Original Article

AYUSH-64 as an add-on to standard care in asymptomatic and mild cases of COVID-19: A randomized controlled trial

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Abstract

Background: The evidence on the efficacy and safety of Ayurveda interventions as an add-on to the standard conventional care for coronavirus disease-2019 (COVID-19) is limited. **Aim and objective:** This study was planned to explore the potential of AYUSH-64 as an add-on to conventional care in improving the clinical recovery and negative reverse transcription–polymerase chain reaction (RT-PCR) conversion in asymptomatic and mild COVID-19 cases. **Materials and methods:** An open-label randomized controlled study was conducted at Government Medical College, Nagpur, Maharashtra, India, with a sample size of 60 participants. In this study, asymptomatic or mild COVID-19 patients were randomized and allocated into intervention and control groups (CG) in a 1:1 ratio. AYUSH-64 two capsules (500 mg each) were administered thrice daily, after food with water for 30 days along with standard care in the intervention group (IG), while the CG received only standard care. The primary outcome was the proportion of participants who turned RT-PCR negative for COVID-19 at 7th, 15th, 22nd and 30th days. Secondary outcomes were the proportion of participants who attained clinical recovery at 7th, 15th, 22nd and 30th days, change in laboratory parameters on the 30th day and incidence of adverse drug reactions/adverse events. The data were compared within group using paired sample *t*-test/Wilcoxon signed-rank test and between group using independent sample *t*-test/Mann–Whitney test. **Results:** Statistically significant difference was not observed in the proportion of participants who turned RT-PCR negative during each of the follow-ups ($P = 0.134$) and both groups demonstrated comparable efficacy. The clinical recovery in terms of complete relief in symptoms in the symptomatic participants was 60% and 37% on day 15 ($P = 0.098$) and 100% and 85.2% on day 30 ($P = 0.112$) in the intervention and CG, respectively. The improvement in the inflammatory markers such as interleukin (IL)-6, tumor necrosis factor-α (TNF-α), and D-dimer was statistically significant ($P < 0.05$) in the IG, whereas in the CG, it was statistically significant for D-dimer only. None of the participants developed any complications nor were any significant ADR/AE observed in the groups. **Conclusions:** In patients with asymptomatic and mild COVID-19, AYUSH-64, as add-on to standard conventional care, contributed to improved clinical recovery and demonstrated potential in reducing the levels of pro-inflammatory markers such as IL-6 and TNF-α. Further, both the groups demonstrated comparable efficacy regarding negative RT-PCR for COVID-19.

Keywords: Ayurveda, AYUSH-64, coronavirus disease-2019, pandemic, SARS-CoV-2

Introduction

Coronavirus disease-2019 (COVID-19) has affected more than 181 million people around the world and around 3.9 million deaths have been reported globally as of 30th June 2021.[1] The physical, psychological, social and economic consequences of the pandemic have been very severe and have affected the world in the most unprecedented manner. Potential therapeutic and prophylactic agents should ideally have antiviral properties against SARS-CoV-2, immunomodulatory properties, and therapeutic adjuvant activity with drugs used while being safe and tolerable.[2] Although several therapeutic interventions such as hydroxychloroquine, corticosteroids, and antivirals have been suggested and tried, the outcomes have not been much promising. The current medical strategy for the

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prophylaxis and management of COVID-19 is broadly based on the repurposing and repositioning of existing medications and deploying them with symptomatic support. Drugs such as antiviral, antimalarial, anti-inflammatory, and monoclonal antibodies have undergone trials, based on published empirical evidence. One of the published systematic reviews reported that corticosteroids are most frequently used to treat patients with COVID-19, followed by lopinavir/ritonavir and oseltamivir.[3]

In view of the unchecked morbidity and mortality rates, the scientific community needs to also consider pluralistic traditional medicine systems used globally. *Ayurveda*, the Indian traditional medicine system, has a lot to offer in this ongoing COVID-19 pandemic. It may provide a much-needed effective and safe alternative or bridge the existing gaps in conventional medicine, leading to reduce disease burden.[4] Integrating Ayurveda interventions with conventional medicine could offer a novel, safe, and cost-effective strategy to effectively manage the COVID-19 pandemic. Ayurveda interventions can be repurposed for the prophylaxis and treatment of COVID-19 since their traditional use has established safety, and experimental studies have demonstrated their immunomodulating, anti-inflammatory, antioxidant properties, and antiviral activity.[5-12] In a recent development, the Government of India has also incorporated the Ayurveda interventions in the national COVID management protocol.[13]

