment. For continuous variables p-value calculated using Analysis of Variance (ANOVA) and for categorical variables using Chi square test/Fisher exact test if cell frequency is less than 5.

Table 2: Participants demographics.

Efficacy Parameters

The efficacy analysis was performed for 30 subjects who completed the study.

Total WBC count

A statistically significant increase in WBC count was observed on day 60 (p-value= 0.0455) whereas no significant difference was seen on day 30 (p>0.05) for PHB group over placebo (Supplementary Table 1a; Figure 2a).

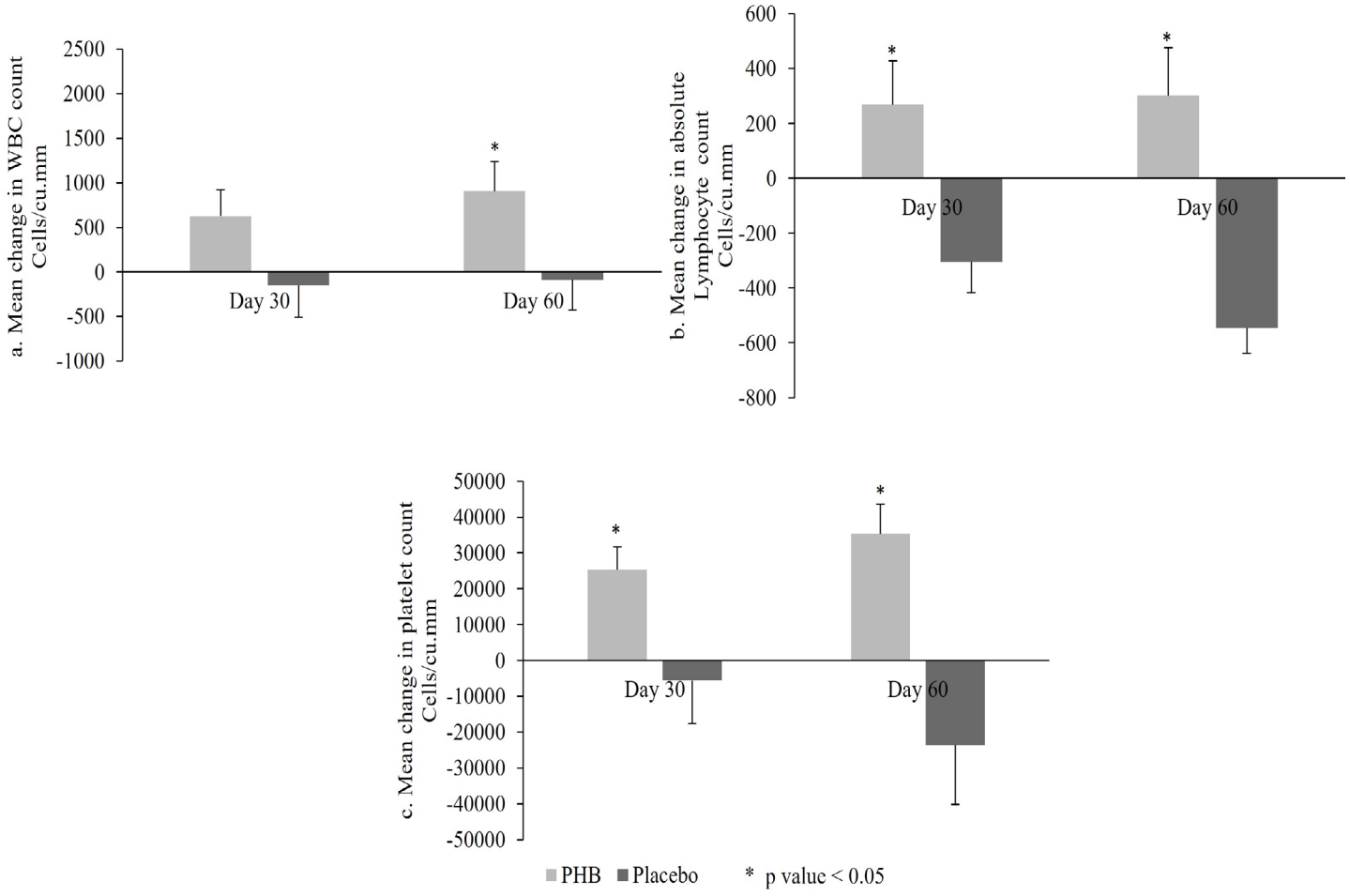

Figure 2: Mean change in WBC, Absolute Lymphocyte, and Platelet counts.

