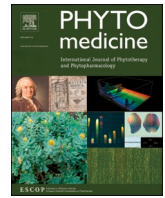




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## Original article

## Randomized placebo-controlled pilot clinical trial on the efficacy of ayurvedic treatment regime on COVID-19 positive patients

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## ABSTRACT

**Background:** Specific treatment for COVID-19 is still an unmet need. Outcomes of clinical trials on repurposed drugs have not been yielding success. Therefore, it is necessary to include complementary approaches of medicine against COVID-19.

**Purpose:** This study was designed to evaluate the impact of traditional Indian Ayurvedic treatment regime on asymptomatic patients with COVID-19 infection.

**Study design:** It is a placebo controlled randomized double-blind pilot clinical trial.

**Methods:** The study was registered with Clinical Trial Registry-India (vide Registration No. CTRI/2020/05/025273) and conducted at the Department of Medicine in National Institute of Medical Sciences and Research, Jaipur, India. 1 g of Giloy Ghanvati (*Tinospora cordifolia*) and 2 g of Swasari Ras (traditional herbo-mineral formulation) and 0.5 g each of Ashwagandha (*Withania somnifera*) and Tulsi Ghanvati (*Ocimum sanctum*) were given orally to the patients in treatment group twice per day for 7 days. Medicines were given in the form of tablets and each tablet weighed 500 mg. While, Swasari Ras was administered in powdered form, 30 min before breakfasts and dinners, rest were scheduled for 30 min post-meals. Patients in the treatment group also received 4 drops of Anu taila (traditional nasal drop) in each nostril every day 1 h before breakfast. Patients in the placebo group received identical-looking tablets and drops, post randomization and double blinded assortments. RT-qPCR test was used for the detection of viral load in the nasopharyngeal and oropharyngeal swab samples of study participants during the study. Chemiluminescent immunometric assay was used to quantify serum levels of interleukin-6 (IL-6), tumor necrosis factor alpha (TNF- $\alpha$ ) and high sensitivity C-reactive protein (hs-CRP) on day 1 and day 7 of the study.

**Results:** By day 3, 71.1 % and 50.0 % patients recovered in the treatment and placebo groups, respectively. Treatment group witnessed 100 % recovery by day 7, while it was 60.0 % in the placebo group. Average fold changes in serum levels of hs-CRP, IL-6 and TNF- $\alpha$  in treatment group were respectively, 12.4, 2.5 and 20 times lesser than those in the placebo group at day 7. There was 40 % absolute reduction in the risk of delayed recovery from infection in the treatment group.

**Conclusions:** Ayurvedic treatment can expedite virological clearance, help in faster recovery and concomitantly reduce the risk of viral dissemination. Reduced inflammation markers suggested less severity of SARS-CoV-2 infection in the treatment group. Moreover, there was no adverse effect observed to be associated with this treatment.

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## Introduction

The COVID-19 pandemic continues to rampage across the globe even after a year from its first emergence in December 2019. Several attempts have been made to repurpose already known drugs to treat COVID-19 infection. Despite, its initial hype, hydroxychloroquine, has been eventually found to be ineffective against SARS-CoV-2. Recovery and post-exposure prophylaxis (PeP) clinical trials of hydroxychloroquine (HCQ) did not show promising outcomes (Boulware et al., 2020; Geleris et al., 2020). No specific antiviral drug has been proven effective for treatment of patients infected with SARS-CoV-2 virus. Known anti-viral drugs, like Remdesivir has been put to clinical trials on patients with moderate to severe COVID-19 infections. However, the results have been non-conclusive, suggesting that Remdesivir is not the best medicine for COVID-19 (Beigel et al., 2020; Studemeister et al., 2020; Wang et al., 2020). With no definite treatment identified, there exists a requirement for interventions from alternative systems of medicines. The average recovery time for mild to moderate cases are 2-3 weeks. Although, once diagnosed, patients are strictly quarantined, still, a period of 14-30 days exists when these patients can act as potential spreaders of the infection. Due to a D614G mutation in the spike (S) protein of SARS-CoV-2 coronavirus, infectivity has increased although, symptoms have become milder (Korber et al., 2020). Unfortunately, this has increased the chances of viral dissemination manifold particularly through the asymptomatic patients, where, the incubation and disease progression periods are difficult to recognize. As such, 3 weeks is a long time given the current desperate situation of the medical conditions around world. Additionally, this silent viral dissemination would mean increased community transmission with extremely high chances of crippling the already overwhelmed medical infrastructure and facilities. A cure or intervention that can reduce the time to recovery, and thereby, reduce the risks of community transmission, will not only be a relief for the patients but is likely to significantly reduce the current burden on the global medical system. Shorter time to recovery means lesser chances of the infection spreading. In addition, if the medicine, besides reducing the recovery time, is also without side-effects, it would be an instant respite in these difficult times.

History of Traditional Medicine (TM) is being recognized by World Health Organization (WHO) as an inherited legacy of repertoire of knowledge, skills and practices, indigenous to a culture. Several studies have reported the efficacy of Traditional Chinese Medicine (TCM) against COVID-19 (Koe, 2020; Li et al., 2020; Lim, 2020; Ren et al., 2020; Xu and Zhang, 2020; Yang et al., 2020). Such reports on the potentials of Ayurveda against COVID-19 is absent. Interestingly, epidemics are recognized in Charak Samhita, an ancient treatise on Ayurveda, as *janapadodhwamsa vikara*, literally translating into 'a disease that can destroy human habitations'. Therefore, COVID-19 is an epidemic from classical Ayurvedic perspective as well. Nevertheless, there is no contemporary scientific evidence supporting the potential role of Ayurveda against COVID-19 (Girija and Sivan, 2020). However, Ayurvedic medicines were effective against 2006 outbreak of Chikungunya Epidemic in India (Girija and Sivan, 2020).