The trial drug, AYUSH-64, was repurposed based on the report of a clinical study in which AYUSH-64 was found effective in influenza-like illness and molecular docking study which revealed that 35 phytoconstituents isolated from AYUSH-64 demonstrated antiviral activity against SARS-CoV-2.[14,15] AYUSH-64 is a polyherbal formulation developed by Central Council for Research in Ayurvedic Sciences (CCRAS), Ministry of AYUSH, Government of India, through extensive pharmacological, toxicological, and clinical studies. Its efficacy and safety have already been proven in infective febrile conditions such as malaria, microfilaremia, chikungunya, and influenza as per the published clinical studies.[14,16-19] Furthermore, previous experimental studies have suggested that the constituents of AYUSH-64 might exert immunomodulating, anti-inflammatory, and antioxidant activities.[20-25] These effects could halt the intense inflammatory responses in COVID-19 that cause progression to significant morbidity.

To date, evidence on the efficacy and safety of including Ayurveda interventions as an add-on to standard care for COVID-19 is limited.[26-29] Therefore, the present open-label, randomized controlled study was planned to test the hypothesis that adding the Ayurveda intervention, AYUSH-64 to standard care was superior to standard care alone in improving the clinical status of patients with asymptomatic and mild COVID-19.

Materials and Methods

This study was an open-label, randomized controlled trial. The study was conducted at Government Medical College, Nagpur, Maharashtra, India, which was a designated COVID-19 referral center notified by the State Government of Maharashtra. The study was conducted from June 10, 2020, to November 2, 2020. Participants aged 18–60 years with positive reverse transcription-polymerase chain reaction (RT-PCR) assay for COVID-19, and categorized under stage I-mild (early infection) as per the Maharashtra Health Services treatment protocol for confirmed COVID-19 hospitalized patients were included in the study. As per the protocol, Stage I include asymptomatic and mild symptomatic patients with or without co-morbidities.[30]

Participants with severe COVID-19 or acute respiratory distress syndrome or severe disease as per 8-point ordinal score,[31] i.e., hospitalized, on invasive mechanical ventilation or extracorporeal membrane oxygenation, with chronic kidney disease, with alanine transaminase or aspartate transaminase more than two times the upper limit of normal, pregnant or lactating women, and any other clinical condition, which would jeopardize the outcome of the study, were excluded from the study.

Detailed information about the study was provided to the eligible patients and written informed consent in the participant's language was obtained before recruiting them in the study.

In the initial phase of the study, it was planned in such a way that the enrolled participants would be kept under observation in the in-patient department of the Government Medical College, Nagpur, till discharge. However, as per the revised guidelines of the Ministry of Health and Family Welfare (MoHFW), Government of India, dated July 2, 2020, to manage asymptomatic and mild COVID-19 patients in home isolation, patients enrolled from that date were kept in home isolation and followed up on 7th, 15th, 22nd, and 30th days.[32] All the RT-PCR diagnosed eligible patients were enrolled very next day of their test. The study participants who remain RT-PCR positive at the end of the study period were discharged as per the existing MoHFW, Government of India guidelines.[33]

Study intervention

AYUSH-64 two capsules (500 mg each) were administered thrice daily after food with water to the participants for 30 days along with standard care in the intervention group (IG) and the control group (CG) received only standard conventional care. The standard care provided was as per the national as well as the state government guidelines for COVID-19 management.[30] The standard care included paracetamol, Vitamin C, zinc, hydroxychloroquine, doxycycline, azithromycin, amoxycillin with potassium clavulanate and favipiravir as per the clinical condition of the patient along with the infection prevention and control practices.

AYUSH-64 is a patent polyherbal formulation developed by the CCRAS, Ministry of AYUSH, Government of India. AYUSH-64 consists of *Saptaparna* (*Alstonia scholaris* R. Br.), *Katuki* (*Picrorhiza kurroa* Royle ex. Benth), *Kiratatikta* (*Swertia chirata* Pexbex. Karst), and *Kuberaksha* (*Caesalpinia*

crista L.). The details and quality standards of the trial drug are given in Tables 1 and 2.

AYUSH-64 was procured from Indian Medicines Pharmaceutical Corporation Limited, Ministry of AYUSH, Government of India. Quality control and safety parameters of the ingredients and the formulation complied with the Ayurveda Pharmacopoeia limits/in-house limits as appropriate.

Outcomes measures

Primary outcome measure

Time to negative RT-PCR conversion (from the day of randomization) was the primary outcome measure. Real-time RT-PCR test was done on the planned follow-up visits, scheduled on the 7th, 15th, 22nd and 30th day of the study.