Visit	PHB (N=15)		Placebo (N=15)		P-value by ANOVA for mean difference - PHB vs Placebo
	Mean ± SE	Mean Change from Baseline ± SE	Mean ± SE	Mean Change from Baseline ± SE	
a. WBC Counts					
Baseline	7453.33 ± 392.29	0	7686.67 ± 385.56	0	-
Day 30±2	8080.00 ± 388.67	626.67 ± 293.01	7671.43 ± 447.95	-150.00 ± 362.29	0.1117
Day 60+2	8360.00 ± 442.59	906.67 ± 329.14	7600.00 ± 560.95	-86.67 ± 341.83	0.0455*
b. Absolute Lymphocyte Count					
Baseline	2314.07 ± 141.06	0	2608.13 ± 167.19	0	-
Day 30±2	2582.40 ± 185.85	268.33 ± 160.76	2334.64 ± 119.43	-303.86 ± 113.95	0.0086*
Day 60+2	2616.20 ± 219.43	302.13 ± 174.46	2063.07 ± 161.54	-545.07 ± 93.74	0.0002*
c. Neutrophils					
Baseline	55.31 ± 1.93	0	54.03 ± 2.17	0	-
Day 30±2	55.40 ± 2.23	0.09 ± 1.4	56.69 ± 2.04	2.22 ± 1.22	0.2714
Day 60+2	55.23 ± 1.88	-0.08 ± 1.2	58.65 ± 2.23	4.62 ± 1.80	0.0385*
d. Lymphocytes					
Baseline	32.05 ± 1.93	0	35.27 ± 2.04	0	.
Day 30±2	33.07 ± 2.07	1.02 ± 1.34	32.75 ± 1.95	-2.26 ± 1.02	0.0679#
Day 60+2	33.31 ± 1.80	1.25 ± 1.11	32.01 ± 1.90	-3.26 ± 1.45	0.0199*
e. Monocytes					
Baseline	7.37 ± 0.34	0	7.02 ± 0.34	0	-
Day 30±2	6.60 ± 0.24	-0.77 ± 0.42	6.64 ± 0.43	-0.21 ± 0.33	0.3159
Day 60+2	6.55 ± 0.18	-0.82 ± 0.31	5.89 ± 0.51	-1.13 ± 0.47	0.5909
f. Eosinophils					
Baseline	4.31 ± 0.86	0	2.86 ± 0.47	0	-
Day 30±2	4.30 ± 0.79	-0.01 ± 0.31	3.39 ± 0.59	0.57 ± 0.35	0.2311
Day 60+2	4.34 ± 0.86	0.03 ± 0.30	2.88 ± 0.32	0.02 ± 0.45	0.9902
g. Basophils					
Baseline	0.95 ± 0.08	0	0.81 ± 0.09	0	-
Day 30±2	0.63 ± 0.08	-0.33 ± 0.10	0.53 ± 0.04	-0.32 ± 0.10	0.9708
Day 60+2	0.57 ± 0.06	-0.38 ± 0.07	0.56 ± 0.09	-0.25 ± 0.11	0.3417
h. Platelet Count					
Baseline	264733.33 ± 20003.68	0	296933.33 ± 19615.95	0	-
Day 30±2	290133.33 ± 19329.19	25400.00 ± 6282.86	288142.86 ± 17843.44	-5642.86 ± 11861.86	0.0296*
Day 60+2	300133.33 ± 17333.11	35400.00 ± 8135.64	273400.00 ± 18650.37	-23533.33 ± 16625.24	0.0035*
i. IgG					
Baseline	1288.93 ± 81.61	0	1355.27 ± 63.67	0	-
Day 30±2	1315.53 ± 88.93	26.60 ± 21.83	1328.79 ± 69.66	-34.29 ± 18.90	0.0485*
Day 60+2	1343.53 ± 86.04	54.60 ± 23.30	1279.60 ± 73.50	-75.67 ± 40.23	0.0091*
j. IgM					
Baseline	111.20 ± 19.62	0	100.13 ± 7.48	0	-
Day 30±2	112.93 ± 18.95	1.73 ± 2.28	101.43 ± 9.14	0.79 ± 0.79	0.7838
Day 60+2	129.13 ± 23.50	17.93 ± 5.92	102.27 ± 10.14	2.13 ± 2.13	0.0514#
k. CD4 Cell Count					
Baseline	902.40 ± 68.73	0	1039.00 ± 70.23	0	-
Day 30±2	1007.00 ± 73.26	104.60 ± 48.51	1004.00 ± 65.83	-42.93 ± 44.54	0.0367*
Day 60+2	1108.93 ± 93.37	206.53 ± 68.33	883.53 ± 64.62	-155.47 ± 52.67	0.0002*
l. CD8 Cell Count					
Baseline	710.47 ± 93.52	0	849.93 ± 90.91	0	-
Day 30±2	758.67 ± 95.33	48.20 ± 44.56	752.21 ± 56.71	-111.79 ± 58.94	0.0417*
