WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 10, 1038-1048. Research Article ISSN 2277– 7105

AN INTERVENTIONAL, PROSPECTIVE PILOT CLINICAL TRIAL TO EVALUATE SAFETY AND EFFICACY OF PIHF IN IMPROVING IMMUNITY IN HEALTHY ADULTS

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Article Received on
21 June 2021,

Revised on 12 July 2021,
Accepted on 02 August 2021

DOI: 10.20959/wjpr202110-20926

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ABSTRACT

Objective: The COVID-19 pandemic has imposed an excessive damage to the world as it is spreading with high rate and simultaneously with a high mortality**.** Within this pandemic situation, it is of high importance to strengthen immune system. **Material and methods:** 30 subjects were enrolled in the study and were treated with the test product for 30 days. Immunity parameters, subjective scales were assessed on baseline and day 30. **Results:** PFHI formulation was effective in increasing the GHQ-28 score by 152.5 % after 30 days. The energy audit frequency score distribution depicted that the "very high energy" and "high energy scores" were increased and the "very low energy" and "low energy" scores were decreased as compared to the baseline. The serum immunoglobulin levels in treated subjects were found to be increased by 39.93 % from the baseline. The digestive complaints were reduced. Occasional abdominal pain was reduced by 44.6%, heartburns were reduced by 34.2 %, and regurgitation and flatulence were decreased by 28.1 % and 25.8 % respectively. Other complications like bloating were reduced by 19.0 %, constipation was decreased by 14.1 % and post prandial fullness was reduced 17.1 %, in span of 30 days. All the parameters in the complete Hemogram were within normal range. There were no episodes of illness, infections, etc. **Conclusion:** This explains PFHI is an effective

and safe formulation. PFHI is effective in enhancing the general health. It has potential action in increasing energy and reducing digestive complications.

KEYWORDS: COVID-19, Immunity, Ayurvedic formulation, Digestive complications.

INTRODUCTION

The World has suffered an excessive damage when the novel COVID-19 pandemic emerged and started spreading with a high rate. The COVID 19 has affected a vast number of population and also has threatened with the death rate.[1] We all are surviving the Pandemic situation with an extreme fear, even after strict measures like shutting down the cities, we are not able to control the spread of infection and mortality. Amidst all chaos improving immunity and health status of every individual has become a key factor. Thus, there is a great need to improve and strengthen the immune system.[2] Immunity is a condition of being able to resist a disease or to reduce the severity of infections and the complications.[3] The common measures of boosting immunity is to supplement diet with nutrition of vitamins, minerals and antioxidants etc.[4] There is researched correlation between stress and immune system, adaptogenic and antistress activity could prove important therapeutic goals to improve immunity overall.[5]

Indigenous systems of medicines like Ayurveda have been instrumental since years to understand health in wholesome way and improve immune status for healthier life. The Ayurveda pays larger emphasis on building strength of mind and body to cope with various stressors, including infection. In Ayurveda several treatment options are available for enhancing immunity against respiratory illnesses, these include certain immunomodulator (known as Rasayana).[6,7] The ministry of Ayush, has released set of guidelines for boosting immunity and measures to be taken to for self-care by using Ayurvedic principles.[8] Ayurved acts by Multimodal mechanism including reduction of oxidative stress, anti-inflammatory mechanisms. Several therapeutic targets from the past incidences of SARS and MERS may help in joining the links and can suggest that Ayurvedic formulations can prove effective in infections of corona virus.

To meet the need of boosting immunity, Naturedge Beverages Pvt. Ltd. has developed PIHF decoction which is in unit dosage form and easy to incorporate in the lifestyle and in a very palatable and tasty formulation. The aim of present research was to evaluate the safety and efficacy of Ayurveda based herbal decoction for improving immunity in healthy subjects.

MATERIALS AND METHODS

Materials

PFHI, an herbal decoction was used for study as an investigational product. It is ready to drink unit pack of 60 ml.

Methods

After getting approval from the ethics committee, the study was registered on the CTRI website. The CTRI registration number is CTRI/2021/03/032208. Subjects were enrolled in the study only after registration of study on the CTRI website. The subjects were recruited after screening for compatibility with inclusion and exclusion criteria. Total 30 subjects were recruited. Each subject received 60 ml unit pack of PFHI test product. The test product was to be administered for 30 days.

The primary objectives of the study were to evaluate the changes in immunoglobulin levels, number of episodes, severity and duration of illness during the study period, to evaluate the changes in blood cell count through complete hemogram and to evaluate changes in symptoms like cough, breathlessness, etc.

The secondary objectives of the study were to evaluate the digestive behavior questionnaire score, to assess the energy audit questionnaire score and to assess quality of life score.