Based on the principles of Ayurveda that can symptomatically remedy COVID-19, a prescription including Swasari Ras, Giloy Ghanvati, Ashwagandha, Tulsi Ghanvati and Anu Taila was developed. The poly herbo-mineral formulation - Swasari Ras- one of the medicines in the studied consortium, is a classical ancient Ayurvedic remedy recommended for respiratory disorders. It is frequently used in Indian traditional medicinal system against asthma and other severe distressful respiratory conditions, like, excessive mucous formation, bronchitis and rhinitis (Balkrishna et al., 2020c). Giloy Ghanvati and Tulsi Ghanvati, the two other medicines used in the treatment regime are aqueous extracts from *Tinospora cordifolia* and *Ocimum sanctum*, respectively. Both these herbs are known for their anti-inflammatory and immunomodulatory properties. Besides, an active component from *O. sanctum*, Scutellarein, has been found through computational studies, to bind to the

enzymatic active site of viral RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2 (unpublished data). Ashwagandha tablets are made from extracts of *W. somnifera*. We have experimental evidence showing withanone, one of the active compounds of *W. somnifera*, to be capable of inhibiting the interaction between Receptor Binding Domain (RBD) of SARS-CoV-2 spike (S) protein and host ACE2 receptor (Balkrishna et al., 2020a). This ACE2-RBD complex formation is crucial for viral entry into host cell. Inhibition of this complex formation may lead to failure of the attachment of the viral particles to respiratory epithelia and may be the cause of early virological clearance seen in our study subjects. In a similar study, we observed that tinocordiside, one of the active compounds of *T. cordifolia* is also capable of disrupting the interaction between ACE2 and RBD of viral S protein (Balkrishna et al., 2020b). In addition to oral administration of medicines, Ayurvedic treatment also included application of nasal drops to ameliorate inflammation of the nasal passages and reduction in mucus formation that contribute to congestion. The herbal components of Anu taila (the nasal drop used in this study) are rich in anti-inflammatory phytochemicals that keep the pro-inflammatory cytokines in check (Dalvi et al., 2015; Patil and Sawant, 2012).

The current study was designed to evaluate the clinical efficacy of the above-mentioned prescription of Ayurvedic medications in moderating the effects of SARS-CoV-2 infection, through a randomized controlled clinical trial on COVID-19 positive patients, under clinical observation. The primary objective of this study was to assess the effect of Ayurvedic medication on the rate of recovery from SARS-CoV-2 through RT-PCR. The secondary objective was to evaluate the effect of this treatment regime on disease pathogenesis through assessment of various inflammatory markers. Here, we report the outcomes of this controlled clinical trial conducted to establish the efficacy of a treatment regime developed on the principles of Ayurveda. The overall observation of this study reveals that the employed traditional treatment has the potential for expedited virological clearance in asymptomatic spreaders, with clinical implications for strategizing against community transmission, particularly, of mutant strain, like D614 which is the major variant of SARS-CoV-2 in circulation currently (Korber et al., 2020).

## Material and methods

### Trial design

A treatment regime was developed on the principles of Ayurveda (ancient Indian medicinal system) with the intention to reduce the time to recovery in asymptomatic cases of COVID-19, whose efficacy was evaluated through a randomized, placebo controlled double blind clinical trial on 100 patients in a dedicated COVID-19 ward at the Department of Medicine in National Institute of Medical Sciences and Research, Jaipur, India. The study was registered with Clinical Trial Registry-India (vide Registration No. CTRI/2020/05/025273) (<http://ctri.nic.in/Clinicaltrials/pmaindet2.php?trialid=43900>), which is affiliated with International Clinical Trials Registry Platform (ICTRP) of World Health Organization. The study was conducted according to the protocol approved by the Institutional Ethical Committee (IEC) (Ref. No. NIMSUR/IEC/2020/036). Patients were all positive for COVID-19 on RT-PCR and were enrolled with their formal consents. A detailed study plan is provided in Fig. 1.

### Procedure

#### Case detection and subject selection

Patients who had no or mild symptoms and were positive on RT-PCR were referred to the clinical trial test site. These patients, if between 15-80 years of age and non-pregnant (in case of females), were screened for the study. Patients' health history data was collected by competent authority of the trial site, assigned to treat the patients with mild to moderate symptoms having no comorbidities like hypertension,

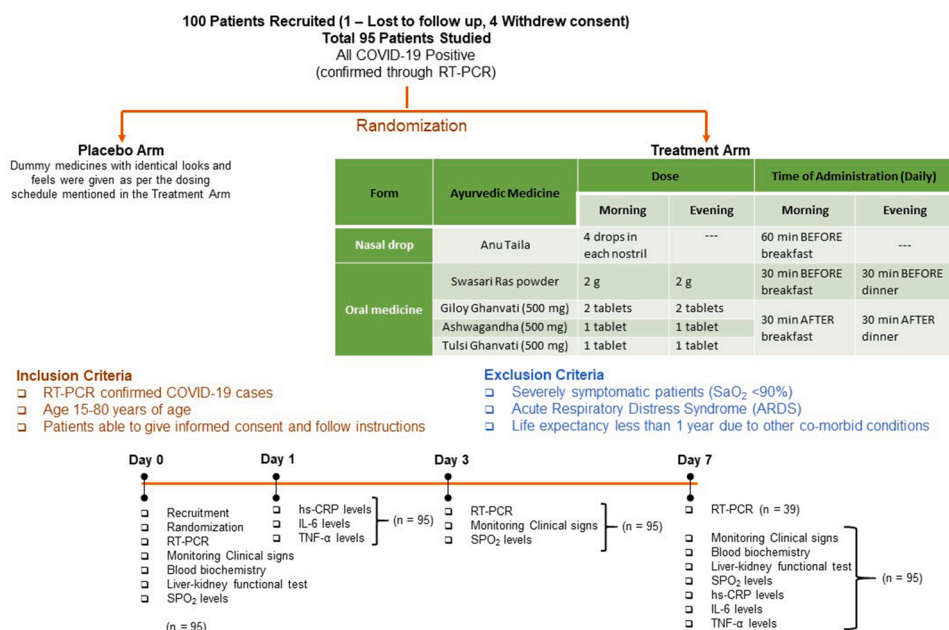


Fig. 1. Schematic depicting the detailed study plan.

diabetes and chronic kidney disease. All patients, who were admitted in this facility were identified for the symptomology of chronicity and any history of past disease and its treatment. These were documented in the medical case files, reviewed through Disease Activity Score (DAS) before entering into the study proforma. Study proforma was routinely checked by the coordinator, to fill the missing data of the patient history and addressed queries. General and systemic physical examinations were done as a focused approach. Vitals were checked and recorded in the medical case files. The laboratory data was collected from the hospital EMR and patient case files. The inclusion and exclusion screening criteria used for this study are in Fig. 1. Patients fulfilling all the criteria, were made aware of the study and informed consent was taken from those who were willing to participate. Subsequently, the patients were randomized into the treatment and placebo arms.

#### Randomization

The patients were randomized into the treatment and the placebo groups in 1:1 ratio. The randomization was done with the help of computer-generated random number tables. Patients assigned with odd numbers were given placebo (Placebo Arm) and those assigned with even numbers received Ayurvedic medicine (Treatment Arm).

#### Baseline investigation

Various parameters like, total leukocyte count, differential leucocyte count and eosinophil sedimentation rate (ESR) were determined and liver and kidney function tests [Alanine transaminase (ALT), Aspartate aminotransferase (AST), serum albumin, serum creatinine, total bilirubin, direct bilirubin] were done at the time of enrolment to serve as baseline reference. All these biochemical parameters were measured according to routinely employed procedures in pathological laboratories. All the patients enrolled in the trial were evaluated clinically for the symptoms of the disease, such as, sore throat, fever, myalgia and arthralgia, cough, nausea and vomiting. The symptoms were documented in the study proforma. Baseline blood pressure (mmHg), pulse rate, respiratory rate and oxygen saturation were measured and documented. Nasopharyngeal swab samples were collected and subjected to RT-PCR analysis for SARS-CoV-2.