Secondary outcome measures

The proportion of participants who attained clinical recovery at 7th, 15th, 22nd and 30th day; improvement in laboratory parameters such as total and differential leukocyte count, absolute lymphocyte count, and erythrocyte sedimentation rate, inflammatory markers such as Interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and D-dimer; proportion of patients who progressed to severe stage of COVID-19 (with the onset of complications and requiring invasive or noninvasive oxygen therapy); and change in score of Perceived Stress Scale were the secondart outcome measures.

Safety assessment

Safety assessment involved incidence of adverse drug reaction/adverse event (ADR/AE) and change in liver function test and kidney function test at the end of the study period, i. e., 30th day.

Sample size

The sample size for the study was calculated assuming that 85% of the participants will turn RT-PCR negative within 15 days in the IG, while this change will be observed in only 50% of the participants in the CG. With a 95% confidence level, power of 80%, and assuming the attrition rate of 20%, the number of participants to be enrolled in each group was estimated to be 30. Hence, a total of 60 participants were enrolled in the two groups of the study.

Randomization

Sixty eligible participants were randomized into two parallel groups in the ratio of 1:1. Statistical Package for Social Sciences SPSS 15.0 for Windows, 233 South Wacker Drive, 11th Floor, Chicago, Illinois, U.S.A. was used to generate the random number sequences.

Ethical consideration

The study was conducted in accordance with the principles of the Declaration of Helsinki and the ICMR's National Ethical Guidelines for Biomedical and Health Research on Human Participants (2017). The study was reviewed, approved, and monitored by the Institutional Ethics Committee of Government Medical College, Nagpur, Maharashtra, India. The clinical trial was registered prospectively at the Clinical Trial Registry of India (CTRI/2020/05/025156). The study was monitored by Data and Safety Monitoring Board. The CONSORT guidelines were followed while reporting the study results.

Statistical analysis

The categorical variables in the study data have been summarized as numbers (percentage) and compared using the Chi-square test. The continuous data have been represented as mean (standard deviation) and median (min-max) for data not following a normal distribution. Parametric data were analyzed by paired *t*-test and independent sample *t*-test for within and between-group analysis, respectively, whereas nonparametric

Table 1: Composition of AYUSH-64 (each 500 mg capsule)

Name of the ingredient	Botanical name	Part used	Quantity (mg)
Saptaparna (aqueous extract)	*Alstonia scholaris*	Bark	100
Kutaki (aqueous extract)	*Picrorhiza kurroa*	Rhizome	100
Kiratatikta (aqueous extract)	*Swertia chirata*	Whole plant	100
Latakaranja (seed powder)	*Caesalpinia crista*	Seed	200

Table 2: Specifications for quality control analysis of AYUSH-64 and its ingredients

Test parameters	Ingredients (%)				Formulation (%)
	Saptaparna (aqueous extract)	*Kutaki* (aqueous extract)	*Kiratatikta* (aqueous extract)	*Latakaranja* (seed powder)	
Loss on drying	NMT: 9	NMT: 6	NMT: 8	-	NMT: 6
pH (1% Sol)	4.5-6.5	4.0-7.0	5.0-7.0	-	4.0-6.5
Total Ash	NMT: 12	NMT: 5	NMT: 15	NMT: 5	NMT: 25.0
Acid-insoluble ash	NMT: 2	NMT: 1	NMT: 2	NMT: 1	NMT: 8.0
Alcohol soluble extractive	NLT: 3	NLT: 3	NLT: 12	NLT: 26	NLT: 5.0
Water soluble extractive	NLT: 85	NLT: 80	NLT: 80	NLT: 4	NLT: 30.0
Heavy metals			Comply with API limits		
TBC and YMC			Comply with API limits		
Specific pathogens			Comply with API limits		
Aflatoxins			Comply with API limits		
Pesticide residue#			Comply with API limits		

NMT: Not more than; NLT: Not less than; API: Ayurvedic Pharmacopoeia of India; TBC: Total Bacterial Count; YMC: Yeast & Mould Count

data were compared by Wilcoxon signed-rank test and Mann-Whitney test for within and between-group analysis, respectively. $P < 0.05$ has been considered as significant. The per-protocol method was used for data analysis. All the data analyses were done using the Stata/MP 16.1 for Windows, Stata Corp, 4905 Lakeway Drive, College Station, Texas, US.

Observation and Results

Patients' enrolment

A total of 81 RT-PCR-confirmed COVID-19 patients were screened for study eligibility from June 10, 2020. Twenty-one patients were not included in the study as they were moderate or severe cases of COVID-19 ($n = 13$), below 18 years ($n = 2$), lactating women ($n = 4$), and not willing to participate in the study ($n = 2$). Sixty participants who met the inclusion criteria were included in the study. The recruitment, allocation, follow-up, and analysis of the study participants are shown as a CONSORT flow diagram in Figure 1. The clinical condition of one participant deteriorated just after the enrolment in the IG and the participant did not receive the allocated intervention. The data of 25 participants in the IG and 27 in the CG were included for final analysis as four participants in the IG and three participants in the CG did not come for the first follow-up visit on the 7th day from randomization and dropped out of the study.