Day 60+2	711.67 ± 78.60	1.20 ± 45.94	754.40 ± 71.76	-95.53 ± 39.35	0.121
m. CD4/CD8 Ratio					
Baseline	1.50 ± 0.17	0	1.41 ± 0.17	0	-
Day 30±2	1.56 ± 0.19	0.06 ± 0.07	1.45 ± 0.16	0.05 ± 0.05	0.8993
Day 60+2	1.89 ± 0.34	0.39 ± 0.22	1.32 ± 0.16	-0.09 ± 0.06	0.0421*
n. CD45 Cell Count					
Baseline	2286.73 ± 158.01	0	2614.27 ± 176.99	0	-
Day 30±2	2633.27 ± 191.85	346.53 ± 163.16	2410.57 ± 144.26	-235.71 ± 138.74	0.0129*
Day 60+2	2806.20 ± 220.65	519.47 ± 174.65	2199.33 ± 156.91	-414.93 ± 110.77	0.0001*
o. CD3 Cell Count					
Baseline	1635.27 ± 140.17	0	1924.67 ± 117.38	0	-
Day 30±2	1863.13 ± 167.60	227.87 ± 92.45	1810.14 ± 83.51	-137.00 ± 103.25	0.0152*
Day 60+2	1998.40 ± 193.06	363.13 ± 112.10	1638.40 ± 112.73	-286.27 ± 91.06	0.0001*
p. Natural killer cell count					
Baseline	188.08 ± 16.96	0	156.87 ± 19.25	0	-
Day 30±2	268.34 ± 32.76	80.26 ± 27.86	131.28 ± 13.46	-27.99 ± 22.14	0.0061*
Day 60+2	254.51 ± 31.81	66.43 ± 27.56	137.16 ± 23.65	-19.71 ± 21.13	0.0194*
q. CRP					
Baseline	2.14 ± 0.59	0	2.72 ± 0.73	0	-
Day 30±2	1.86 ± 0.52	-0.28 ± 0.33	2.74 ± 0.63	0.05 ± 0.30	0.4685
Day 60+2	1.60 ± 0.58	-0.54 ± 0.38	4.33 ± 1.26	1.61 ± 0.89	0.0345*
r. Perceived Stress Scale (PSS)					
Baseline	14.07 ± 1.95	0	13.60 ± 2.39	0	-
Day 30±2	13.80 ± 1.30	-0.27 ± 1.74	16.33 ± 2.49	2.73 ± 2.04	0.2727
Day 60+2	11.40 ± 1.66	-2.67 ± 2.81	16.73 ± 2.48	3.13 ± 1.99	0.1032
s. Pittsburgh Sleep Quality Index PSQI – Total PSQI Score					
Baseline	3.33 ± 0.86	0	5.00 ± 1.00	0	-
Day 30±2	2.53 ± 0.32	-0.80 ± 0.74	5.20 ± 0.79	0.20 ± 0.87	0.3877
Day 60+2	1.80 ± 0.40	-1.53 ± 0.96	5.60 ± 0.95	0.60 ± 0.80	0.0976#
t. Common Cold Questionnaire CCQ – Total Scores					
Baseline	4.33 ± 1.00	0	3.33 ± 0.71	0	-
Day 30±2	1.00 ± 0.53	-3.33 ± 0.85	3.53 ± 1.46	0.20 ± 1.55	0.0553#
Day 60+2	0.33 ± 0.19	-4.00 ± 0.97	2.20 ± 0.63	-1.13 ± 0.69	0.0225*
u. Common Cold Questionnaire CCQ – General Symptoms					
Baseline	0.87 ± 0.34	0	0.53 ± 0.29	0	-
Day 30±2	0.27 ± 0.15	-0.60 ± 0.34	1.07 ± 0.56	0.53 ± 0.58	0.1037
Day 60+2	0.00 ± 0.00	-0.87 ± 0.34	0.40 ± 0.16	-0.13 ± 0.26	0.0935#
v. Common Cold Questionnaire CCQ – Nasal Symptoms					
Baseline	2.07 ± 0.45	0	2.00 ± 0.35	0	-
Day 30±2	0.47 ± 0.40	-1.60 ± 0.38	1.40 ± 0.51	-0.60 ± 0.54	0.1404
Day 60+2	0.20 ± 0.14	-1.87 ± 0.39	1.40 ± 0.38	-0.60 ± 0.45	0.0409*
w. Common Cold Questionnaire CCQ – Throat Symptoms					
Baseline	0.53 ± 0.19	0	0.47 ± 0.13	0	-
Day 30±2	0.13 ± 0.09	-0.40 ± 0.16	0.47 ± 0.24	0.00 ± 0.26	0.2011
Day 60+2	0.07 ± 0.07	-0.47 ± 0.19	0.00 ± 0.00	-0.47 ± 0.13	1
x. Common Cold Questionnaire CCQ – Chest Symptoms					
Baseline	0.87 ± 0.24	0	0.33 ± 0.16	0	-
Day 30±2	0.13 ± 0.09	-0.73 ± 0.25	0.60 ± 0.25	0.27 ± 0.30	0.0159*
Day 60+2	0.07 ± 0.07	-0.80 ± 0.24	0.40 ± 0.16	0.07 ± 0.25	0.0188*