#### Treatment and drug administration

Ayurvedic treatment comprised of oral administrations of 1 g of

Giloy Ghanvati (*Tinospora cordifolia*) and 2 g of Swasari Ras (traditional herbo-mineral formulation) and 0.5 g each of Ashwagandha (*Withania somnifera*) and Tulsi Ghanvati (*Ocimum sanctum*) twice per day for 7 days. Medicines were given in the form of tablets and each tablet weighed 500 mg. While, Swasari Ras was administered in powdered form, 30 min before breakfasts and dinners, rest were scheduled for 30 min post-meals. Patients in the treatment group also received four drops of Anu Taila (traditional nasal drop) daily 60 min before breakfast, in each nostril. Placebo group received tablets with identical looks and feels, composed of wheat flour as major ingredient along with excipients and binders, like, gum acacia, talcum and magnesium stearate. Placebo for nasal drop Anu taila was refined palm oil. The treatment regime is provided as a part of Fig. 1. Giloy Ghanvati, Tulsi Ghanvati and Ashwagandha were compressed into tablets. Swasari ras was in the form of powder. The medications were provided by Divya Pharmacy, Haridwar, Uttarakhand, India. The doses were determined based on the suggestions available for each of these herbs in Pharmacopeia of Ayurvedic Medicine. As this was a double-blind trial, both patients and caregivers were blinded regarding the intervention. The “packets” containing the active ingredients/placebo were given to the patients. These hospitalized patients were kept admitted through the entire course of the study. Apart from the above-mentioned treatment, no other intervention was provided to the patients.

#### Follow up monitoring and investigations

Some of the parameters were investigated on day 1 of administering the medicines. These included the serum levels of interleukin-6 (IL-6), C-reactive protein (hs-CRP) and tumor necrosis factor-alpha (TNF-α). On day 3, RT-PCR was repeated to assess the virological clearance. Patients who turned out negative for day 3 RT-PCR were excluded from further assessment of virological clearance through RT-PCR. Such patients were labelled as RT-PCR negative in the study proforma. For rest of the patients, nasopharyngeal swabs were again subjected to RT-PCR analysis for monitoring the virological clearance on day 7. In order to evaluate the effect of the treatment on the inflammatory response, serum levels of IL-6, TNF-α and hs-CRP and ESR were also assessed on day 7. Blood biochemical parameters, like, total leukocyte and differential leucocyte counts and liver and kidney function tests (AST, ALT, serum albumin, serum creatinine, total bilirubin, direct bilirubin) were repeated on day 7. Blood pressure, pulse rate, respiratory rate and oxygen saturation

were monitored daily during the treatment. However, for study purpose, day 3 and day 7 vitals were recorded in the proforma.

#### Data collection

Data was collected at National Institute of Medical Sciences, Jaipur, Rajasthan, India. A proforma was made to collect the data. Data was cross-checked to ensure error proofing.

#### Investigations

##### Primary outcomes

Patient testing negative for SARS-CoV-2 in the RT-PCR analysis was the primary outcome of this study. The PCR for SARS-CoV-2 was done with the ICMR approved RT-PCR methodology (ICMR registration number DBLCL001). Three genes (E gene, N gene, ORF1ab) were used to identify the SARS-CoV-2 virus in the nasopharyngeal and oropharyngeal swab.

##### Secondary outcomes

Serum levels of pro-inflammatory markers, like, highly sensitive C-reactive protein (hs-CRP), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- $\alpha$ ) were the secondary outcomes of this study. Blood from the patients was collected under 8 h fasting condition on day 1 and day 7, for measuring pro-inflammatory markers. IL-6 level was assessed according to manufacturer's protocol through electrochemiluminescence immunoassay (ECLIA) using Cobas (Roche Diagnostics GmbH, Sandhofer Strasse, Mannheim, Germany) a fully automated, one-step direct immunoassay using chemiluminescent technology, with a detection limit of 1.5 pg/ml. Likewise, the serum C reactive protein and TNF- $\alpha$  were quantified through quantitative chemiluminescent immunometric assay (CLIA) using Immulite 1000 (Siemens Healthcare GmbH, Henkestr, Erlangen, Germany) with analytical sensitivities of 0.1 mg/ml and 1.7 pg/ml, respectively.

#### Statistical analysis

Data was analyzed through SPSS ver 23 (released 2015 IBM SPSS Statistics for windows, version 23.0. Armonk, NY: IBM Corp). All continuous variables were checked for the normality by the shapiro wilk test. Parametric continuous variables were presented by mean and SD. Non parametric continuous variables were depicted as a median. The nominal variables were analyzed by chi-square test. Independent student t tests were used to compare the means of the parametric continuous variables. Mann-Whitney U test was used to compare means of the non-parametric continuous variables. Kaplan Meir analysis was used for the event analysis. Treatment effect parameters, like, Experimental Event Rate (EER), Control Event Rate (CER), Absolute Risk Reduction (ARR) and Relative Risk Reduction (RRR) and number(s) needed to treat (NNT) were calculated using online tool (<https://med.mercer.edu/libraries/mobile-ebm/calculators.htm>). Odds ratio was calculated using MedCalc ([https://www.medcalc.org/calc/odds\\_ratio.php](https://www.medcalc.org/calc/odds_ratio.php)). Statistical significance of the treatment playing a role behind the difference in the recovery frequencies observed between the two groups was determined using two-way ANOVA in GraphPad Prism (version 7.0, CA, USA).

#### Results

During May-June 2020, 100 patients were screened and enrolled for this study. Out of these 100 patients, 1 lost to follow-up and 4 withdrew their consents. Therefore, the observations reported in this study are from 95 patients (Fig. 1).