Baseline clinical characteristics of study participants

The baseline characteristics of the study participants such as age, gender, symptomatic status, comorbidities, appetite, and bowel habits are shown in Table 3. There was no statistically significant difference in the baseline distribution of demographic and clinical characteristics between the two groups ($P > 0.05$). The majority of participants in both groups were male and the mean age of the participants in the intervention and CGs was 43.68 ± 9.97 and 35.22 ± 11.80 years, respectively. In the present study, 64% and 70.4% of patients were found symptomatic in the IG and CG, respectively. Comorbidities such as diabetes mellitus, hypertension, bronchial asthma, chronic obstructive pulmonary disease, cardiovascular disease, and thyroid dysfunction were present in the IG ($n = 05$) and CG ($n = 10$).

Efficacy outcomes

The efficacy outcome was evaluated through the proportion of participants who attained negative RT-PCR conversion and clinical recovery in the scheduled follow-up on the 7th, 15th, 22nd and 30th days. The proportion of participants who turned

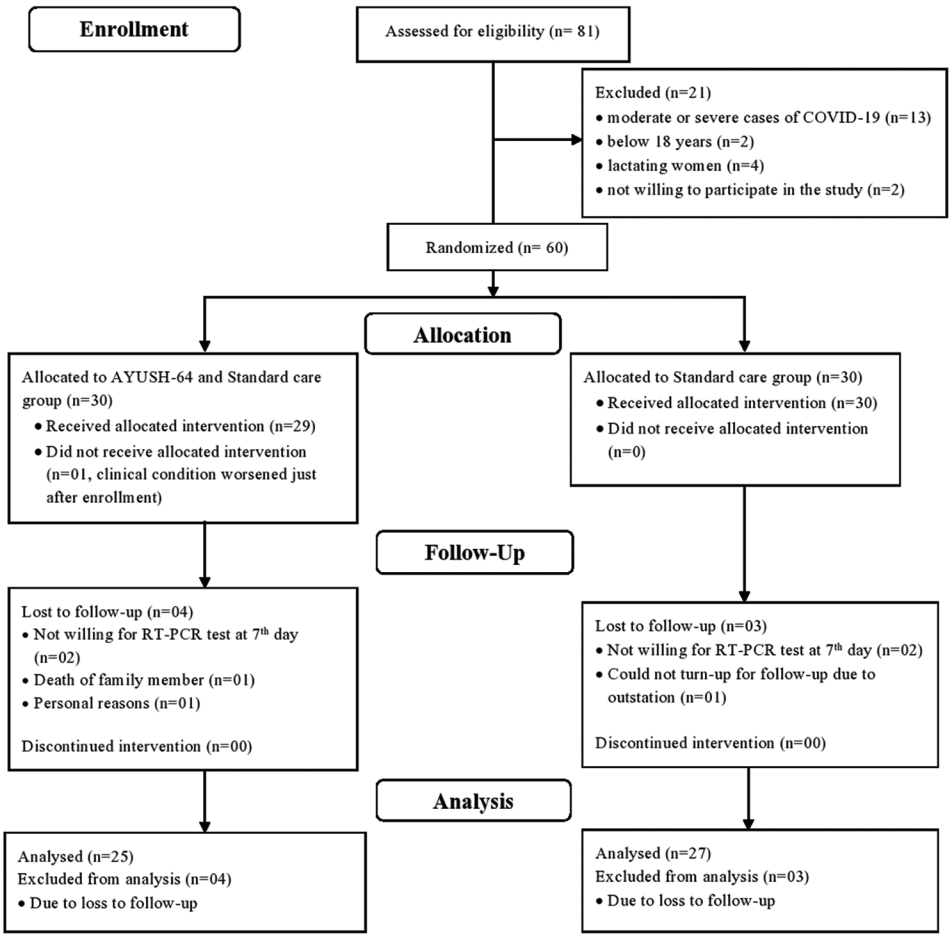

Figure 1: CONSORT flow diagram

Table 3: Baseline characteristics of the participants in both the groups

Variables	Parameters	IG (*n*=25)	CG (*n*=27)	*P*$
Age:Mean±SD		43.68±9.97	35.22±11.80	0.448
Gender	Male	18 (72.0)	18 (66.7)	0.677
	Female	7 (28.0)	9 (33.3)	
Clinical features	Asymptomatic	9 (36.0)	8 (29.6)	0.625
	Symptomatic	16 (64.0)	19 (70.4)	
Stage of disease	Group A	8 (32.0)	8 (29.6)	0.530
	Group B	14 (56.0)	14 (51.9)	
	Group C	3 (12.0)	5 (18.5)	
Comorbidities	COPD	1 (4.0)	0	-
	Bronchial asthma	2 (8.0)	2 (7.4)	0.936
	Diabetes mellitus	3 (12.0)	2 (7.4)	0.575
	Hypertension	2 (8.0)	5 (18.5)	0.267
	Cardiovascular disease	0	2 (7.4)	-
	Thyroid dysfunction	0	3 (11.1)	-
Bowel habits	Regular	22 (88.0)	22 (81.5)	0.515
	Irregular	3 (12.0)	5 (18.5)	
Appetite	Normal	23 (92.0)	23 (85.2)	0.442
	Disturbed	2 (8.0)	4 (14.8)	
Stool consistency	Normal	22 (88.0)	20 (74.1)	0.203
	Constipated	3 (12.0)	7 (25.9)	

$Compared using Chi-square/Fisher's exact test. Values have been expressed as *n* (%) for all variables except age. IG: Intervention group, CG: Control group, SD: Standard deviation, COPD: Chronic obstructive pulmonary disease