Inclusion criteria

Volunteer of either sex of age between 16 to 80 years were included in the study. The subjects ready to provide written informed consent form were considered in the study. The subjects with or without propensity of seasonal cough, cold and allergic episodes were included in the study. The subject willing to come for the follow-up visits for evaluation were enrolled to participate in the study.

Exclusion criteria

The subjects with known abnormal laboratory, ECG or X-ray finding during screening were excluded from the study. Subjects with known uncontrolled diabetes mellitus with HbA1C more than 7.5, subjects with hypertension, congestive heart failure, unstable angina pectoris and myocardial infarction were not included in the study. Subjects with current medical history of any major illness such as cancer, heart disease, COPD, Asthma, etc. in the past were not considered eligible to be considered in the study. Subjects who had participated in

another clinical trial within 3 months were excluded from the study. Vulnerable population like pregnant, breast-feeing women and women of child bearing age refusing to use contraceptive measures were excluded from the study. Immunologically compromised subjects, subjects with past history of addiction abuse, rehabilitation, subjects consuming 1 cigarette per day were excluded from the study. Subjects which were found as not eligible as per Investigator's opinion were excluded from the study.

Study procedure

The study was sponsored by Naturedges Beverages Pvt. Ltd. and the proposal was applied to Institutional ethics committee for review and approval. After approval from institutional EC clinical study was registered on CTRI website and study was conducted at Atharva Ayurved and Research Centre, Pune.

Male and female subjects of age between 18 to 60 years (both inclusive) attending study site(s) were screened for eligibility criteria. On screening visit, a written informed consent was obtained from subjects for their participation in the study. Subject's demographic details were recorded. Subject clinical examination was performed. Subject's medical, surgical and treatment history was recorded. Subject's current medication if any was noted in the case record from (CRF). Subject's vitals were recorded. The subjects were considered for further evaluation as per the inclusion and exclusion criteria.

On baseline visit i.e., Day 0, subject fitting in all inclusion criteria and showing absence of all exclusion criteria were randomized. Subjects were on PIHF 60 ml per day till 30 days.

Subject undergone clinical examination. Subject's vitals were recorded. The subjective questionnaire and symptoms grading were recorded.

The record of concomitant medication was kept in the CRF. All subjects were advised to follow their diet routine as per designed by study center dietician during the entire study period.

The presence of any adverse events was strictly monitored and reported. The treatment was followed on subsequent days till day 30. On day 15 the questionnaire, symptom grading and adverse events were checked on telephonic call and day 30 was the physical visit and blood samples were collected for hematological tests and assessment of other scales, scores etc. were performed.

RESULTS AND OBSERVATIONS

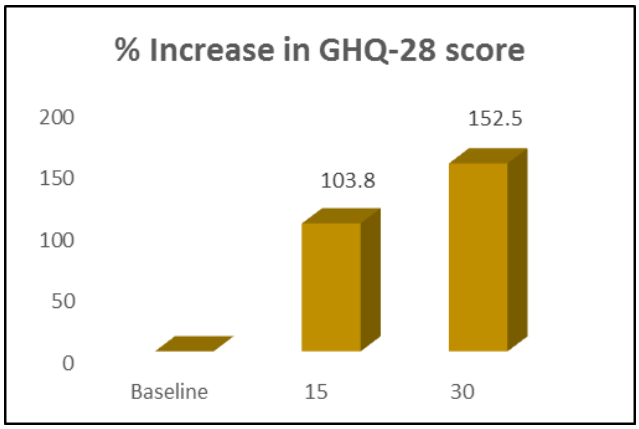
Ddemographic details

In the present study, 35 subjects were screened. Out of 35 subjects, 2 lost to follow up and 3 were screen failure. 30 subjects were considered evaluable cases at the end of the study. The mean age of subjects was 35.2± 6.83 years. There were 15 male and 15 female subjects.

Efficacy assessments

Assessment of General Heath Questionnaire-28 (GHQ-28) Score

There was significant increase GHQ-28 score in PIHF treated group. After 15 days there was 103% and on 30 days around 1.5 times increase in GHQ-28 score in improving Quality of Life. (Graph 1) In case of intergroup analysis, marketed product treated group demonstrated significant increase in GHQ-28 score ($p<0.001$) at day 30, 60 and 90 when compared to baseline value of the same group.



Graph 1: Assessment of General Heath Questionnaire-28 (GHQ-28) Score between the groups.

Assessment of Energy Audit Questionnaire

The frequency distribution of energy events depicted difference in “Very High” score is increased significantly in PIHF treated group from baseline to day 30.

The frequency distribution of energy events depicted difference in “High” score is increased significantly in PIHF treated group from baseline to day 30.

The frequency distribution of energy events depicted gradually after treatment the number of subjects representing “High” and “Very High” energy level scores were increased and “Moderate” to “Very Low” energy levels were decreased.