PHB – Poly Herbal Blend. N - Number of subjects in specified treatment. SE - Standard Error. Between-group analysis with ANOVA. *P-value <0.05 #P-value<0.1.

Supplementary Table 1: Summary of efficacy endpoints between PHB and placebo groups.

Absolute Lymphocyte count

PHB group showed a significant increase in absolute lymphocyte count on day 30 (p-value = 0.0086) and day 60 (p-value = 0.0002) as compared to placebo (Supplementary Table 1b; Figure 2b).

Differential WBC

Placebo showed a significant increase in the % of Neutrophils on day 60 (p=0.0385) and no significant difference on day 30 (p>0.05) as compared to PHB group (Supplementary Table 1c). Further, PHB group showed a significant increase in % of Lymphocytes on day 60 (p=0.0199) and a trend on day 30 (p=0.0679) as compared to placebo (Supplementary Table 1d). There was no significant difference in % of monocytes, eosinophils, and basophils between PHB and placebo groups on day 30 and 60 (Supplementary Table 1e-g).

Platelet Count

PHB group showed a significant increase in platelet count on day 30 (p-value = 0.0296) and day 60 (p-value = 0.0035) as compared to placebo (Supplementary Table 1h; Figure 2c).

IgG and IgM

A significant increase in IgG was observed for PHB on day 30 (p-value = 0.0485) and day 60 (p-value = 0.0091) as compared to placebo (Supplementary Table 1i; Figure 3a). An increasing trend in IgM was observed on day 60 (p=0.0514) and no significant difference on day 30 (p>0.05) for PHB group as compared to placebo (Supplementary Table 1j; Figure 3b).

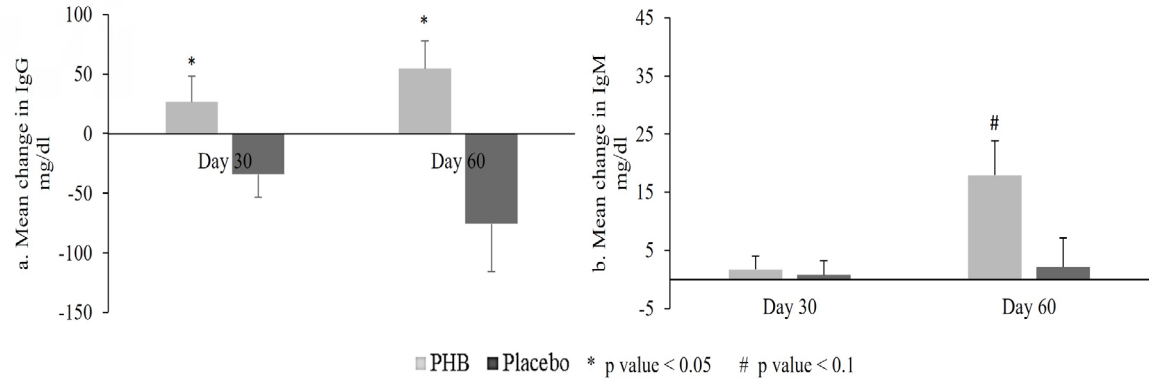

Figure 3: Mean change in IgG and IgM.

CD4, CD8 cell count and CD4/CD8 ratio

PHB group showed a significant increase in CD4 cells on day 30 (p=0.0367) and day 60 (p=0.0002) as compared to placebo (Supplementary Table 1k; Figure 4a). Further, PHB group showed a significant increase in CD8 cells on day 30 (p=0.0417) and no significant difference on day 60 (p>0.05) as compared to placebo (Supplementary Table 1l; Figure 4b). Also, PHB group showed a significant increase in CD4/CD8 ratio on day 60 (p=0.0421) and no significant difference on day 30 (p>0.05) as compared to placebo (Supplementary Table 1m; Figure 4c).