Table 4: Effect on outcome parameters in both the groups

Outcome Parameters	IG (*n*=25), *n* (%)	CG (*n*=27), *n* (%)	*P*$
Primary outcome measure			
Negative RT-PCR			
7th day	16 (64.0)	19 (70.4)	0.625
15th day	20 (80.0)	24 (88.9)	0.375
22nd day	23 (92.0)	26 (96.3)	0.507
30th day	23 (92.0)	27 (100.0)	0.134
Secondary outcome measures			
Clinical recovery			
7th day	9 (36.0)	7 (25.9)	0.432
15th day	15 (60.0)	10 (37.0)	0.098
22nd day	18 (72.0)	15 (55.6)	0.219
30th day	25 (100)	23 (85.2)	0.112
Perceived Stress Scale Score: Median (minimum-maximum)			
Baseline	21 (0-32)	16 (0-32)	0.205
30th day	0 (0-18)	0 (0-12)	0.181

$Compared using Chi-square/Fisher's exact test. IG: Intervention group, CG: Control Group, RT-PCR: Reverse transcription-polymerase chain reaction

RT-PCR negative on the 7th, 15th and 30th day were 64%, 80% and 92% in the IG and 70.3%, 88.8% and 100% in the CG, respectively [Table 4]. The difference observed between the intervention and CG is statistically insignificant ($P = 0.134$). The remaining two RT-PCR-positive patients in the IG were also asymptomatic before the end of the study period.

The clinical recovery was 60% and 37% on 15th day ($P = 0.098$) and 100% and 85.2% at the end of the study period, i.e., 30th day ($P = 0.112$) in the intervention and CG, respectively [Table 4]. Clinical features such as fever, chest pain, and anorexia relieved within 7 days in all the IG participants and cough, expectoration, and breathlessness were also absent before the 15th day. Other symptoms such as sore throat, nasal discharge, bodyache, headache and nausea persisted in a very few participants till the 22nd day in the IG [Table 5]. In the CG, nasal discharge and expectoration relieved by the 15th day while nausea, bodyache and headache persisted throughout the study period. The major symptoms such as fever and breathlessness persisted in the CG till the 15th day and complete relief in cough was achieved at 22nd day in the CG.

The levels of inflammatory markers/cytokines such as IL-6, TNF-α, and D-dimer are shown in Table 6. The reduction in

