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Research Article

THE ROLE OF HERBAL IMMUNOMODULATORS AS ADJUVANT THERAPY FOR ASYMPTOMATIC AND MILDLY SYMPTOMATIC COVID-19: AN EXPLORATORY CLINICAL STUDY

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ABSTRACT

Objectives: The objective of the study is to evaluate herbal immunomodulators (Septilin and Bresol) as a possible adjuvant therapy for the treatment of asymptomatic and mildly symptomatic COVID-19.

Methods: Randomized, open-label, comparative clinical study. Subjects were randomized to either arm I [Septilin and Bresol+standard of care (SOC)] or arm II (SOC). This study was registered on CTRI (CTRI/2020/06/025801).

Results: Subjects in arm I showed a greater reduction in levels of interleukin-6 tumor necrosis factor-α following treatment than in arm II. Subjects in arm I showed a greater increase in levels of interferon (IFN)-β and IFN-λ than those in arm II. There was a greater reduction in D-dimer in arm I than in arm II subjects (64.28% vs. 35.59%) and all arm I subjects had D-dimer values in the normal range compared to 70% of arm II subjects. There were statistically significant reduction in lactate dehydrogenase and neutrophil-to-lymphocyte ratio in arm I ($p<0.016$ and $p<0.013$, respectively). Clinical assessments during the post-illness convalescence period showed significant improvements in fatigue assessment scores and quality of life.

Conclusion: This herbal combination as an adjuvant to SOC may provide additional long-term benefits in COVID-19 infection by reducing inflammation. This treatment may offer a good addendum for the management of post-COVID-19 illness.

Keywords: COVID-19, Herbal immunomodulators, Tablet septilin, Tablet bresol, Post-COVID-19 illness.

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INTRODUCTION

After it was first reported in Wuhan, China, the infectious coronavirus disease 2019 (COVID-19; nCoV or SARS-CoV-2) spread rapidly to more than 200 countries within 3 months. On March 11, 2020, the World Health Organization (WHO) declared this disease a pandemic because of its global spread, rapid transmission, and criticality.

The treatments available for COVID-19 are largely supportive and exploratory in nature. Research is underway with several existing antiviral drugs that have been repurposed as frontline therapies for COVID-19. In the absence of strong evidence in modern medical interventions, herbal medicines may help to reduce the burden of COVID-19 which has proved to be recurrent in nature. Ayurvedic principles emphasize the maintenance of physical and mental health through preventative, curative, and rehabilitative approaches. Alternative medicine may play an important role in the present crisis [1]. Health-promoting immunomodulatory herbs (rasayana) can improve host defense and could therefore be an effective approach to the management of COVID-19 [2]. In recent years, individual herbs and some polyherbal combinations that promoteimmune and respiratory health have undergone rigorous scientific scrutinyin clinical trials to establish their efficacy and safety. Some of these formulations could be added successfully to current treatment regimens for COVID-19.

Septilin, a polyherbal formulation, is a clinically tested, potent immunomodulator that helps to build the body's defense mechanisms and protect against infections. Septilin comprises herbs with proven anti-inflammatory [3] and immunomodulatory [4,5] properties viz. Guduchi (*Tinospora cordifolia*), Yashtimadhu (*Glycyrrhiza glabra*), Amalaki (*Emblica officinalis*), Manjishta (*Rubia cordifolia*), Guggulu (*Commiphora mukul*), Shigru (*Moringa pterygosperma*).

The principal ingredient of Bresol, another clinically tested polyherbal combination, is Haridra (*Curcuma longa*). It possesses anti-inflammatory [6,7], mucolytic [8-10], and bronchodilator properties and is commonly used to treat respiratory disorders. The other ingredients are Tulasi (*Ocimum sanctum*), Vasaka (*Adhatoda vasica*), Trikatu, Triphala, Vidanga (*Embelia ribes*), Musta (*Cyperus rotundus*), Tvak (*Cinnamomum zeylanicum*), Ela (*Elettaria cardamomum*), and Nagakesara (*Mesua ferrea*). Both are proprietary products of Himalaya Wellness Company, India, and are manufactured at Himalaya's Good Manufacturing Practice-certified facilities in accordance with the WHO's guidelines for Good Agricultural and Collection Practices. The standardization of production protocols minimizes batch-to-batch variations and ensures the safety, efficacy, and quality of the products. Both products have been subject to rigorous preclinical and clinical studies with multiple indications [11-22]. This study explores the possible use of Septilin and Bresol as adjuvant treatments in the management of COVID-19 infection. Levels of laboratory parameters related to inflammation and immunity are evaluated and clinical assessments in the post-illness convalescence period are conducted to assess the overall well-being (in terms of quality of life [QOL] and fatigue assessment scores [FAS]) of patients after treatment with Septilin and Bresol.

METHODS

This was anopen label, randomized, comparative, exploratory clinical study conducted at the Victoria Hospital, Bangalore Medical College and Research Institute (Bengaluru, India). The study aimed to evaluate

adjuvant treatment with Septilin and Bresol (herbal immunomodulators and respiratory wellness support) compared with standard of care (SOC) alone in patients confirmed with COVID-19 and at low clinical risk (asymptomatic and mildly symptomatic). Subjects who tested positive for COVID-19 based on reverse transcription-polymerase chain reaction (RT-PCR) analyses were considered for screening (SARS-CoV-2 RT-PCR-confirmed COVID-19–positive cases within 72 h of the onset of symptoms/48 h of a positive RT-PCR result for COVID-19). The main inclusion criteria were subjects who were COVID-19 positive, age 18–60 years, asymptomatic or mildly symptomatic, and at low clinical risk as assessed by the 0–4 point National Early Warning Score (NEWS) system [23]. Overall well-being of the subject was assessed by using 0-5point WHO-5, FAS was assessed by 1-5 point Checklist Individual Strength, World Health Organization QOL assessment instrument, and Fatigue scale.

The main exclusion criteria were those with a NEWS score ≥5; acute respiratory distress presenting with a respiratory rate >24/min, oxygen saturation (SaO2/SPO2) ≤94% in air-conditioned rooms, or an arterial oxygen partial pressure to fractional inspired oxygen (PaO_2/FiO_2)ratio <300 mmHg; a recent significant pulmonary condition, such as asthma or chronic obstructive lung disease; inability to take oral medication; ailments related to absorption; an immunocompromised state or on immunosuppressive therapy; severe and uncontrolled metabolic, endocrinal, cardiac, renal, or hepatic disease; and pregnant or breastfeeding women.