CD45 and CD3 cell count

PHB group showed a significant increase in CD45 cells on day 30 (p=0.0129) and day 60 (p=0.0001) as compared to placebo (Supplementary Table 1n; Figure 4d). Further, PHB showed a significant increase in CD3 cells on day 30 (p=0.0152) and day 60 (p=0.0001) as compared to placebo (Supplementary Table 1o; Figure 4e).

Natural Killer (CD 16/56) cell count

PHB group showed a significant increase in Natural Killer (CD 16/56) cells on day 30 (p=0.0061) and day 60 (p=0.0194) as compared to placebo (Supplementary Table 1p; Figure 4f).

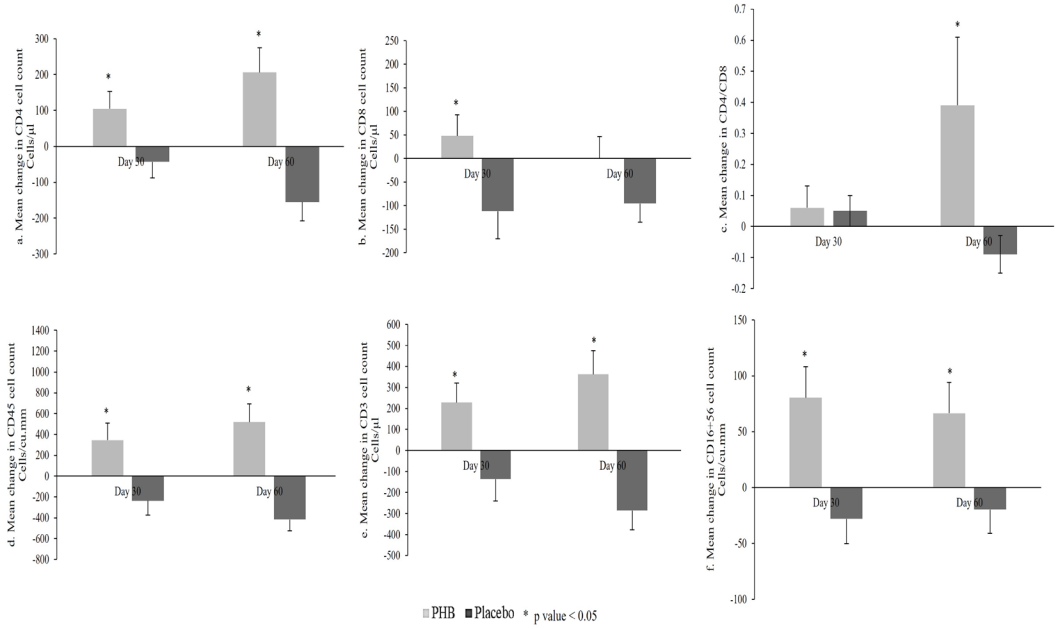

Figure 4: Mean change CD cell count.

C-Reactive Protein

PHB group showed a significant decrease in inflammatory marker CRP on day 60 (p=0.0345) and no significant difference on day 30 (p>0.05) as compared to placebo (Supplementary Table 1q; Figure 5a).