The same results are evident from Mean Score of Energy Audit. (Table 1)

Table 1: Assessment of Energy Audit Questionnaire Mean Score between the groups.

Energy levels	Mean Score of Energy Audit Questionnaire		
	Baseline	Day 15	Day 30
Very High	0.91±1.11	7.36±6.06	23.11±12.34
High	7.91±3.39	34.07±7.13	25.88±11.98
Moderate	38.53±7.15	33.47±6.11	13.81±7.10
Neutral	43.11±7.39	22.64±5.47	10.36±7.10
Low	17.32±4.29	10.80±3.29	5.38±3.10
Very low	9.36±3.61	8.93±4.10	4.11±2.16

Assessment of serum immunoglobulin (IgG)

The serum immunoglobulin levels were increased in 39.93 % on day 30. The results are depicted in Table 2.

Table 2: Assessment of serum immunoglobulin (IgG).

	Serum IgG (mg/dl) Mean ± SD			
	Baseline	Day 30	% increase	P value
Treatment group	850.9 ± 128.0	1190.7 ± 207.5	39.93	P<0.05

By Student's' Test, P > 0.05 Not Significant, Significant p< 0.05

Assessment of Digestive Behaviour Score

There was significant reduction in digestive complaints after incorporating PIHF treatment to the subjects. The % reduction is expressed in the table 3.

Table 3: Assessment of Digestive Behaviour Score Mean between the groups.

Parameters	Mean Score ± SD		% reduction
	Baseline	Day 30	
Occasional Abdominal Pain	1.21±0.24	0.67±0.71	**44.6**
Heartburn	5.44±0.66	3.58±0.69	**34.2**
Regurgitation	5.62±0.61	4.04±0.80	**28.1**
Flatulence	5.34±0.80	3.96±0.85	**25.8**
Bloating	7.07±0.86	5.73±0.89*	**19.0**
Constipation	4.11±0.96	3.53±0.79*	**14.1**
Loss of Appetite	7.36±0.48	5.69±0.92*	**22.7**
Post Prandial Fullness	**7.56±0.50**	**6.27±0.96***	**17.1**

By Student's' Test, P > 0.05 Not Significant, Significant p< 0.05

Changes in Mean parameters of Complete Hemogram

All the parameters in the complete Hemogram were within normal range. Parameters of complete hemogram were comparable at baseline and at day 30, mean value of parameters of

complete hemogram did not show any significant change from baseline and the difference was statistically insignificant. The results are depicted in Table 4.

Table 4: Changes in Mean parameters of Complete Hemogram between the groups.

Parameters	Mean ± SD	
	Baseline	Day 30
Total Leukocyte Count (X 10^3 / µL)	7.21±2.01	6.98 ±1.97
Neutrophils (%)	53.40±13.70	52.83±11.34
Lymphocytes (%)	30.08±6.51	29.64±6.76
Monocytes (%)	4.32±1.97	5.02±2.33
Eosinophils (%)	4.27±3.84	4.38±2.13
Basophils (%)	0.40±0.28	0.48±0.37
Total RBC Count (X 0^6/µL)	4.80±0.56	4.50±0.57
Haemoglobin (g/dL)	14.13±1.78	13.98±1.49
Haematocrit (%)	43.15±3.86	41.58±2.78
Platelets (X 10^3 / µL)	280.14±85.20	281.18±64.86
Platelet Distribution Width (fL)	12.31±1.72	12.09±1.96
Mean Platelet Volume (fL)	10.08±1.59	10.10 ±2.22
ESR (mm/hr)	**7.11± 2.00**	7.10±4.19

By Student 't' Test, P > 0.05 Not Significant

Changes in number of episodes, severity and duration of illness during the study period

There were no subjects reported any episodes of illness, infections etc. in the study population with no events of cough, breathlessness and fever etc.

DISCUSSION

The World has suffered an excessive damage when the novel COVID-19 pandemic emerged and started spreading with a high rate. The COVID 19 has affected a vast number of population and also has increased death rate to an extreme high level.[9] Due to undefined treatment in COVID-19, the only way to survive in this Pandemic situation is to boost immunity, to stay uninfected from this deadly virus. In strengthening immunity, Ayurvedic formulation have been found trust-worthy and effective from past centuries or long.[10] To extend the trust of population and serve as an efficient source of enhancing immunity, Naturedges beverages Pvt. Ltd. has developed an herbal decoction (PIHF). The current study aims at clinical validation of safety and efficacy of PIHF in improving immunity. The present clinical study represents data of 30 healthy individuals who received PFHI. The dose of test product was 60 ml PIHF daily orally for 30 days. Following are the broad outcomes of the present study of PFHI in healthy individuals. The General health questionnaire-28 (GHQ-28) is a self-administered screening questionnaire, designed for use in diagnosing and assessing