Subjects who fulfilled the eligibility criteria were randomized into two treatment arms, arm I and arm II, at a 1:1 ratio. Subjects assigned to arm I received Septilin and Bresol orally at a dose of one tablet, twice daily, along with SOC for the specified duration of treatment (from day 1 to 14 days after hospital discharge, as applicable); subjects in arm II (the control arm) received only SOC, according to institutional guidelines. Subjects were followed up daily until their discharge from the hospital (visit 2) according to the treatment guidelines for the management of such subjects and were assessed subsequently by telephone 7±1 days after the hospital discharge (visit 3) and 14±3 days after hospital discharge (visit 4).

Statistical analyses

Statistical analyses of the data generated in this trial were performed using GraphPad Prism software, V.6.07 for Windows (San Diego, CA, USA). Means and standard deviations (SD) were calculated for continuous variables, and frequencies and percentages were calculated for categorical variables. After screening, eligible subjects were randomized into arm I or arm II using a list produced by computer-generated randomization at a 1:1 ratio. The randomization list had blocks of the same length (block size: 4), was not stratified, and was generated by a biostatistician using sealed envelope online software. The two study authors were responsible for assigning the subjects to groups according to the randomization list. Study site staff members and a study monitor were responsible for data collection and verification and ensured that the randomization was based on the sequencing.

Continuous variables with normal distributions were subjected to within-group comparisons using repeated-measures analysis of variance, followed by a *post-hoc* test and a paired t-test. Variables with non-normal distributions were compared using the nonparametric Friedman test, followed by a *post-hoc* test.

For between-group comparisons, continuous variables with normal distributions were compared using unpaired t-tests, whereas variables with non-normal distributions were compared using the MannWhitney test. The significance level for all tests was set at $p \leq 0.05$. All analyses were two-tailed.

RESULTS

Study subjects

A total of 59 subjects were enrolled in the study (arm I=30 subjects and arm II=29 subjects); however, 19 subjects withdrew their consent. Therefore, a total of 40 subjects (20 in each group) completed the study and were available for laboratory investigations and final analyses. The detailed baseline demographics for all subjects recruited and those who completed the study are provided in Table 1a and b. A flow diagram of the study based on the Consolidated Standards of Reporting Trials guidelines is depicted in Fig. 1.

Clinical assessments

The subjects in the study were evaluated during the time taken for the positive CoV-RT-PCR result to become negative (days) and the time taken to be discharged from hospital (days). Based on the guidelines of the institute for assessments on specific days, these parameters could not be differentiated, especially with this small sample size (Table 2). There was no increase in the severity of symptoms in any of the subjects. Further, there was no clinical failure of treatment and no recurrence of the RT-PCR-positive result for COVID-19.

Inflammatory markers (interleukin [IL]-6 and tumor necrosis factor [TNF]-α)

Slight reductions in levels of IL-6 and TNF-α were seen in both arms. This was statistically significant when compared with the baseline values. Arm I demonstrated slightly greater reductions than arm II (IL-6: 9.35% vs. 9.17%, TNF-α: 8.63% vs. 7.91%, respectively). Additionally, for the IL-6 level, 10 subjects (50%) in arm I were almost within the reference range at the time of discharge compared to six subjects (30%) in arm II (Tables 3 and 4).

Immunological markers (interferon [IFN]-β and IFN-λ)

Increased IFN levels (both β and λ) were seen in both arms. However, the increase was greater in arm I than in arm II subjects at the end of

Table 1a: Summary of demographic characteristics of enrolled subjects

Variables	Arm I (n=30)	Arm II (n=29)
Gender		
Male	16	19
Female	14	10
Age (years)		
Mean±SD	38.27±11.33	36.93±12.09
Median	38.5	33
Min–Max	18–59	20–59
Weight (kg)		
Mean±SD	63.3±7.06	63.31±8.73
Median	64	62
Min–Max	48–76	46–79
Height (cm)		
Mean±SD	162.2±5.84	163.7±5.89
Median	161.5	165
Min–Max	152–176	152–175

Table 1b: Summary of demographic characteristics of subjects who completed the study

Variables	Arm I (n=20)	Arm II (n=20)
Gender, n (%)		
Male	11 (55%)	16 (80%)
Female	9 (45%)	4 (20%)
Age (years)		
Mean±SD	38.9±9.22	39.65±11.58
Median	38.5	37
Min–Max	23–59	20–57
Weight (kg)		
Mean±SD	63.6±8	65.45±9.33
Median	65	66.5
Min–Max	48–76	46–79
Height (cm)		
Mean±SD	162.5±6.66	164.7±5.92
Median	161.5	165
Min–Max	152–176	152–175