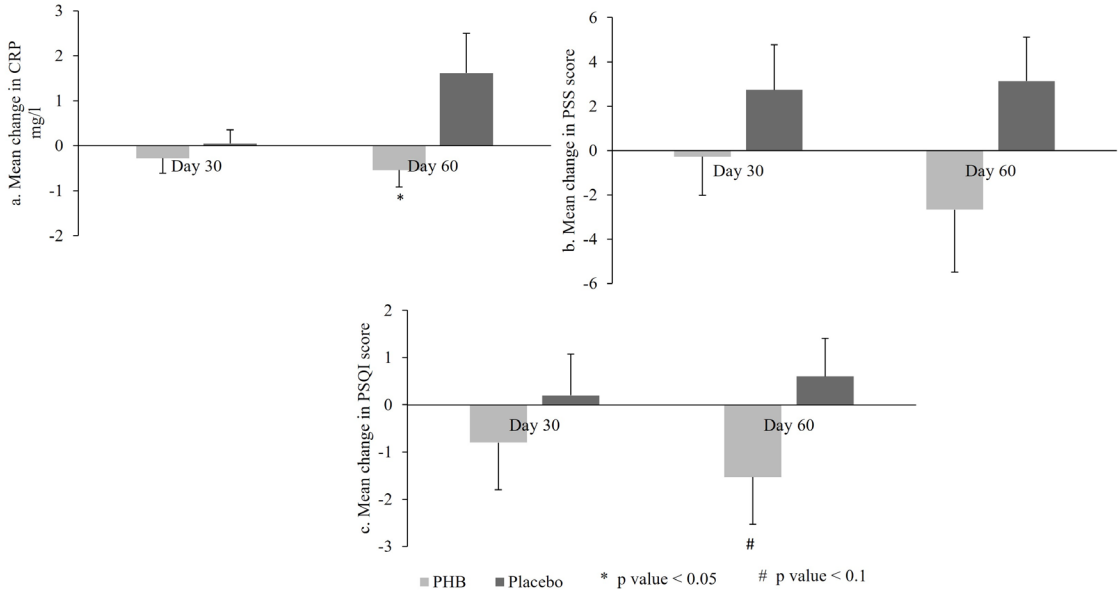

Figure 5: Mean change in CRP, PSS and PSQI scores.

Perceived Stress Score

No significant difference (p>0.05) was observed between PHB and placebo groups on perceived stress levels on days 30 and 60 (Supplementary Table 1r, Figure 5b).

PSQI Sleep Score

PHB group showed a trend in improving overall sleep quality on day 60 (p=0.0976) and no significant difference on day 30 (p>0.05) as compared to placebo (Supplementary Table 1s; Figure 5c).

CCQ

PHB group showed a decreasing trend in symptoms of common cold on day 30 (p=0.0553) and significant decrease on day 60 (p=0.0225) as compared to placebo (Supplementary Table 1t; Figure 6a). Further evaluation of individual sub-questionnaires showed a decreasing trend in general symptoms on day 60 (p=0.0935) for PHB group over placebo (Supplementary Table 1u; Figure 6b). PHB group showed significantly decreased nasal symptoms on day 60 (p=0.0409) as compared to placebo (Supplementary Table 1v; Figure 6c). There was no significant difference observed for throat symptoms between PHB and placebo groups (Supplementary Table 1w; Figure 6d). PHB group showed significantly decreased chest symptoms on day 30 (p=0.0159) and day 60 (p=0.0188) as compared to placebo (Supplementary Table 1x; Figure 6e).

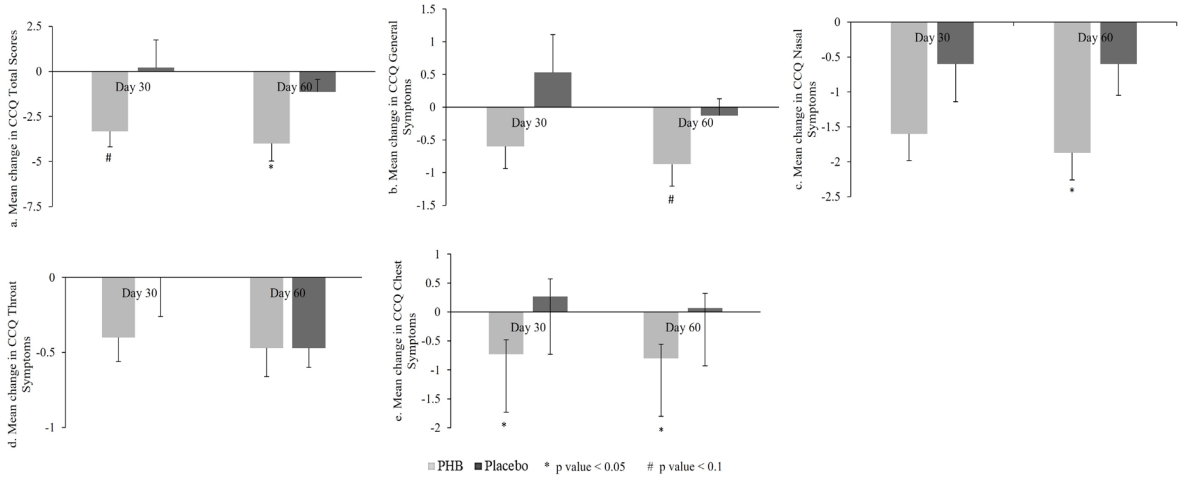

Figure 6: Mean change CCQ.

Safety

The composition of PHB supplement was found to be safe and well-tolerated and there was no incidence of treatment-related adverse events. A total of 7 subjects reported adverse events, 2 subjects in the PHB group and 5 subjects in the placebo group (Table 4). Though the AEs were reported after the consumption of study products, none of the adverse events were found to be related to the study supplements, no AEs were severe, and all the AEs recovered and were of mild intensity.