##### Baseline characteristics

All baseline characteristics are summarized in Table 1. Most of the

**Table 1**  
Demographics and clinical characteristics.

Characteristic	Placebo (N=50)	Treatment (N=45)	p-value
<b>Demographics</b>			
Age (mean $\pm$ SD)	35.4 $\pm$ 10.4	33.4 $\pm$ 9.4	0.38
Sex (Male/Female)	42/8	35/10	
<b>Age Category (in years) (<math>\delta</math>/<math>\varphi</math>)</b>			
< 18	0/1	1/0	
18 - 30	13/3	13/4	
31-55	26/4	20/6	
> 55	3/0	1/0	
<b>Vitals [median (range)]</b>			
Blood pressure systolic (mmHg)	120 (110, 138)	120 (100, 130)	0.60
Blood pressure diastolic (mmHg)	79 (70, 94)	80 (70, 90)	0.40
Pulse rate (/minute)	81 (70, 96)	80 (70, 90)	0.78
Respiratory rate (/minute)	17 (12, 20)	17 (12, 20)	0.41
Oxygen saturation (%)	99 (97, 100)	99 (96, 100)	0.42
<b>Hematology (mean <math>\pm</math> SD)</b>			
<b>Hemoglobin (Hb) (g/dl) <sup>1</sup></b>			
Male ( $\delta$ )	14 $\pm$ 1.0	15 $\pm$ 1.0	0.46
Female ( $\varphi$ )	12.9 $\pm$ 0.7	11.8 $\pm$ 1.4	0.09
Total Leukocyte Count (TLC) ( $\times 10^6$ cells/l) <sup>2</sup> [median (range)]	8200 (4300, 16000)	8100 (5400, 13800)	0.88
Lymphocyte ( $\mu$ l) <sup>3</sup> [median (range)]	2944 (1118, 6500)	3053 (1680, 5829)	0.94
Eosinophil sedimentation rate (ESR) (mm/hr) <sup>4</sup> (median)	20.5 (0, 42)	22 (0,42)	0.15
<b>Liver-kidney Functional Test (mean <math>\pm</math> SD)</b>			
Creatinine (mg/dl) <sup>5</sup>	0.9 $\pm$ 0.2	0.9 $\pm$ 0.1	0.37
Alanine transaminase (ALT) (U/l) <sup>6</sup>	39.3 $\pm$ 28.4	35.0 $\pm$ 28.0	0.47
Aspartate aminotransferase (AST) (U/l) <sup>7</sup>	32.3 $\pm$ 12.7	30.7 $\pm$ 9.7	0.51
Total Bilirubin (mg/dl) <sup>8</sup>	0.9 $\pm$ 0.5	0.9 $\pm$ 0.4	0.94
Direct Bilirubin (mg/dl) <sup>9</sup>	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.68
Albumin (g/dl) <sup>10</sup>	4.8 $\pm$ 0.6	4.8 $\pm$ 0.6	0.82
<b>Co-existing medical conditions</b>			
Diabetes	1	0	
Hypertension	0	1	
Asthma	0	0	

<sup>1</sup> Normal Hb range: 13.5-17.5 g/dl (male); 12-15.5 g/dl (female)

<sup>2</sup> Normal TLC value: 4500-11000 ( $\times 10^6$ ) cells/l

<sup>3</sup> Normal lymphocyte value: 1000-4800/ $\mu$ l

<sup>4</sup> Normal ESR: 0-23 mm/hr

<sup>5</sup> Normal serum level of creatinine: 0.84-1.21 mg/dl

<sup>6</sup> Normal serum level of ALT: 7-56 U/l

<sup>7</sup> Normal serum level of AST: 5-40 U/l

<sup>8</sup> Normal serum level of total bilirubin: 0.1-1.2 mg/dl

<sup>9</sup> Normal serum level of direct bilirubin: < 0.3 mg/dl

<sup>10</sup> Normal serum level of albumin: 3.4 - 5.4 g/dl

cases were in the age bracket of 31-55 years. In present study, enrolment of male patients was more in numbers. Patients were comparably distributed in terms of age, clinical characteristics and gender among both the arms (Fig. 2; Table 1).

Out of 50 patients in the placebo group, 1 was reported to have Diabetes. 1 out of 45 patients in the treatment group was hypertensive. Average vitals for both placebo and treatments groups were comparable

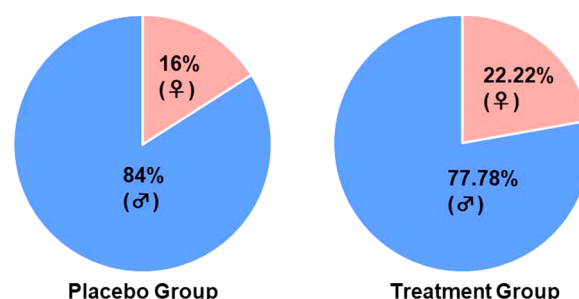


Fig. 2. Gender distribution in the placebo and treatment arms.



and within the acceptable range. Likewise, the blood biochemical parameters and serum levels of creatinine and alanine transaminase (ALT) (reflecting normalcy of kidney functioning) and those of aspartate aminotransferase (AST), total and direct bilirubin (suggestive of normal liver function) of the both groups were similar. These values were found to be within their respective normal ranges. Comparison between the baseline hematological parameters, liver-kidney function tests and their corresponding values measured on day 7 showed no significant change, suggesting that there was no undesirable effect due to the treatment (Table 1). From these observations, it is deducible that the administered treatment did not exert any observable adverse side effects.

### Outcome of the study

#### Primary outcome

The primary outcome of this clinical trial was reduction in the time taken by the patients treated with Ayurvedic medicines in testing negative in the RT-PCR analysis, indicating expedited recovery in the treatment group. By day 3, 71.1% patients in the treatment group tested negative for RT-PCR compared to 50% patients exhibiting same outcome in the placebo group. Treatment group witnessed 100% recovery by day 7, while it was 60% in the placebo group. (Fig. 3A). Details of the observations for the calculations of the percent recovery after day 3 and day 7, are provided in Table 2. We subjected our data to two-way ANOVA analysis and found that the p value for column factor (treatment) is 0.0282. This means that the treatment has an effect that is statistically significant. In other words, the reduction in time to recovery in the treatment group is statistically significant (25 out of 50 in placebo versus 32 out of 45 patients in treatment group were found negative on RT-PCR conducted on day 3). Kaplan Meier graphs are used to represent clinical data to show the effect on survival in response to treatments in comparison to controls. Here, the Kaplan Meier plot shows that the recovery proportion was more in the treatment group (Fig. 3B). Log rank (Mantel-Cox) test showed this observation to be statistically significant ( $p=0.0054$  at 95% CI). Critical parameters of Evidence Based Practice (EBP) were calculated for our primary outcome, that is, observed reduction in time to recovery in the treatment group for day 3 and day 7 and listed in Table 3. These parameters, particularly, Experimental Event Rate (EER), Control Event Rate (CER), Absolute Risk Reduction (ARR) and Relative Risk Reduction (RRR) reflect that this reduction in time to recovery is likely to reduce the risk of adverse disease prognosis. Number(s) needed to treat (NNT), particularly, with the small size of the given sample, indicates that this medical intervention is effective compared to the control placebo. Odds ratios were calculated to check the likelihood of the reduction in time to recovery in the treatment group. We observed odds ratios to be 0.41 (at 95% CI 0.17-0.95,  $p = 0.04$ ) and 0.05 (at 95% CI 0.003-1.02,  $p = 0.05$ ), respectively, for treatments for 3 and 7 days. An odd ratio  $< 1$  indicates that lesser chance of delayed

recovery in the treatment group is a more likely event.