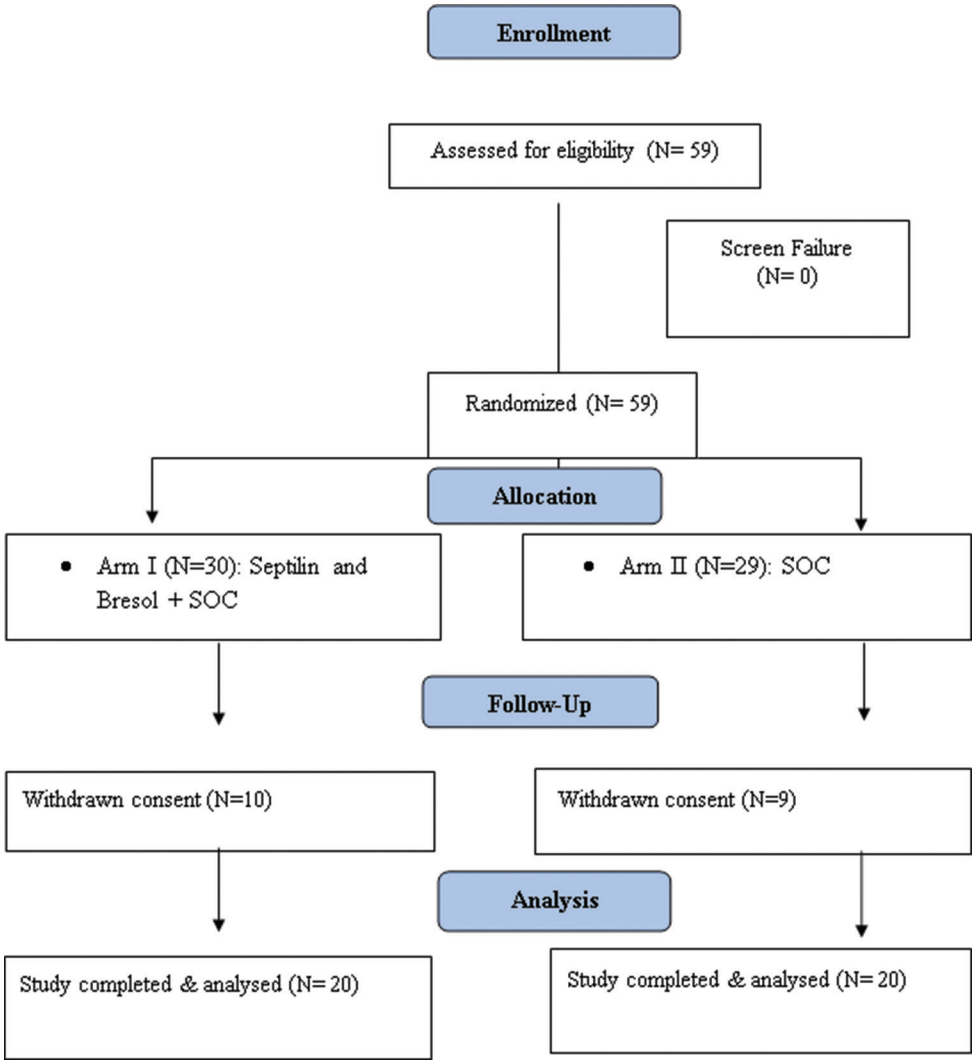

Fig. 1: Flow diagram of the study design as per the Consolidated Standards of Reporting Trials guidelines

Table 2: Time to convert 2019 nCoV RT-PCR to negative and time to discharge from hospital

Time to convert 2019 nCoV RT-PCR to negative (days)			Time to discharge from hospital (days)		
n	**Arm I**	**Arm II**	**n**	**Arm I**	**Arm II**
	20	**19**		**20**	**19**
Median (days)	9	9	Median (days)	9	10
Min–Max	7–12	7–16	Min–Max	7–12	7–15
Mean±SD	9.55±1.39	9.63±2.01	Mean±SD	9.05±1.39	9.26±1.88

RT-PCR: Reverse transcription-polymerase chain reaction

the study (EOS; visit 4 [IFN-β: 74.31% vs. 67.26%; IFN-λ: 187.50% vs. 167.94%, respectively) (Figs. 2 and 3).

Other markers (D-dimer, lactate dehydrogenase [LDH], Neutrophil-to-lymphocyte ratio [NLR])

D-dimer

D-dimer levels were evaluated at visits 1, 2, and 4. In arm I subjects, the baseline mean value was 0.56±0.91 μg/ml. This had reduced to 0.2±0.1 μg/ml at 14 days post-discharge (visit 4). In arm II, the mean value was 0.59±0.58. This had reduced to 0.38±0.39 (which is still borderline high). These results are indicative of a higher reduction in D-dimer levels in arm I than in arm II subjects by the end of the study (64.28% vs. 35.59%, respectively). All arm I subjects had D-dimer levels within the normal range at visit 4. However, 30% of arm II subjects had D-dimer levels outside the normal range at visit 4 (Fig. 4).

LDH

In arm I, LDH reduced from 234.5±62.45 U/L at baseline to 189±36.81 U/L at 14 days post discharge (visit 4), with a significance of $p<0.016$. A reduction in the LDH level was also seen in arm II subjects, from 304.07±188.6 U/L at baseline to 202.75±55.25 U/L at visit 4 with a significance of $p<0.026$.

However, approximately 40% of subjects (8/20) still had LDH values that were borderline high or above the normal reference range in arm II, whereas only 15% (3/20) subjects had values that were borderline high or above the normal reference range in arm I (Fig. 5).

NLR

In arm I, the NLR at baseline was 2.75±1.52, which reduced to 2.03±0.77 at visit 2 (discharge visit), with a significance of $p<0.013$. In arm II, the NLR at baseline was 2.56±1.46, which reduced to 2.14±0.68 at visit 2. This change was not significant. A greater reduction (26.18%) was observed in arm I than in arm II subjects (16.40%) during the critical inflammation period (until the RT-PCR results for COVID-19 were negative) (Fig. 6).

Post-illness convalescence symptoms

This study evaluated symptoms commonly seen during convalescence from COVID-19. A statistically significant improvement was seen in energy levels, appetite, and ability to concentrate in arm I subjects at visit 2 and 3 as compared with these factors at baseline. There was also a significant difference between these improvements in arm I and arm II subjects. A significant improvement was also seen in body pain, joint movement, sleep quality, recurrent respiratory tract infection, and shortness of breath in arm I subjects. This was also true for arm II, but the improvements were greater in arm I (Table 5).

QOL

This study evaluated QOL using the WHO-5 Well-Being Index (WHO-5) and the FAS. The FAS included a questionnaire comprising 10 questions on fatigue, individual strength, and QOL. Answers to the questions are scored on a 5-point scale ranging from 1 to 5 (where 1=never, 2=sometimes, 3=regularly, 4=often, and 5=always). A total FAS score <22 indicates no fatigue; a score ≥ 22 indicates fatigue. Arm I subjects demonstrated significant improvements in FAS scores between baseline and later assessments. Improvements in arm I subjects were also significantly greater than those in arm II subjects. All (100%) arm I subjects were relieved of fatigue after treatment, whereas 35% of arm II subjects continued to experience fatigue (Tables 6-7 and Fig. 7).