Table 5: Effect on chief complaints in both the groups

Chief complaints	Baseline, *n* (%)	7th day, *n* (%)	15th day, *n* (%)	22nd day, *n* (%)	30th day, *n* (%)
Fever					
IG	11 (44.0)	0	0	0	0
CG	15 (55.6)	3 (11.1)	1 (3.7)	0	0
P$	0.405	-	-	-	-
Cough					
IG	13 (52.0)	6 (24.0)	0	0	0
CG	15 (55.6)	6 (22.2)	4 (14.8)	1 (3.7)	0
P$	0.797	0.879	-	-	-
Breathlessness					
IG	10 (40.0)	3 (12.0)	0	0	0
CG	8 (29.6)	3 (11.1)	2 (7.4)	0	0
P$	0.432	0.920	-	-	-
Sore throat					
IG	13 (52.0)	6 (24.0)	2 (8.0)	1 (4.0)	0
CG	14 (51.9)	7 (25.9)	3 (11.1)	2 (7.4)	0
P$	0.991	0.873	0.704	0.599	-
Expectoration of sputum					
IG	2 (8.0)	2 (8.0)	0	0	0
CG	4 (14.8)	2 (7.4)	0	0	0
P$	0.442	0.936	-	-	-
Nausea					
IG	4 (16)	6 (24)	1 (4)	0	0
CG	5 (18.5)	3 (11.1)	1 (3.7)	3 (11.1)	1 (3.7)
P$	0.810	0.220	0.956	-	-
Bodyache/myalgia					
IG	11 (44.0)	8 (32.0)	4 (16.0)	5 (20.0)	0
CG	10 (37.0)	6 (22.2)	9 (33.3)	6 (22.2)	2 (7.4)
P$	0.609	0.427	0.149	0.845	-
Abdominal pain					
IG	2 (4.8)	0	0	0	0
CG	2 (7.4)	1 (3.7)	1 (3.7)	0	0
P$	0.936	-	-	-	-
Nasal discharge/nasal congestion					
IG	3 (12.0)	0	3 (12.0)	0	0
CG	1 (3.7)	1 (3.7)	0	0	0
P$	0.262	-	-	-	-
Chest pain					
IG	2 (4.8)	0	0	0	0
CG	2 (7.4)	1 (3.7)	1 (3.7)	0	0
P$	0.936	-	-	-	-
Anorexia					
IG	1 (4.0)	0	0	0	0
CG	2 (7.4)	2 (7.4)	1 (3.7)	2 (7.4)	0
P$	0.599	-	-	-	-
Headache					
IG	15 (60.0)	9 (36.0)	4 (16.0)	1 (4.0)	0
CG	15 (55.6)	9 (33.3)	6 (22.2)	7 (25.9)	4 (14.8)
P$	0.746	0.273	0.569	0.051	-

$Compared using Chi-square/Fisher's exact test. Values have been represented as *n* (%). IG: Intervention group (*n*=25), CG: Control group (*n*=27)

the levels of IL-6 and TNF-α at the end of the study period was statistically significant in the IG ($P < 0.05$), whereas it was statistically insignificant in the CG. The D-dimer levels significantly reduced after the treatment in both the groups ($P < 0.05$). The Perceived Stress Scale score also improved at the end of the study period in both the groups [Table 4].

Vital parameters such as SpO_2, pulse rate, respiratory rate, and blood pressure were within normal limits in both groups, during

Table 6: Effect on laboratory parameters in both the groups

Laboratoryparameters	IG (*n*=25)	CG (*n*=27)	*P*#
Total leucocyte count (10^3/μL)			
Baseline	6.04±1.78	5.89±1.42	0.736
30th day	6.56±1.57	6.93±1.98	0.459
P$	0.091	0.008*	
Neutrophils(%)			
Baseline	57.4±11.16	58.7±8.83	0.641
30th day	60.28±8.76	58.44±6.60	0.396
P$	0.194	0.871	
Lymphocytes(%)			
Baseline	34.04±11.54	32.85±8.67	0.675
30th day	31.32±8.71	33.51±6.73	0.311
P$	0.203	0.681	
Eosinophils(%)			
Baseline	2.84±1.06	3.29±1.97	0.311
30th day	3.6±2.23	3.37±1.64	0.673
P$	0.103	0.854	
Absolute lymphocyte count (per mm^3)			
Baseline	1992.88±669.35	1897.03±540.39	0.571
30th day	2099.4±673.12	2300.5±693.93	0.295
P$	0.451	<0.001*	
ESR (mm/h)			
Baseline	17.6±9.97	20.55±11.90	0.338
30th day	21.44±9.62	18.51±10.68	0.307
P$	0.070	0.348	
D-dimer (μg/mL)[a]			
Baseline	233.8 (173.9-628.3)	250.0 (157.8-293.7)	0.782
30th day	185.0 (129.4-263.7)	132.0 (84.7-213.5)	0.055
P$	0.015*	0.005*	
IL-6 (pg/mL)[a]			
Baseline	5.6 (2.3-13.8)	3.6 (1.7-4.8)	0.067
30th day	1.9 (0.15-5.3)	0.4 (0.2-6.6)	0.905
P$	0.037*	0.361	
TNF-α (pg/mL)			
Baseline	5.70±2.15	5.58±1.32	0.809
30th day	4.13±1.85	6.48±4.14	0.012*
P$	0.014*	0.318	

*$P<0.05$ has been considered as significant, [a]Data have been reported as median (Q1-Q3), #Between group *P* value, compared using independent sample *t*-test/Mann-Whitney test, $Within group *P* value, compared using paired sample *t*-test/Wilcoxon signed-rank test. Values have been represented as mean±SD. IG: Intervention group, CG: Control Group, ESR: Erythrocyte sedimentation rate, IL-6: Interleukin-6, TNF-α: Tumor necrosis factor-α, SD: Standard deviation

the study period. None of the participants required invasive or noninvasive oxygen therapy or developed complications such as pneumonia, acute respiratory distress syndrome, sepsis, arrhythmia, etc. during the study period in both groups.