#### Secondary outcome

Effects of Ayurvedic treatment on the levels of pro-inflammatory markers, hs-CRP, IL-6 and TNF- $\alpha$  in the serum were the secondary outcomes of this study. The serum levels of hs-CRP, IL-6 and TNF- $\alpha$  were measured on day 1, after randomization and commencement of treatment, and on day 7. Increase in hs-CRP, IL-6 and TNF- $\alpha$  in response to SARS-CoV-2 infection is indicative of the severity of disease prognosis (Del Valle et al., 2020; Fara et al., 2020; Sharifpour et al., 2020). Average serum level of hs-CRP was significantly increased in the placebo group on day 7 compared to day 1. However, hs-CRP levels in treatment group on day 1 and 7 were comparable and significantly lower than that in the placebo group on day 7. hs-CRP levels in placebo and treatment groups on day 1 were comparable. (Fig. 4A). It was found that the fold change in hs-CRP was 12.4 times lesser in the treatment group when compared to that in the placebo group (Fig. 4B). This indicated a better disease prognosis in the treatment group. Likewise, the fold change in IL-6 was also lesser on day 7 in the treatment group suggesting lesser chances of ensuing of cytokine storm, the main reason behind acute phase prognosis (Fig. 5). Representative values of IL-6 serum levels on day 1 and day 7 from both placebo and treatment groups are provided in Table 4. Serum levels of TNF- $\alpha$  were also measured on day 1 and day 7 and were found to be comparable on day 1 for the placebo and treatment groups, ruling out any bias at the baseline. Placebo group experienced a significant rise ( $p < 0.001$ ) in the serum TNF- $\alpha$  level by day 7. However, the mean serum level of TNF- $\alpha$  was reduced in the treatment group ( $p < 0.05$ ) relative to the placebo group, suggesting that, the treatment might have, most likely, prevented the TNF- $\alpha$  level from rising, in the first place (Fig. 6A). It is noteworthy that the fold change in TNF- $\alpha$  level in the treatment group was 20 times lesser than the placebo group (Fig. 6B).

### Discussion

This placebo-controlled randomized trial was conducted on asymptomatic to mildly symptomatic patients who were positive for SARS-CoV-2 on RT-PCR. The primary outcomes indicated a reduction in the time to recovery in response to the treatment. Although the patients were recovering in the placebo group, their speed of recovery was slower than those in the treatment group. Among other outcomes, the fold increase in the serum levels of hs-CRP and pro-inflammatory markers, IL-6 and TNF- $\alpha$ , were reduced. The average level of serum TNF- $\alpha$  in treatment group after seven days of treatment was comparable to that observed in placebo and treatment groups on day 1, suggesting that the treatment might have modulated this pro-inflammatory marker.

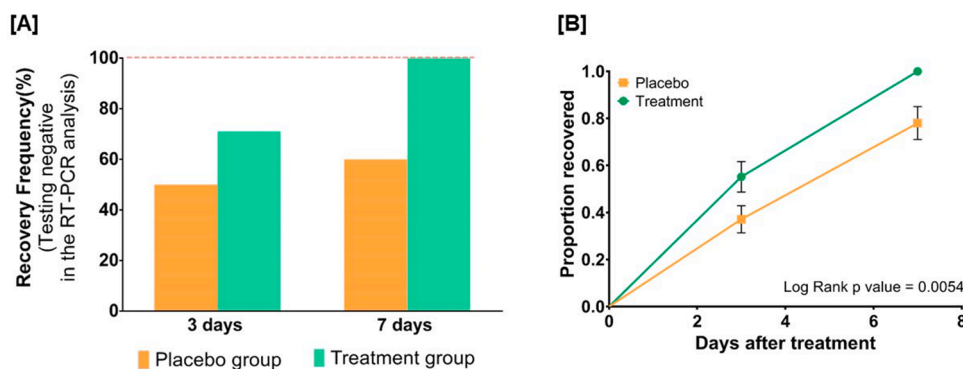


Fig. 3. Effect of Ayurvedic treatment on the recovery time.

[A] Recovery efficiencies in the placebo and treatment arms after 3 and 7 days of treatments. [B] Kaplan Meier Curve showing proportion of recovery.

**Table 2**  
Primary outcomes of the study.

Counts	Placebo (N = 50)				Treatment (N = 45)			
<b>Statistics</b>								
	<b>Day 3</b>		<b>Day 7</b>		<b>Day 3</b>		<b>Day 7</b>	
Valid	50		25		45		13	
Missing	0		25		0		32	
<b>Day 3 RT-PCR</b>								
	<b>Frequency</b>	<b>Percent</b>	<b>Valid Percent</b>	<b>Cumulative Percent</b>	<b>Frequency</b>	<b>Percent</b>	<b>Valid Percent</b>	<b>Cumulative Percent</b>
RT-PCR Negative	25	50.0	50.0	50.0	32	71.1	71.1	71.1
RT-PCR Positive	24	48.0	48.0	98.0	13	28.9	28.9	100
Test not done <sup>1</sup>	1	2.0	2.0	100.0	—	—	—	—
Total	50	100	100		45	100	100	
<b>Day 7 RT-PCR</b>								
	<b>Frequency</b>	<b>Percent</b>	<b>Valid Percent</b>	<b>Cumulative Percent</b>	<b>Frequency</b>	<b>Percent</b>	<b>Valid Percent</b>	<b>Cumulative Percent</b>
RT-PCR Negative	15	30	60 <sup>2</sup>	60	13	28.9	100	100
RT-PCR Positive	10	24.7	40	100	0	0	0	100
Total	25	40	100		13	28.9	100	
Missing	25	60			32	71.1		
System Total	50	100			45	100		

<sup>1</sup> This sample was negative for RT-PCR on day 7.

<sup>2</sup> Average recovery time without specific treatment: 2-3 weeks (14-21 days) (<https://www.forbes.com/sites/brucelee/2020/05/05/how-long-does-it-take-to-recover-from-covid-19-coronavirus-and-return-to-work/#3a74a58f4347>)(8,9)

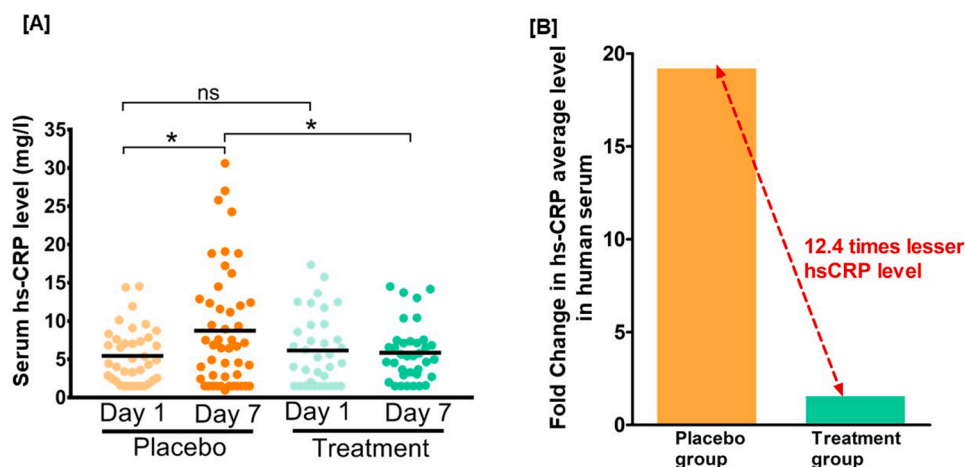
**Table 3**  
Size of the treatment effect.