Table 3: Inflammatory markers (IL-6 and TNF-α)

Parameters	Visits		Arm II (n=20)	Arm II (n=20)
IL-6 (pg/ml)	Visit 1	Mean±SD	18.82±1.72	19.39±1.88
		Median	18.9	18.85
		Min–Max	14.4–22.6	15.35–22.38
		IQR	18.01–19.6	17.96–21.1
		95% CI	18.02–19.62	18.51–20.27
	Visit 2	Mean±SD	17.06±1.82	17.61±1.52
		Median	16.85	17.87
		Min–Max	12.8–20.5	15.4–20.64
		IQR	15.99–18.33	16.2–18.8
		95% CI	16.21–17.91	16.9–18.32
		%CFB↓	9.35	9.17
		p-value (WG)	<0.0001	<0.0001
TNF-α (pg/ml)	Visit 1	Mean±SD	14.01±3.59	13.77±3.15
		Median	12.6	12.9
		Min–Max	10.2–23.5	11.0–22.25
		IQR	11.75–15.45	11.6–14.4
		95% CI	12.33–15.69	12.3–15.24
	Visit 2	Mean±SD	12.8±3.21	12.68±2.95
		Median	11.9	11.65
		Min–Max	8.9–21.29	10.2–20.4
		IQR	11.0–13.8	11.08–12.93
		95% CI	11.3–14.3	11.3–14.06
		%CFB↓	8.63	7.91
		p-value (WG)	<0.0018	<0.0001

Statistical tests: Paired t-tests were used for within-group comparisons and unpaired t-tests for between-group comparisons. The level of significance was fixed at P<0.05. WG: Within-group, BG: Between-group, CFB: Change from baseline, CI: Confidence interval, IQR: Interquartile range, SD: Standard deviation, IL: Interleukin, TNF: Tumor necrosis factor

The QOL was further evaluated using the WHO-5, which utilizes a 6-point Likert scale (ranging from 0 to 5). The well-being of subjects was evaluated at visits 1, 2, and 4. Subjects in arm I demonstrated a substantially greater improvement in all the parameters evaluated than those in arm II (Tables 8-9 and Fig. 8).

Laboratory investigations

Most of the hematology and blood biochemistry parameters were within the normal reference ranges. Values outside the reference range were not considered clinically significant.

Adverse event (AE) profile

A total of five AEs occurred in the study. Two mild AEs were reported in arm I (indigestion). These subjects recovered from the AE without treatment or any sequelae. There were three mild AEs (sore throat) reported in arm II. However, none of these were serious enough for subjects to be withdrawn from the study. No serious AEs were reported or observed in any of the subjects. Neither group raised any significant safety concerns, indicating the safety profile of Septilin and Bresol tablets.

DISCUSSION

COVID-19 is a highly contagious infection, with no definitive treatment available. Currently available treatments are largely supportive. In the absence of evidence-based modern interventions, it is possible that herbal ayurvedic medicines can aid value in the overall management of COVID-19. Rasayana ayurvedic botanicals have been used for centuries to strengthen immunity and may be considered for COVID-19 prophylaxis and as an add-on treatment [2]. This study affirmed the potential of Ayurveda as a useful adjuvant treatment in the management of COVID-19. Integrating Ayurveda with modern medicine will offer a novel and effective means of prevention, cure, and rehabilitation from COVID-19 [24].

This study was an open-label, comparative clinical study that explored the efficacy and safety of Septilin and Bresol tablets as adjuvants to SOC, according to applicable guidelines related to the management of the COVID-19 pandemic. Clinical assessments were conducted to measure factors (hospital stay, conversion to negative test, severity) relevant to the treatment of COVID-19 infection. However, these assessments could not demonstrate large differences, given the small sample size.

The inflammatory markers IL-6 and TNF-α are known to be produced in response to COVID-19 infection. Severity is believed to be due to inflammatory storms caused by an over-reaction of cytokines [25]. Several studies have found inflammation to be an important indicator of COVID-19, which is closely related to its severity. Therefore, IL-6 might be a promising therapeutic target [26,27]. In this study, subjects treated with Septilin and Bresol (arm I) demonstrated a greater reduction in IL-6 levels after treatment than subjects in the control arm (arm II). More subjects in the experimental group than in the control group had IL-6 values close to the reference range at the time of discharge (50% vs. 30%, respectively). This indicates that adjuvant therapy with Septilin and Bresol could confer both short- and long-term benefits by reducing inflammation, thus contributing to recovery from COVID-19 and its associated symptoms.

Table 4: Range of inflammatory markers (pre- and post-treatment)

Parameters	Outcome	Arm I (n=20)		Arm II (n=20)	
		Pre-treatment	Post treatment	Pre-treatment	Post treatment
		n (%)			
IL-6 (0.00–16.4 pg/ml)	Normal range	2 (10)	10 (50)	1 (5)	6 (30)
	Not in normal range	18 (90)	10 (50)	19 (95)	14 (70)
TNF-α (0.00–9.8 pg/ml)	Normal range	0 (0)	1 (5)	0 (0)	0 (0)
	Not in normal range	20 (100)	19 (95)	20 (100)	20 (100)