Safety outcomes

ADRs or serious AEs were not observed/reported by any of the study participants in both groups. Liver function test and kidney function test were found to be within the normal limits throughout the study period in both groups [Table 7].

Discussion

In the present open-label randomized controlled study, the proportion of participants who attained negative RT-PCR conversion on the scheduled follow-up visits was the primary outcome measure to evaluate the efficacy of the study intervention. The difference observed in the proportion of participants who turned RT-PCR negative for COVID-19 during each of the follow-ups is statistically insignificant between the intervention and CG. Incidentally, the status of RT-PCR in patients who have clinically recovered from COVID-19 bears little relevance as it is evident that SARS-CoV-2 virus can rarely be cultured in respiratory samples after 9 days of symptom onset, especially in patients with mild disease.[34]

The clinical endpoint of the study was the time to attain clinical recovery within 30 days after randomization and was observed to be not significantly different between groups, but there was trend of good symptomatic response in the IG than the

Table 7: Effect on liver and kidney function in both the groups

Laboratory parameters	IG (n=25)	CG (n=27)	$P^{\#}$
Blood urea (mg/dl)			
Baseline	16.87±7.67	20.14±13.38	0.290
30th day	15.34±6.08	16.40±4.18	0.462
$P^{\$}$	0.147	0.118	
Serum uric acid (mg/dl)			
Baseline	4.94±1.43	4.99±1.70	0.920
30th day	4.63±1.20	5.11±1.30	0.170
$P^{\$}$	0.205	0.625	
Serum creatinine (mg/dl)[a]			
Baseline	0.7 (0.55-0.85)	0.6 (0.7-0.9)	0.317
30th day	0.7 (0.6-0.9)	0.6 (0.7-0.8)	0.679
$P^{\$}$	0.119	0.085	
SGOT (U/L)			
Baseline	24.36±15.08	22.18±8.19	0.517
30th day	21.36±8.59	23.48±11.01	0.445
$P^{\$}$	0.316	0.552	
SGPT (U/L)			
Baseline	23.40±15.08	18.62±13.36	0.184
30th day	20.68±11.08	23.18±25.12	0.648
$P^{\$}$	0.168	0.283	
Serum alkaline phosphatase (IU/L)			
Baseline	82.68±29.03	79.88±15.44	0.664
30th day	77.28±25.67	80.96±22.19	0.582
$P^{\$}$	0.029*	0.682	
Total protein (g/dl)			
Baseline	6.99±0.39	7.10±0.40	0.323
30th day	7.06±0.41	7.16±0.43	0.425
$P^{\$}$	0.521	0.497	
Serum albumin (g/dl)			
Baseline	4.40±0.28	4.44±0.41	0.717
30th day	4.53±0.28	4.56±0.34	0.694
$P^{\$}$	0.172	0.126	
Serum globulin (g/dl)			
Baseline	2.58±0.31	2.63±0.38	0.620
30th day	2.54±0.39	2.59±0.31	0.627
$P^{\$}$	0.675	0.442	
Serum bilirubin conjugated (mg/dl)			
Baseline	0.18±0.07	0.17±0.08	0.565
30th day	0.19±0.13	0.19±0.08	0.869
$P^{\$}$	0.671	0.176	
Serum bilirubin unconjugated (mg/dl)			
Baseline	0.41±0.47	0.24±0.15	0.085
30th day	0.32±0.24	0.26±0.17	0.294
$P^{\$}$	0.282	0.538	

*P<0.05 has been considered as significant, [a]Data have been reported as median (Q1-Q3), [$]Within group P value, compared using paired sample t-test/Wilcoxon signed-rank test, [#]Between group P value, compared using independent sample t-test/Mann-Whitney test. Values have been represented as mean±SD. IG: Intervention group, CG: Control group, SD: Standard deviation, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase

CG. Further, only mild cases of COVID-19 were selected, so the number of participants having symptoms at baseline was low and not sufficient for statistically significant difference. Furthermore, the enrolled participants were kept in isolation at home after baseline evaluation. Hence, evaluation of clinical recovery was done only when the patients attended the follow-ups and due to recall bias, patient-reported duration of symptomatic relief could not be recorded in all the participants. Hence, the exact proportion of participants who attained clinical recovery on each day following the enrolment could not be elicited. Hence, the absence of statistical difference could not be used for limiting the possible role of the study intervention on clinical outcomes.