Treatment Effect Parameter	Treatment Duration		Information Conveyed
	3 days	7 days	
Experimental Event Rate (EER)	0.71	1.00	Therapeutic benefit gained
Control Event Rate (CER)	0.50	0.60	Therapeutic benefit missed
Absolute Risk Reduction (ARR)	-0.21	-0.40	Provide a quantitative idea of the reduction in the probability of bad outcome
Relative Risk Reduction (RRR)	-0.42	-0.67	
Number Needed to Treat (NNT)	~ 5 (4.7)	~ 3 (2.5)	Conveys the effectiveness of the medical intervention
Odds Ratio (OR)	0.41 ( $p = 0.04$ , CI = 0.17-0.95)	0.05 ( $p = 0.05$ , CI = 0.003-1.02)	OR < 1 reflects lesser chance of delayed recovery in treatment group

### Principal findings

This randomized, placebo-controlled trial is a first of its kind which evaluated the effect of Ayurvedic medicines on COVID-19. In this trial, both placebo and treatment groups were comparable in clinical characteristics and disease severity, which removed the confounding at baseline itself. The sample size was of convenience as no previous data is available to derive the variables to calculate sample size. This trial showed that Ashwagandha (*W. somnifera*), Tulsi (*O. sanctum*), Giloy (*T. cordifolia*), Swasari (traditional herbo-mineral formulation) and Anu Taila (traditional nasal drop) are capable of promoting faster clearance of the virus. The reduction in serum levels of hs-CRP, TNF- $\alpha$  and IL-6 suggested that these Ayurvedic medicines can possibly control inflammatory cascade.

In our study cohort, we had young patients (mean age 34.4, SD 9.9, range-15 to 65 years), with male predominance (Male/Female-77/18). As our center is designated for asymptomatic/mildly symptomatic patients who are less than 80 years of age, we could enroll middle aged patients who were either asymptomatic or had mild symptoms. This is concordant with the national data of India in which around 80% of the patients are asymptomatic (Ghosh, 2021).



**Fig. 4.** COVID-19 prognosis based on hs-CRP levels.

[A] Serum levels of hs-CRP on day 1 and day 7 after commencement of treatment in placebo and treatment groups. Statistical significance of the differences between two groups was determined by unpaired two tailed *t*-test. [B] Fold change in serum hs-CRP in placebo and treatment groups.

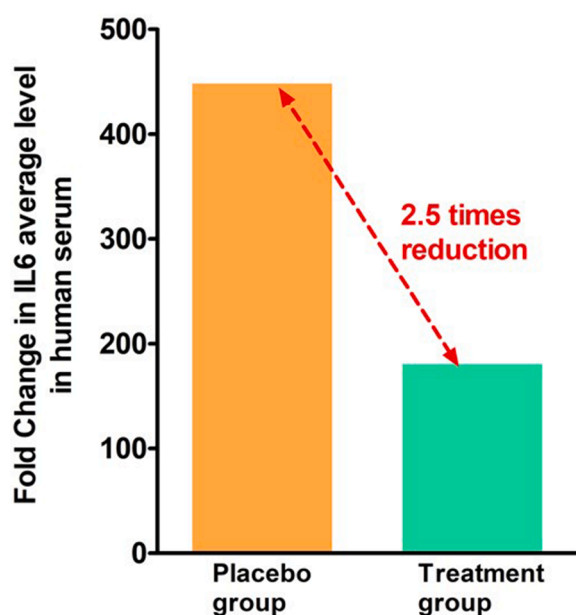


Fig. 5. Effect of Ayurvedic treatment on serum levels of IL-6. Fold changes in serum IL-6 levels in placebo and treatment groups.

Table 4

Indicative IL-6 concentrations (pg/ml)<sup>#</sup> on days 1 and 7 of patients in placebo and treatment groups.

Placebo		Treatment	
Day 1	Day 7	Day 1	Day 7
1.81	253.7	284.80	7.55
1.5	14.49	221.00	1.50
1.5	11.57	113.70	1.50
1.53	81.9	50.14	1.58
1.5	12.87	34.32	22.81
6.75	16.23	29.30	20.86
6.82	19.07	23.13	18.04
4.43	12.33	22.78	13.04
7.03	27.01	22.26	14.51
7.33	11.15	17.35	2.00

<sup>#</sup> Selected top ten levels of IL-6 showing substantial changes from day 1 values in each study group.

Our study showed that all the patients who received Ayurvedic treatment, were negative for RT-PCR test of nasopharyngeal swab samples collected on day 7, while, 40 % of the patients in the placebo group was still positive for COVID-19. The mortality and morbidity of the COVID-19 disease are caused by inflammation and tissue damage inflicted by the host's immune system. This was clearly evident from the recovery trial in which 6 mg per day dexamethasone led to reduction of mortality in severe COVID-19 patients (Horby et al., 2020). The cytokine release syndrome, resulting from robust immune activation, leads to multiorgan dysfunction and death (Del Valle et al., 2020). Inflammatory markers, like, IL-6, TNF- $\alpha$  and hs-CRP are used as predictors for risk stratification of COVID-19 cases (Del Valle et al., 2020; Fara et al., 2020; Sharifpour et al., 2020). Anti-IL-6 antibodies, like, tocilizumab, is being used to treat severe SARS-CoV-2 infections (Guaraldi et al., 2020). In our study, we monitored the serum levels of IL-6, TNF- $\alpha$  and hs-CRP to assess the effect of the Ayurvedic regime on inflammation. IL-6 was found to be reduced in the treatment group, although the reduction was not statistically significant in comparison to the placebo group, most likely, due to small sample size. Further research involving larger sample size is required to clearly establish this trend. Elevated IL-6 levels are associated with increased hs-CRP levels, increased disease severity and reduced chances of recovery (Fara et al., 2020). Increased hs-CRP levels are negatively correlated to oxygen saturation and are associated with aggravation in asymptomatic and mildly symptomatic cases and mortality in severe COVID-19 patients, indicating lung injury linked disease course (Ali, 2020; Sharifpour et al., 2020). In our study, hs-CRP was also found to be reduced significantly in the treatment group by day 7. As reported earlier, Serum hs-CRP levels rapidly increases within 6 to 8 h of contracting the infection and decreases during the healing phase when the inflammation subsides (Ali, 2020). Thus, comparable hs-CRP levels from day 1 and day 7 sampling in the treatment group indicates healing among the patients in this group. In contrast, the significantly increased hs-CRP level in the placebo group after 7 day suggests sustained inflammation. The study by Meng et al on asymptomatic COVID-19 patients in China, showing that out of 58 patients, 55 patients (94%) had GGO (Ground glass opacity) of lungs in CT scan, indicates that asymptomatic patients with no or minimal symptoms, had remarkably high prevalence of lung involvement (Meng et al., 2020). Lung lesions and hs-CRP levels are positively correlated with each other (Wang, 2020). Therefore, the reduced hs-CRP level in our study, may act as an indirect marker of improved lung pathology, if any. Patients with high degree of lung inflammation are likely to have higher residual lung scarring. Any intervention targeted to reduce scarring of the lung can improve Quality of Life (QoL) in the long term. So, all interventions which are directed to reduce inflammation, can lead to reduction in