IL: Interleukin, TNF: Tumor necrosis factor

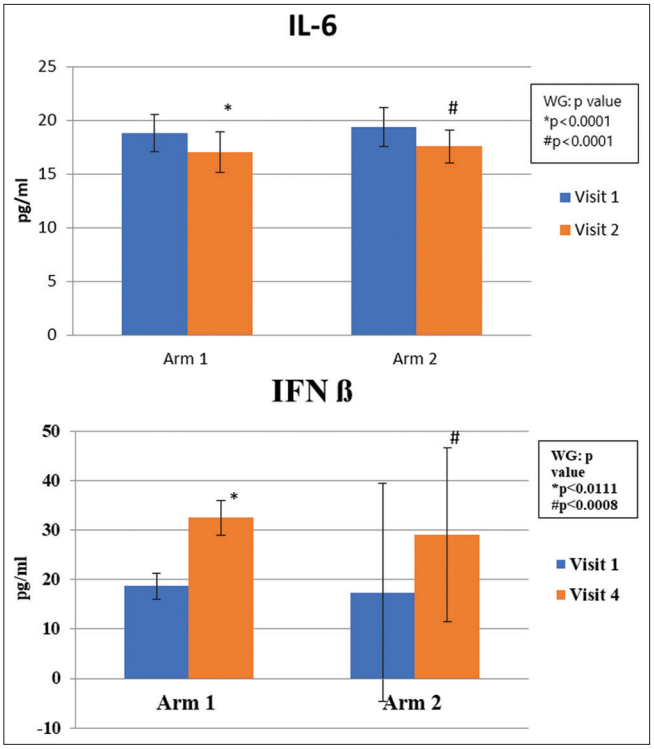

Fig. 2: Interferon-ß (pg/ml) values

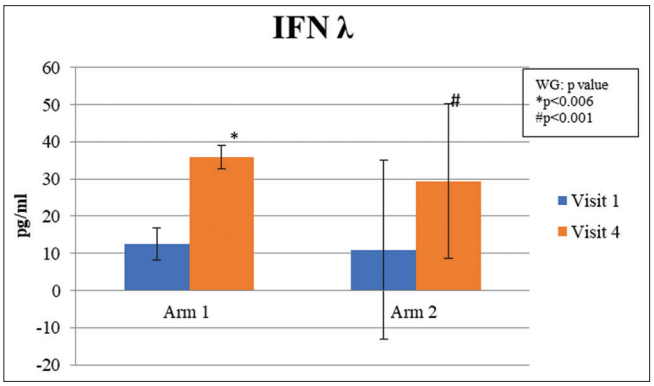

Fig. 3: Interferon-λ (pg/ml) values

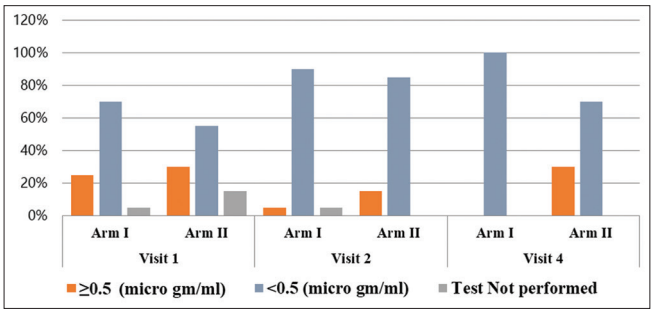

Fig. 4: D-Dimer ranges (%) for different visits

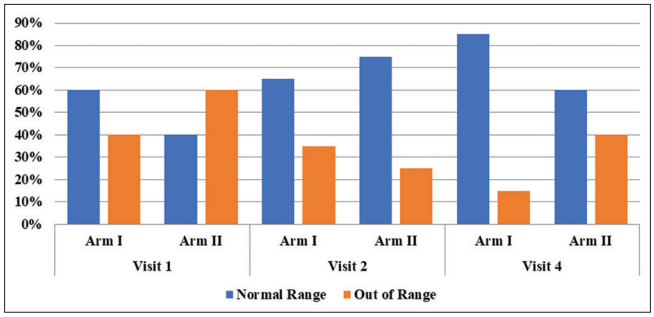

Fig. 5: Lactate dehydrogenase ranges (%) for different visits

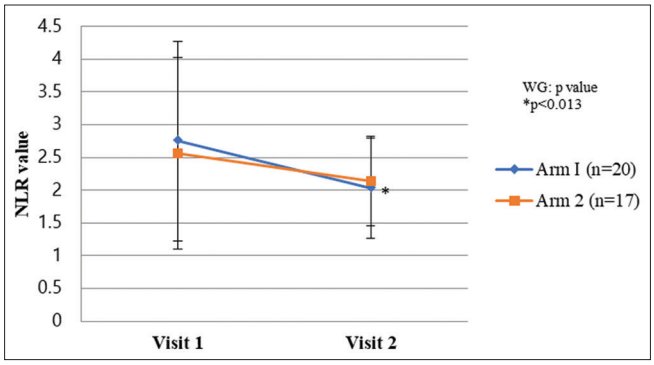

Fig. 6: Neutrophil-to-lymphocyte ratio values

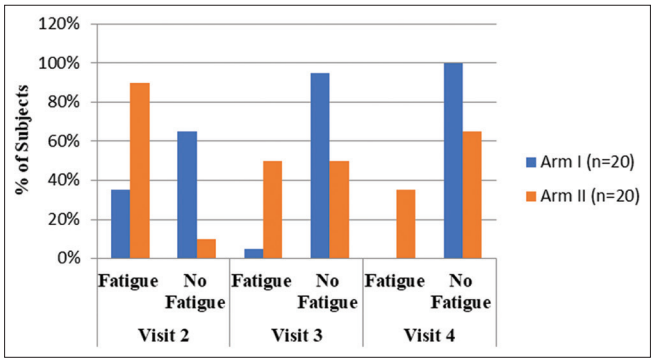

Fig. 7: Fatigue assessment scores (%)

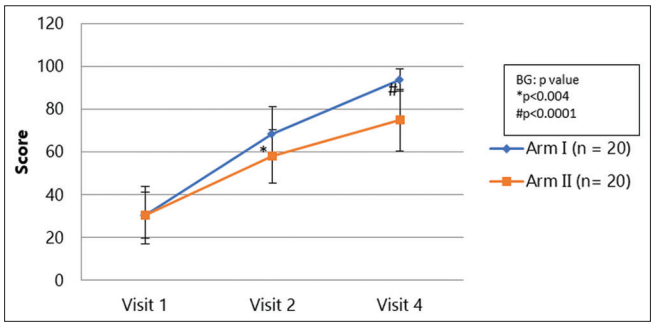

Fig. 8: WHO-5 well-being index scores

The immunological markers IFN-β and IFN-λ are the most specific and relevant to protection from COVID-19. Various preclinical studies have shown IFNs to be potent inhibitors of replication of the coronavirus and they can play an important role in the treatment of COVID-19. Thus, enhancing IFN activity helps fight COVID-19 infection (and other viral infections of the respiratory tract) more efficiently [28,29,30]. Increased levels of IFN-β and IFN-λ were observed in the experimental group of this study, favoring the use of Septilin and Bresol as adjuvants. Further, this could aid in the prevention of the severe and critical stages of viral infections.