Adverse Event (AE)	**PHB** (N=15)	**Placebo** (N=15)	**Total** (N=30)
	Total AE (%)	Total AE (%)	Total AE (%)
Number of subjects with at least one related AE	2 (13.33)	5 (33.33)	7 (23.33)
General disorders and administration site conditions (Pain)	0 (0.00)	2 (13.33)	2 (6.67)
Infections and infestations (Cold)	1 (6.67)	1 (6.67)	2 (6.67)
Nervous system disorders (Headache)	1 (6.67)	2(13.33)	3(10.00)

AE – Adverse Event; N- Number of subjects in specified treatment group; PHB - Poly-Herbal Blend.

Table 4: Adverse events.

Discussion

Optimal functioning of immune system is critical to protect the body from diseases or infections [1,2,4-9]. Phytochemicals from medicinal plants with their immunomodulatory properties have been extensively used in traditional medicine as well as pharmaceuticals as a source of therapeutics with proven safety [28,29]. Phytochemical contents of medicinal herbs such as flavonoids, lactones, alkaloids, and glycosides target immune system at multiple stages and hence are more efficient in providing overall health benefits [15,30]. We evaluated a poly herbal blend consisting of standardized extracts of Ashwagandha, Boswellia, Neem, Star Anise and a formulated turmeric extract (Ultrasol curcumin®) with their established science and safety from published literature. We measured multiple clinically validated immunological parameters of innate and adaptive immune response in healthy human subjects frequently susceptible to cold and flu. Our study outcome indicate that administration of PHB for 60 days has significant effect on various parameters of immune response as compared to placebo with a statistically significant increase in cell count for total WBCs, as well as sub-population of WBCs including lymphocyte %, natural killer cells and platelets that mediate innate immune response. We could not demonstrate any specific effect of PHB on neutrophils as the placebo group showed significant increase in % neutrophils on day 60 as compared to PHB group. Similarly, there was a statistically significant increase in adaptive immune response as measured through increased CD45, CD3, CD4, CD8 cell count and CD4/CD8 ratio as well as increased IgG and IgM serum antibody levels. The above improved immune response was associated with significantly decreased CRP levels which is an acute inflammatory marker and decrease in symptoms of common cold in subjects receiving PHB as compared to placebo. Further PHB was found to be safe with no treatment related adverse events reported throughout the study period.

White blood cells play an important role in destruction of infectious microbes and overall maintenance of immune response [3]. We measured total WBC count as well as sub-population of WBCs including neutrophils, lymphocytes, monocytes, eosinophils, and basophils. We found a statistically significant ($p < 0.05$) increase in WBC count for PHB over placebo group on day 60. This was further evident from our cell count for cells positive for CD45 marker which is found in all nucleated blood cells which showed a significant increase on day 30 ($p < 0.05$) and day 60 ($p < 0.05$) as compared to placebo. Similarly, we also found a significant increase in absolute lymphocyte count as well as cells positive for CD3 marker that represent T lymphocytes on day 30 ($p < 0.05$) and day 60 ($p < 0.05$) as compared to placebo for subjects receiving PHB. Lymphocytes population include NK cells, B-cells and T-cells and are essential components of innate and adaptive immunity. NK cell subsets play an important role in overall surveillance, recognize and kill abnormal cells in the body as part of innate immune response. We found that PHB increased Natural Killer (CD 16/56) cells on day 30 ($p < 0.05$) and day 60 ($p < 0.05$) as compared to placebo group. T lymphocytes regulate cell mediated immunity, proliferate upon antigen stimulus and differentiate into different type of effector cells such as CD4 or CD8 cells. Further, T lymphocytes also modulate function of B lymphocytes to differentiate into antibody producing plasma cells in a highly interconnected network and maintain healthy and functional immune system both during normal and disease conditions [31]. CD4 T lymphocytes are subsets of lymphocytes that regulate immune response by stimulating other immune cells, such as macrophages, B cells, and CD8 lymphocytes and reduced number of CD4 cells in blood indicate immune suppression as seen in AIDS condition. We observed a significant increase in CD4 T lymphocyte count in blood on day 30 ($p < 0.05$) and day 60 ($p < 0.05$) in subjects receiving PHB as compared to placebo. Similarly we observed increase in CD8 T lymphocyte blood count on day 30 ($p < 0.05$) as compared to placebo. CD8 T lymphocytes are a sub-population of lymphocytes that regulate adaptive immune response and provide protection against viral infections. This is further validated by increased CD4/CD8 ratio in subjects receiving PHB with significant increase in CD4/CD8 ratio on day 60 ($p < 0.05$) as compared to placebo. A low or inverted CD4/CD8 ratio is an indicator of underlying immune suppression and extensively used to monitor patient management in clinics.