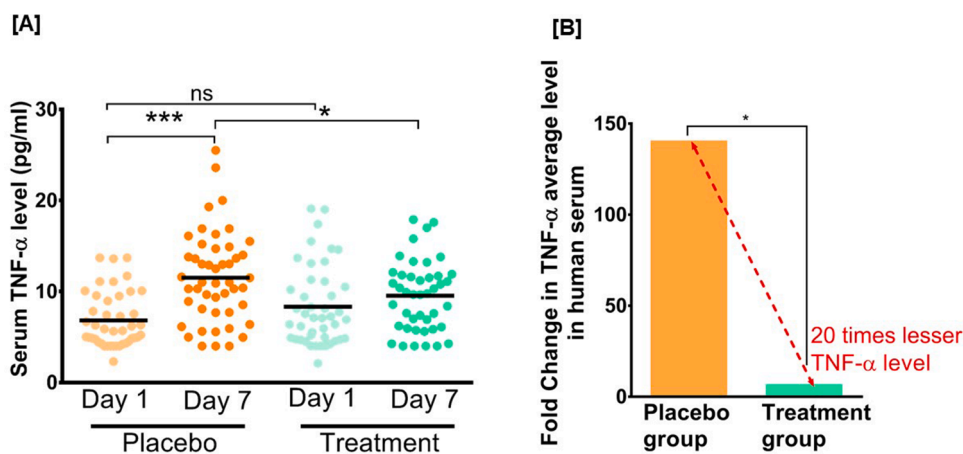


Fig. 6. Changes in levels of TNF- $\alpha$  in response to treatment.

[A] Serum levels of TNF- $\alpha$  on day 1 and day 7 after commencement of the treatment in placebo and treatment groups. [B] Comparison between the fold changes in serum TNF- $\alpha$  in placebo and treatment groups. Statistical significance of the differences between two groups was determined by unpaired two tailed *t*-test



organ injury and scarring. Therefore, we can infer that the Ayurvedic treatment regime tested in this study potentially offers long-term benefits to lung health.

Observational clinical data shows that increased TNF- $\alpha$  level is a predictor of disease severity and chance of survival (Del Valle et al., 2020; Fara et al., 2020). Anti-TNF- $\alpha$  therapy and TNF- $\alpha$  inhibitors could be correlated with reduction in severity of COVID-19 cases (Robinson et al., 2020). In our study, TNF- $\alpha$  exhibited a significant reduction in fold change (~20 times) following Ayurvedic treatment relative to the placebo group. This implicates that the tested Ayurvedic treatment regime is capable of moderating one of the important inflammatory markers, which is a recognized predictor of disease severity and survival.

Taken together, this placebo-controlled trial of Indian traditional medicine on COVID-19 patients showed that the Ayurvedic treatment was associated with early virological clearance. For better patient compliance, Giloy Ghanvati, Ashwagandha and Tulsi Ghanvati have been combined into a single formulation called Coronil tablet. Pre-clinical study with Coronil has shown that this medicine moderated the SARS-CoV-2 spike (S) protein induced COVID-19 symptoms reproduced in humanized zebrafish model of the disease (Balkrishna et al., 2020d).

#### Implications for clinical practice

The two significant implications of this study are: (1) the scope of reducing or preventing community transmission by virological clearance of super-spreaders, and (2) generation of knowledge base on asymptomatic patients in India, which, has been significantly lacking till now. Super-spreaders are the young patients enrolled in our study who were detected by contact tracking and case identification. They had minimal symptoms, and if not tested might not have sought medical attention. In India where the healthcare system is still under development, decreasing the viral load in these super-spreaders can dramatically reduce community transmission. With this evidence of promising potential of virological clearance in the super-spreaders, a community-based approach for preventing/reducing community transmission can be designed. Fortunately, Ayurvedic drugs have minimal or no side effects therefore, these could be distributed *en mass*. Besides, this approach can be used along with all standard precautions and preventive measures. The second implication of this study, is the insight into the asymptomatic patient population in India. Although patients were asymptomatic, they still had raised levels of the hs-CRP suggesting ongoing inflammation. The age group of our asymptomatic cohort is comparable to another cohort study from China on clinical course of asymptomatic cases of COVID-19 (Meng et al., 2020). The priority amidst the current COVID-19 pandemic is to find an evidence-based treatment or intervention. The clinical study reported here establishes the efficacy of the Ayurvedic treatment regime in healing COVID-19. However, this pilot study was conducted upon asymptomatic and mildly symptomatic patients, therefore it cannot predict the extrapolation of results on severe patients, or those with comorbidities. Although, COVID-19 vaccine has been designed, developed and being administered, it has its some limitations (Pratt, 2021). Our current study does not indicate whether the Ayurvedic treatment regime can overcome the vaccine associated limitations. Nevertheless, the importance of the present study is not diminished. This study has the potential to pave the path for developing Ayurvedic regimes to assist the treatment of asymptomatic patients who are not yet vaccinated.

#### Comparisons with other studies

The clinical trials on repurposing drugs for COVID-19 reported so far included studies on Hydroxychloroquine (HCQ), Remdesivir and Dexamethasone (Beigel et al., 2020; Boulware et al., 2020; Geleris et al., 2020; Grein et al., 2020; Horby et al., 2020; Wang et al., 2020). However, despite exhaustive literature search, we could not find any

scientific report on clinical trials of any form of traditional Indian medicines on COVID-19. Therefore, the observations from this study are discussed in the context of the above-mentioned trials.