Other markers of significance to COVID-19 include D-dimer, LDH, and NLR levels, which were also evaluated in this study. There are numerous reports that coagulopathy can develop in subjects with COVID-19,

Table 5: Clinical assessments for post-illness convalescence from COVID-19 infection

Parameters	Value	Arm I (n=20)			Arm II (n=20)		
		Visit 2	Visit 3	Visit 4	Visit 2	Visit 3	Visit 4
Low energy levels	Mean±SD	2.1±0.79	1.3±0.57	1.05±0.22	2.65±0.75	1.9±0.85	1.65±0.75
	Median	2	1	1	2.5	2	1.5
	Min–Max	1–4	1–3	1–2	2–4	1–3	1–3
	p-value (WG)	-	<0.015	<0.003	-	<0.0153	<0.001
	p-value (BG)	-	<0.044	<0.014	-	-	-
Restricted joint movements	Mean±SD	1.75±0.64	1.25±0.55	1.05±0.22	2.25±0.72	1.7±0.73	1.4±0.68
	Median	2	1	1	2	2	1
	Min–Max	1–3	1–3	1–2	1–4	1–3	1–3
	p-value (WG)	-	-	<0.009	-	-	<0.011
	p-value (BG)	-	-	ns	-	-	Ns
Irregular bowel movement	Mean±SD	1.95±0.76	1.25±0.55	1.05±0.22	2.05±0.76	1.75±0.79	1.4±0.6
	Median	2	1	1	2	2	1
	Min–Max	1–3	1–3	1–2	1–3	1–3	1–3
	p-value (WG)	-	-	<0.007	-	-	<0.024
	p-value (BG)	-	-	ns	-	-	Ns
Loss of appetite	Mean±SD	1.95±0.6	1.25±0.55	1±0	2.5±0.69	1.75±0.85	1.55±0.6
	Median	2	1	1	2	1.5	1.5
	Min–Max	1–3	1–3	1–1	2–4	1–3	1–3
	p-value (WG)	-	<0.025	<0.001	-	<0.024	<0.009
	p-value (BG)	-	-	<0.001	-	-	-
Body pain	Mean±SD	1.7±0.73	1.25±0.55	1.05±0.22	2±0.86	1.65±0.81	1.2±0.52
	Median	2	1	1	2	1	1
	Min–Max	1–3	1–3	1–2	1–3	1–3	1–3
	p-value (WG)	-	-	<0.003	-	-	<0.003
	p-value (BG)	-	-	ns	-	-	Ns
Disturbed sleep	Mean±SD	2.05±0.83	1.2±0.52	1.1±0.31	2.55±0.6	1.7±0.66	1.3±0.57
	Median	2	1	1	3	2	1
	Min–Max	1–3	1–3	1–2	1–3	1–3	1–3
	p-value (WG)	-	-	<0.020	-	<0.019	<0.001
	p-value (BG)	-	-	Ns	-	-	Ns
Recurrent respiratory tract infection	Mean±SD	1.85±0.67	1.2±0.52	1.1±0.31	2.05±0.83	1.7±0.92	1.4±0.6
	Median	2	1	1	2	1	1
	Min–Max	1–3	1–3	1–2	1–3	1–4	1–3
	p-value (WG)	-	-	<0.025	-	-	<0.048
	p-value (BG)	-	-	ns	-	-	Ns
Ability to concentrate/focus	Mean±SD	3.65±1.04	4.15±0.81	4.75±0.64	3.1±1.07	3.55±1.1	3.8±1.06
	Median	3.5	4	5	3	3	4
	Min–Max	1–5	2–5	3–5	1–5	1–5	2–5
	p-value (WG)	-	-	<0.020	-	-	-
	p-value (BG)	-	<0.039	<0.038	-	-	-
Shortness of breath	Mean±SD	1.6±0.6	1.1±0.31	1.0±0	1.8±0.62	1.4±0.6	1.15±0.37
	Median	2	1	1	2	1	1
	Min–Max	1–3	1–2	1–1	1–3	1–3	1–2
	p-value (WG)	-	-	<0.032	-	-	<0.024
	p-value (BG)	-	-	ns	-	-	Ns

Statistical tests: Within-group Friedman test, followed by a post-hoc test, between-group Mann–Whitney test; WG: Within-group, BG: Between-group, Level of significance was fixed at p<0.05

Table 6: Fatigue assessment scale (n [%])

Visits	Outcome	Arm I (n=20) (%)	Arm II (n=20) (%)
Visit 2	Fatigue	7 (35)	18 (90)
	No fatigue	13 (65)	2 (10)
Visit 3	Fatigue	1 (5)	10 (50)
	No fatigue	19 (95)	10 (50)
Visit 4	Fatigue	0 (0)	7 (35)
	No fatigue	20 (100)	13 (65)

especially in critical cases. Therefore, monitoring and controlling D-dimer levels is essential in the management of this disease [30-33]. Subjects in the Septilin and Bresol arm (arm I) of our study showed a greater reduction in D-dimer levels from the baseline than those in the control arm; none of the arm I subjects had values outside the normal range at the final evaluation. In arm II, 30% of the subjects had D-dimer levels outside the normal range at the final evaluation. This trend toward the normalization of the D-dimer level indicates that as adjuvants, Septilin and Bresol may help in the prevention of disease progression (in terms of severity and possible complications). They may also help prevent D-dimer-related complications (cardiovascular orthromboembolic events) in COVID-19, even after recovery. Such post-recovery complications are emerging as an alarming characteristic of the COVID-19 virus.