C- Reactive Protein (CRP) is an acute inflammatory marker measured in the blood to monitor infection or disease associated inflammatory conditions. We see a significant ($p < 0.05$) decrease in CRP levels on day 60 in case of subjects receiving PHB as compared to placebo although the change that we observed remained within the normal level. Serum immunoglobulin levels as indicator of humoral immune status are measured in clinical practice routinely and low level serum immunoglobulin indicate humoral immunodeficiency [1]. While serum IgM levels provide a rapid immune response, IgG is long-lasting high-affinity antibodies [1]. We observed a significant increase ($p < 0.05$) in serum IgG levels both on day 30 and 60 and an increasing trend for IgM levels for day 60 in case of PHB as compared to placebo.

Immune modulatory properties of botanical extracts such as *Andrographis paniculata*, Turmeric, Ashwagandha, Boswellia, *Pelargonium sidoides*, Neem has been reported in the past in human and various in vitro and in vivo experimental models. In general these extracts seem to have effect on CD4+ and CD8+ T cell, B cell and NK cell count as well as serum cytokine levels of IFN-γ and IL-4 [3,31-38]. Immunomodulatory effect of *Pelargonium sidoides* extract was seen in subjects with upper tract airway infections [35]. Similarly, neem extract was explored in HIV infected patients with a 30-day regime showed good protection with improved lymphocyte and CD4+ cells count [36]. The botanical extracts used in PHB blend are known to modulate

immune response through elevation of blood CD4, CD8 T cells, NK cells, B cells, increased cytotoxic activity of macrophages and increased levels of serum IgG and IgM antibodies in various experimental models [5,37,39-41]. Further, ingredients used in PHB have also widely demonstrated to have extensive anti-viral properties [25,38,42-50]. Additionally all these ingredients with phytochemical contents such as polyphenols and flavonoids also known for their ability to scavenge free radicals and inhibit lipid peroxidation which further helps to control inflammation [51,52].

Sleep and stress also play key role in acquired immunity. Studies report that poor sleep alters the acquired immunity, increases the risk of infections, and stress [10,53,54]. On the other hand sleep and the circadian system exert a strong regulatory influence on immune functions. Sleep enhances immune defense and chronic sleep deprivation that induce state of chronic stress, also known to impact immune functions and general health [33,55,56]. We see a trend in improving overall sleep quality ($p=0.0976$) and decreasing trend in symptoms of common cold on day 30 ($p=0.0553$) and significant decrease on day 60 ($p=0.0225$) as compared to placebo in our study. This was further accompanied by significantly decreased nasal and chest symptoms on day 60 ($p=0.0409$) and a non-significant reduction ($p > 0.05$) in general symptoms and throat symptoms of CCQ.

Study limitations were as follows:

- The study was done with sample size of 15 subjects in each group. Although the results showed statistical significance for several biomarkers, additional studies with focus on larger sample size would be necessary to re-validate these study findings.
- In this study, subjects were not individually monitored for dietary, occupational, and lifestyle conditions. Future studies to address these limitations will help re-validate these findings.

Conclusions

The active components of medical plants have been used as an important source of clinical therapeutics since they offer a chemical diversity associated with multi-pharmacological activity and widely recognized for their health benefits in traditional medicine. We observed that poly herbal blend used in this study not only improved multiple parameters of innate and adaptive immune response in our study subjects but also has significant improvement in overall quality of life by affecting symptoms of cold and inflammation. We conclude that PHB with multiple plant extracts known for their positive effect on immune homeostasis could provide an effective option to support robust immune response.

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Author contributions: Belliappa CMA, Abhijeet Morde, Muralidhara Padigaru and Sathish Kumar Durairaj designed the study and were involved in the data interpretation. Belliappa CMA helped in the conduct of the study at the site, volunteer recruitment, study procedures and data collection. Sathish Kumar Durairaj helped in statistical analysis and study report preparation. The manuscript was drafted by Belliappa CMA, Abhijeet Morde, Muralidhara Padigaru. All authors read and approved the final manuscript.

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Compliance with ethics guidelines: Institutional ethics approval was obtained from Ethics Committee of Telerad Rxdx Healthcare Private Limited (EC registration number ECR/1494/Inst/KA/2021). This study was conducted in compliance with the ethical principles originating in or derived from the Declaration of Helsinki and in compliance with the International Conference on Harmonization (ICH), Good Clinical Practice (GCP) Guidelines, as well as in strict compliance with the "The New Drugs and Clinical Trial Rules- 2019", the Ministry of Health and the Government of India at all stages of the trial for adherence to protocol and compliance with ethical and regulatory guidelines. The EC was duly apprised of the progress and updates of the trial at regular intervals as per prescribed guidelines.

Data Availability: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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