Three trials on Hydroxychloroquine included an observational, a post-exposure prophylaxis and a pre-exposure prophylaxis study with sample sizes 1146, 821 and 106, respectively (Bhattacharya et al., 2020; Boulware et al., 2020; Geleris et al., 2020). Our study is comparable to the observational one which is conducted on a significantly larger sample size compared to ours. Ours is a pilot study intended to evaluate the suitability of Ayurvedic medicine in treating COVID-19, so that our observations may generate clinical take-home-points for developing strategies against the pandemic. The outcomes of all these three trials have put Hydroxychloroquine in an unfavorable spot with regard to its appropriateness as a treatment for COVID-19.

Clinical trials on Remdesivir as a potential treatment for COVID-19 have given mixed results. A randomized, double-blind, placebo-controlled, multicentric trial on 237 patients showed that Remdesivir was not associated with statistically significant clinical benefits (Wang et al., 2020). This study included patients with 94% or less oxygen saturation, who required assisted breathing. In contrast, the study conducted on 61 patients with similar inclusion criteria offered an inconclusive inference (Grein et al., 2020). The report from a double-blind, randomized, placebo-controlled trial conducted on 1063 patients with moderate symptoms showed reduced chances of lower respiratory tract infection and expedited recovery in hospitalized patients (Beigel et al., 2020). This trial was also designed to evaluate the safety outcomes of using Remdesivir against COVID-19, and the results showed absence of side effects. However, nausea and vomiting are known to be the common side effects of Remdesivir. Time to recovery was reduced to 11 days upon Remdesivir treatment from an average 15 days in case of placebo group. With Ayurvedic treatment, time to 100 % recovery was 7 days, lesser than what has been observed for Remdesivir. However, most of the patients in our study were asymptomatic. Suitability of Remdesivir in preventing community transmission of COVID-19 contributed by asymptomatic patients has not been explored. Despite subclinical disease manifestation, viral shedding duration (~24.5 days) in asymptomatic patients is comparable to the symptomatic ones, thereby, increasing the risks of community transmission (Noh et al., 2020; Zhou et al., 2020). With its known side effects, Remdesivir might not be the choice for treating asymptomatic patients if an alternative with no such side-effects existed. Reducing community transmission would be an added advantage. The Ayurvedic medicines studied in this trial fit well with this requirement: these are safe without any observed side-effects, can be distributed *en mass* and are effective against asymptomatic to mild cases.

Dexamethasone has been found to be promising as an intervention in reducing mortality among patients with severe symptoms, on mechanical ventilation (Horby et al., 2020). These observations were obtained from a randomized adaptive intervention trial where 2104 patients received dexamethasone alone, and 4321 patients were allocated to usual care concurrently with dexamethasone. Apparently, outcomes were favorable for patients with > 7 day symptoms, suggesting that the timing of dexamethasone administration is critical. Dexamethasone being a steroidal immunosuppressant, is helpful when the patient has entered the severe phase, with an overdriven inflammatory response. As concluded by Horby et al (2020), exposure to dexamethasone too early in the illness would suppress the immunity and hence, may facilitate disease progression towards severity. Probably, that is why, patients with symptoms for < 7 days, who are still in the viremic or early pulmonary phase benefit less from dexamethasone (Horby et al., 2020). On the other hand, Ayurvedic medicines, from the outcomes of our study, prove to be effective in preventing the inflammatory surge, detrimental for disease prognosis and thus, suitable for asymptomatic and mildly symptomatic patients.

### Limitations of the study

Our study included asymptomatic or mildly symptomatic patients only, therefore, clinical criteria could not be used to identify disease resolution, although, the exclusion and inclusion criteria put in place were followed in-principle.

Results of this trial cannot be generalized to the severe patients and patients with co-morbidities. As this is a pilot study, we engaged a small sample size. The sample size of 100 is a limitation with this study. However, the robustness of the observations obtained were verified through rigorous statistical analysis to make up for the small sample size.

### Conclusion

SARS-CoV-2 already has a different mutation in circulation and several others have been predicted based on evolutionary benefits (Korber et al., 2020; Padhi and Tripathi, 2020). The kind of epidemiological demands that might arise in case a community transmission of a milder viral strain is swiftly followed by the appearance of a virulent one can be quite challenging. Therefore, a strategy to contain viral dissemination would be a much-preferred course of action. This study reports the first clinical trial done for Ayurvedic treatment for COVID-19. The main outcome of this study is a reduction in the time to recovery in response to the treatment and decreased fold changes in the serum levels of hs-CRP and pro-inflammatory markers, interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- $\alpha$ ). The average level of serum TNF- $\alpha$  in treatment group after seven days of treatment was comparable to that observed in placebo and treatment groups on day 1, suggesting that the treatment had a moderating effect on this pro-inflammatory marker. Likewise, increase in serum level of IL-6 was also under control in the treatment group, indicating reduced likelihood of cytokine response syndrome. In addition, reduced hs-CRP level in the treatment group suggested healing against inflammation. Taken together, the Ayurvedic treatment regime facilitated expedited recovery from SARS-CoV-2 infection without any observed adverse effects. Thus, this study demonstrated the suitability of employing traditional Indian Ayurvedic medicine in treating COVID-19.

### Author contributions

Conceptualization: BST, AV, DN; Data curation: DN, AS, SH; Formal analysis: DN, SH, AV; Funding: BST, AB, AV; acquisition: GD, AS, DB, DN; Investigation: GD, AS, DB, DN; Methodology: GD, AS, DB, DN; Project administration: DN, AV; Resources: BST, AB, AV; Supervision: BST, DN, AV; Validation: DN, SH; Visualization: SH, DN; Writing - original draft: SH, DN; Writing - review & editing: AV, AB, BST. The corresponding authors attest that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

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The collaborative institutions, National Institute of Medical Sciences, Jaipur, India and Patanjali Research Institute, Haridwar, India, have borne their shares of expenditures incurred for conducting their portions of the study from their own internal funds. This study received no specific external funding or grant from any funding agency. No external funding organization was involved in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

### Ethical approval

This study was approved by the Institutional Ethical Committee (IEC) (Ref. No. NIMSUR/IEC/2020/036) of National Institute of Medical Sciences and Research, Jaipur, Rajasthan, India and registered with Clinical Trial Registry-India (CTRI) (CTRI No. CTRI/2020/05/025273). This study has also been enlisted in the International Clinical Trials Registry Platform (ICTRP) of World Health Organization (WHO). Patient consent was acquired as per IEC and CTRI guidelines.

### Consent to participate and publish

Written informed consent of the participants was obtained for participation in the study and publication of the data.

### Declaration of Competing Interest

Authors declare no conflict of interests with regards to the submitted work. The medications were provided by Divya Pharmacy, Haridwar, Uttarakhand, India. Acharya Balkrishna is an honorary trustee in Divya Yog Mandir Trust. Besides, providing the medications, Divya Pharmacy was not involved in any aspect of the clinical trial reported in this study. Clinical trial was conducted at National Institute of Medical Sciences, Jaipur, India.

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### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phymed.2021.153494.

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