psychiatric health.[11] The GHQ-28 score was assessed in subjects with treatment group. It was found to be increased by 103.8 % and 152.5 % on 15th and 30th day respectively. The energy audit questionnaire is used to assess the energy level as represented with frequency scores. The events in the energy scale are very high, high, moderate, low, and very low. In the present study the mean score of energy audit was assessed and the frequency score distribution depicted that the "very high energy" and "high energy scores" were increased as compared to baseline and the very "low energy" and "low energy" scores were decreased as compared to the baseline. In subjects with treatment with the test product, the serum immunoglobulin levels were found to be increased by 39.93 % from the baseline. As correctly quoted "The gut is the place where bacteria and immune system meet" this explain that Digestion and immunity go together. The better the digestion, better is immunity. Digestive complaints are more common in the covid-19 pandemic. Majorly digestive abnormalities are certain early symptoms of the progression of disease. Occasional abdominal pain, flatulence, bloating, constipation, loss of appetite is common in mild to moderate disease.[12] The digestive health questionnaire has been built by health experts so as to assess the digestive complaints in subjects. In the present study, there were significant reduction in digestive complaints after incorporating PIHF treatment to the subjects. Occasional abdominal pain was reduced by 44.6%, heartburns were reduced by 34.2 %, and regurgitation and flatulence were decreased by 28.1 % and 25.8 %. Other complications like bloating were reduced by 19.0 %, constipation was decreased by 14.1 %, loss of appetite by 22.7 %, and post prandial fullness was reduced 17.1 %, in span of 30 days. Hemogram of all the subjects with test product were observed for changes in hemogram, all the parameters in the complete Hemogram were within normal range. Parameters of complete hemogram were comparable at baseline and at day 30. There were no subjects reported any episodes of illness, infections etc. in the study population with no events of cough, breathlessness and fever. The consumption of the decoction is ought to be boosting immunity by multimodal mechanisms (MMM). The formulation is a blend of certain herbal extracts namely Giloy extract (Tinospora cordifolia), Wheat grass extract (Triticum aestivum), moringa extract (Moringa oleifera Lam), Dashmool extract, Amla (Phayllanthus emblica) safed musali (Chlorophytum Borivilianum), lemon dry powder, ginger extract (Zingiber offiacinalis) and many more. The Giloy extract is reported to function by boosting the phagocytic activity of macrophages, production of reactive oxygen species (ROS) in human neutrophil cells, enhancement in nitric oxide (NO) production by stimulation of splenocytes and macrophages indicative of anti-tumor effects.

Aqueous Tinospora extracts has been also reported to influence the cytokine production, mitogenicity, stimulation and activation of immune effector cells.[13]

The wheat grass extract possesses immune-activating properties thorough directly affecting the surface antigen expression and Th1 cytokine secretions in monocytes and then indirectly activating NK and T cells only in the presence of monocytes.[14] Dashmool extract has tremendous benefits as the name suggest Dashmool meaning ten herbs with powerful benefits. This Vata pacifier, Anti-inflammatory, immunomodulatory, adaptogenic. Moreover, it possesses detoxifying, stress reliving properties.[15] Dashmool is an effective uterine tonic and detoxifier and is a boon for uterine disorders, and other diseases like Atherosclerosis, Fatigue, Parkinson's, Gout, and Paralysis. It flushes toxins out of your body, boosting your immune system immensely.[15] Kalmegh is an antioxidant, adaptogenic and appetite stimulant which helps in increasing diet of the subjects and contributes to the overall effects of the formulation.[16] Amla is a rich source of vitamin C, acts a potent antioxidant, also it has beneficial effects in digestion. Amla is rich in enzymes that promotes the absorption of nutrients. Thus, contributing to a healthy gut.[17] The blend of all these herbs, play a role in boosting the immunity and reducing digestive complication when treated with PFHI. Thus, it can be concluded from the present study that PFHI is a safe and effective formulation in boosting immunity in healthy individuals.

CONCLUSION

Boosting immunity is of prime importance in this era of pandemic. Our formulation PFHI developed to strengthen immune system and this study it was tested to evaluate the clinical validation of safety and efficacy of PIHF in improving immunity. From the study we can conclude that PFHI is effective in enhancing the general health. It has potential action in increasing energy and reducing digestive complications. The test formulation is effective in boosting immunity which is demonstrated by an increase in IgG antibodies amongst the subjects with test treatment. It gives claimed beneficial effects without altering the normal hemogram and without causing any episodes of illness and infection. Thus, from the current study it is evident the PIHF is safe and effective formulation.

ACKNOWLEDGEMENTS

The authors would like to acknowledge entire team involved in the trials and the back office team for their contribution in the research work.

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