LDH is an enzyme found abundantly in the heart, liver, lungs, muscles, kidneys, and blood cells. It is a general indicator of acute or chronic tissue damage and an inflammatory marker [34]. LDH levels are known to increase in response to acute and severe lung damage and elevated LDH levels are seen in other interstitial lung infections [35]. In subjects who are COVID-19 positive, LDH levels may be used to measure abnormal inflammation status, which can lead to lung damage and respiratory distress [36]. In this study, a significant reduction in LDH levels (p<0.016) was observed in individuals treated with Septilin and Bresol. Despite the small sample size, it was encouraging that approximately 40% of the subjects (8/20) in arm II showed LDH levels that were borderline high or outside the normal range in arm

Table 7: Fatigue assessment scale (FAS) scores

Visits	Arm I	Arm II
	(n=20)	(n=20)
Visit 1		
Mean±SD	19.75±5.21	25.7±3.76
Median	20	25.5
Min–Max	11–27	19–34
p-value (BG)	<0.001	
Visit 2		
Mean±SD	13.25±4.29	20.05±6.02
Median	12	21
Min–Max	10–25	10–32
% CFB	32.91%	21.98
p-value (BG)	<0.001	Ns
p-value (WG)	<0.002	<0.004
Visit 4		
Mean±SD	11.3±2.36	18.35±6.07
Median	10	18.5
Min–Max	10–19	10–31
% CFB	42.78%	28.59%
p-value (BG)	<0.001	Ns
p-value (WG)	<0.001	<0.001

Statistical tests: Within-group Friedman test, followed by a *post-hoc* test, between-group Mann–Whitney test; level of significance was fixed at p<0.05; ns: not significant, BG: Between-group, WG: Within-group. A total FAS score<22 indicates no fatigue, a score≥22 indicates fatigue

Table 8: WHO-5 well-being index (WHO-5) scores

Visits	Arm I	Arm II
	(n=20)	(N=20)
Visit 1		
Mean±SD	30.6±10.72	30.4±13.45
Median	32	34
Min–Max	4–48	0–48
p-value (BG)	Ns	Ns
Visit 4		
Mean±SD	68.6±12.4	58±12.41
Median	66	58
Min–Max	52–100	36–84
p-value (WG)	<0.001	<0.001
p-value (BG)	<0.004	Ns
Visit 4		
Mean±SD	93.6±5.09	74.8±14.22
Median	96	76
Min–Max	80–100	52–100
p-value (WG)	<0.001	<0.001
p-value (BG)	<0.0001	Ns

Zero (0) represents the worst imaginable well-being and 100 represents the best imaginable well-being. Statistical tests: Within-group Friedman test, followed by a post-hoc test, a between-group Mann-Whitney test; the level of significance was fixed at p<0.05; ns: not significant; BG: between-group; WG: within-group

II compared to only 15% (3/20) of the subjects in arm I at the final evaluation. These findings suggest that adjuvant therapy with Septilin and Bresol can benefit patients withCOVID-19 by helping to prevent lung and muscle damage. These formulations may also contribute to improved post-recovery well-being.

NLR is are liable prognostic tool and marker of inflammation and subsequent COVID-19 complications [37,38]. A greater reduction in NLR was observed in arm I subjects (p<0.013) than arm II subjects during the critical inflammation period (until the RT-PCR result for COVID-19 turns negative). This indicates that the Septilin and Bresol combination has effective anti-inflammatory properties that may be helpful in the hyperinflammatory stage of COVID-19. A lower NLR has been shown to correlate with a more favorable COVID-19prognosis, which is established and proved in multiple studies [37,38].

The emerging evidence of trouble some residual symptoms (e.g., cough, sputum, sore throat, disorders of the gastrointestinal tract, fatigue, and dyspnea, along with reduced mental well-being) after discharge from the hospital (post-illness convalescence symptoms of COVID-19 infection) are of great concern and were therefore clinically assessed and analyzed in this study after the subjects became negative on the COVID-19 test [39-42]. Statistically significant improvements were observed in energy levels, appetite, and ability to concentrate/ focus, supporting the use of Septilin and Bresol for full recovery from COVID-19.

Overall well-being was assessed in this study using the FAS and the WHO-5. Arm I subjects demonstrated significant improvements in their FAS scores between baseline and later evaluations. There was also a significantly greater improvement in FAS scores in arm I subjects than in arm II subjects. All (100%) arm I subjects were assessed as free of fatigue after treatment, whereas 35% of subjects in the control arm still experienced fatigue. Arm I subjects demonstrated significant and substantially greater improvements in all WHO-5parameters than those in arm II subjects. This indicates that a combination of Septilin and Bresol may be beneficial in over coming a range of troubling residual symptoms in the post-illness convalescence period of COVID-19. The favorable data during the active infection phase may be correlated with a positive outcome of the post-illness convalescence phase.

From the encouraging safety profile in this study, it can be inferred that the combination of Septilin and Bresol is a safe and well-tolerated adjuvant in patients with confirmed asymptomatic and mildly symptomatic COVID-19. This is in line with the safety profile of both the products, which have been on the market for decades. In the absence of a standard treatment and management regimen for COVID-19 infections (including active treatment and post-COVID-19 illness), we hope that the results of this exploratory study provide a ray of hope to the scientific community. Adjuvant therapy with Septilin and Bresol for the management of COVID-19 may be a promising addendum to modern medicine armament. However, the sample size is in this study was too small to offer conclusive evidence. A sample size–estimated study may provide a more convincing and acceptable conclusion. Unfortunately, there was a limitation in determining a statistically justified sample size during the initial phase of the pandemic. After many months since the conceptualization of this study, it is still challenging to derive a sample size with numerical assumptions from a large spectrum of assessments. This study was designed to use the herbal treatments tested as open-label and adjuvant in mild cases. The herbal composition of the proprietary drugs, the need to avoid increasing risk in severe cases, and the further ethical need not to use these products as a stand-alone therapy for acute COVID-19 were all kept in mind in the design of this study. As the understanding of the disease course developed over the study period, less impact is expected in clinical scenarios with asymptomatic and mildly symptomatic subjects. However, we could still identify some favorable effects on inflammatory and immunological markers known to be related to the severity of the disease. Considering the intricacies of this condition, this study was planned as an open-label study. Therefore, variations due to subjective assessments cannot be ruled out.