The levels of IL-6 demonstrated within-group statistically significant findings when compared with the baseline, while TNF-α demonstrated within-group and between-group significant findings. The levels of these inflammatory cytokines were within the accepted reference range during the study period in all the recruited participants. Pro-inflammatory cytokines such as IL-6, secreted by the monocytes are implicated in triggering signalling multiple cytokine cascades with higher chances for tissue damage and organ failure, which in COVID-19 implies elevated chances of mortality and morbidity and is associated with disease severity and chances of respiratory failure necessitating mechanical ventilation.[35-37] TNF-α more or less acts as an amplifier of inflammation and TNF-α blockade has been clinically used in the management of many inflammatory diseases. Clinical evidence in this area suggests that modulators of elevated cytokines/chemokines may provide precision management and the findings in the present study show that the interventions have a potential to limit the persistent elevation of plasma cytokines which are often seen, even after attaining negative viral titers. The possible role of the intervention in these highly dynamic inflammatory cytokines would need further exploration in COVID-19 patients with significantly elevated cytokines. D-dimer is a biomarker that reflects fibrin formation and degradation and higher levels have been linked with higher mortality in COVID-19 patients with increased risk of clinically diagnosed thrombotic events, critical illness, and death.[38] In the present study, the D-dimer levels were within the preferred upper reference range throughout the study. However, the depletion in the D-dimer levels within group and between the groups at 30th day was statistically significant.

Ayurveda consider diseases characterized by pyrexia as the cardinal symptom under the spectrum of *Jwara*, the pathogenesis of which is characterized by *Amavastha* (circulating endogenous or exogenous substances including infectious agents, inflammatory products) in the initial stages wherein the disease activity will be profound, followed by a stage where the disease activity limits itself or undergo resolution either as a host response or due to medical intervention. AYUSH-64 is an intervention that was developed for *Vishamajwara* (fever characterized by the onset of symptoms in a paroxysms or cyclical manifestation) and is expected to neutralize the *Ama* at the level of tissues. The baseline disease assessment in the present study shows that the asymptomatic participants do not fit in the category of classical *Jwara* and the laboratory parameters also do not support a profound *Amavastha* or *Dhatugata Avastha* (localization of disease at the level of various tissues).

None of the participants had study intervention prematurely stopped by the investigators because of AEs including gastrointestinal symptoms (anorexia, nausea, and vomiting) and impaired liver function and renal function. It would be prudent to reflect that the Ayurveda intervention was safe to be used with contemporary medicine and less likely to cause any drug-drug interactions when taken together.

Strategies to enhance the potency of AYUSH-64 such as multiple divided doses during the 24-hour period, combination with other Ayurvedic interventions with *Jwarahara* (drugs which alleviate disease conditions with pyrexia) potential, administered with suitable *Anupana* (adjunct administered either along with or just after the principal medicine to enhance its therapeutic action) based on individual constitution or disease state to mitigate immune-pathological host responses shall be adopted focusing on the individualized treatment regimen of Ayurveda. Furthermore, multicentric study with more number of participants should be conducted to further investigate the role of combinational therapy and explore viral dynamics.

Limitations of the study

Despite the carefully designed protocol for the present study, some limitations merit mention. We included only patients with asymptomatic and mild COVID-19, so the study findings cannot be extrapolated to patients with severe disease. Further, the study was designed as open-label and single-center study. Furthermore, the participants were in home isolation, so the exact duration for attaining clinical recovery cannot be assessed, which is a major outcome of interest in the study.

Conclusions

In asymptomatic and mild COVID-19 patients, AYUSH-64, as an add-on to standard conventional care, contributed to improvement in clinical recovery and also demonstrated the potential in reducing the levels of pro-inflammatory markers such as IL-6 and TNF-α. However, the difference observed in the proportion of participants who turned RT-PCR negative for COVID-19 is statistically insignificant between both groups.

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Conflicts of interest

The authors declare that they have no financial conflict of interest related to this study. The authors Govind Reddy, Manisha Talekar, Arunabh Tripathi, Babita Yadav, Amit K Rai, Sophia Jameela, Rakesh Rana, Shruti Khanduri, Bhagwan S Sharma, Bhogavalli Chandrasekhararao and Narayanam Srikanth work in Central Council for Research in Ayurvedic Sciences (CCRAS), Ministry of AYUSH, Government of India, New Delhi.

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