Besides limitations beyond our control during study conceptualization, this research has strong credentials: (i) The study was conducted at a reputed medical institute (the only dedicated COVID-19 treatment facility in the state at the time), after approval from the ethics committee of the institute and registration with the Clinical Trials Registry in India; (ii) Documentation and verification of data were performed according to ICH guidelines, and (iii) data management was performed in compliance with 21 CFR 11 EDC (Oracle Clinical) for data collection (with SDTM-CDISC standards). This ensured the highest level of data credibility. The laboratory involved in the immunological and inflammatory markers assessment is ISO 9001:2008 and NABL-accredited. For all other laboratory assessments, the hospital's

Table 9: Summary of WHO-5 scores [n (%)]

Summary of overall well-being (WHO-5) [n (%)]							
Over the past 2 weeks	**Response**	**Arm I (n=20) (%)**			**Arm II (n=20) (%)**		
		Visit 1	**Visit 2**	**Visit 4**	**Visit 1**	**Visit 2**	**Visit 4**
I have felt cheerful and in good spirits	All the time	0 (0)	2 (10)	17 (85)	0 (0)	0 (0)	4 (20)
	Most of the time	0 (0)	5 (25)	3 (15)	0 (0)	2 (10)	10 (50)
	More than half the time	0 (0)	11 (55)	0 (0)	1 (5)	8 (40)	5 (25)
	Less than half the time	13 (65)	2 (10)	0 (0)	11 (55)	10 (50)	1 (5)
	Some of the time	6 (30)	0 (0)	0 (0)	5 (25)	0 (0)	0 (0)
	At no time	1 (5)	0 (0)	0 (0)	3 (15)	0 (0)	0 (0)
I have felt calm and relaxed	All the time	0 (0)	2 (10)	8 (40)	0 (0)	0 (0)	3 (15)
	Most of the time	0 (0)	8 (40)	11 (55)	0 (0)	8 (40)	7 (35)
	More than half the time	2 (10)	9 (45)	1 (5)	1 (5)	8 (40)	10 (50)
	Less than half the time	7 (35)	1 (5)	0 (0)	12 (60)	4 (20)	0 (0)
	Some of the time	10 (50)	0 (0)	0 (0)	5 (25)	0 (0)	0 (0)
	At no time	1 (5)	0 (0)	0 (0)	2 (10)	0 (0)	0 (0)
I have felt active and vigorous	All the time	0 (0)	1 (5)	15 (75)	0 (0)	0 (0)	5 (25)
	Most of the time	0 (0)	8 (40)	5 (25)	0 (0)	5 (25)	6 (30)
	More than half the time	0 (0)	10 (50)	0 (0)	2 (10)	8 (40)	7 (35)
	Less than half the time	11 (55)	1 (5)	0 (0)	9 (45)	6 (30)	2 (10)
	Some of the time	8 (40)	0 (0)	0 (0)	7 (35)	1 (5)	0 (0)
	At no time	1 (5)	0 (0)	0 (0)	2 (10)	0 (0)	0 (0)
I woke up feeling fresh and rested	All the time	0 (0)	3 (15)	15 (75)	0 (0)	0 (0)	4 (20)
	Most of the time	0 (0)	5 (25)	5 (25)	0 (0)	3 (15)	9 (45)
	More than half the time	1 (5)	11 (55)	0 (0)	0 (0)	12 (60)	5 (25)
	Less than half the time	11 (55)	1 (5)	0 (0)	11 (55)	4 (20)	2 (10)
	Some of the time	6 (30)	0 (0)	0 (0)	6 (30)	1 (5)	0 (0)
	At no time	2 (10)	0 (0)	0 (0)	3 (15)	0 (0)	0 (0)
My daily life has been filled with things that interest me	All the time	0 (0)	2 (10)	15 (75)	0 (0)	1 (5)	4 (20)
	Most of the time	0 (0)	4 (20)	4 (20)	0 (0)	3 (15)	9 (45)
	More than half the time	1 (5)	12 (60)	1 (5)	2 (10)	11 (55)	5 (25)
	Less than half the time	11 (55)	2 (10)	0 (0)	10 (50)	5 (25)	2 (10)
	Some of the time	5 (25)	0 (0)	0 (0)	5 (25)	0 (0)	0 (0)
	At no time	3 (15)	0 (0)	0 (0)	3 (15)	0 (0)	0 (0)

laboratory was used. These measures contributed to the robustness of the study.

CONCLUSION

This clinical study shows that a combination of Septilin and Bresol as adjuvant therapy may provide long-term benefits by reducing inflammation, as demonstrated by the reduction and normalization of key COVID-19 inflammatory markers (IL-6, TNF-α, D-dimer, and LDH). The normalization of the levels of these inflammatory markers may prevent further physical damage, contribute to recovery from COVID-19, and minimize symptoms, even after recovery. The enhanced IFN-β and IFN-λ activity observed in the experimental arm of the study indicates an improved immune response to COVID-19.

Reductions were observed in post-COVID-19 symptoms and improvements in QOL after treatment with the polyherbal products tested in this study. Psychological distress and depression can have negative effects on immune responses, so helping those with COVID-19 to overcome these aspects and could contribute greatly to disease recovery. The data obtained in this research using various validated scales support the adjuvant role of these products in individuals struggling to overcome a range of troubling residual symptoms (especially fatigue and weakness) in post-illness convalescence from COVID-19.

Considering the favorable effects on inflammatory and immunological markers, and the improvements in post-illness COVID-19 symptoms and overall well-being (including QOL and fatigue) found in this study, the addition of Septilin and Bresol would seem a good addendum to the treatment of COVID-19 and for those suffering from post-COVID syndrome.

This study has shown that conventional clinical trial parameters may be adopted for research into traditional ayurvedic and herbal medicines that may assist in the control of the COVID-19 pandemic, for which no modern medicines are available (especially for post-COVID illness). In light of the strong credentials of this study, we are confident that these products will help those struggling with this disease by controlling inflammation and reducing overwhelming residual symptoms from post-COVID syndrome. Further research is warranted to corroborate our findings.

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AUTHORS CONTRIBUTIONS

Dr. C. R. Jayanthi has contributed to conducting clinical study, preparation, and review of manuscript. Dr. Avinash HR, Dr. Swetha Sridhar, Dr. Sriya Sridhar and Dr. Akhila K contributed to assisting the clinical study and preparation of manuscript. Dr. Rajesh Kumawat contributed to the revisions and finalization of manuscript.

CONFLICTS OF INTEREST

Dr. C.R. Jayanthi has received clinical trial support from Himalaya Wellness Company. Dr. Rajesh Kumawat is an employee of Himalaya Wellness Company. The authors declare that the financial interests or personal relationships have not influenced the work reported in this paper. Dr. Avinash H. R, Dr. Swetha Sridhar, Dr. Akila K, and Dr. Sruthi Nambiar have no known competing financial interests or personal

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