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Effects of a Natural Extract on Molecular Signature in Post-Acute Sequelae SARS-CoV-2 (PASC) Infection: A Quasi-Experimental Study

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ABSTRACT

Aim: Long COVID becomes an economic and public health challenge that affects the daily activities and quality of life of millions of COVID-19 survivors. Long COVID symptoms, particularly persistent fatigue, appear to be associated with a chronic state of inflammation. Based on the anti-inflammatory property of Tinospora cordifolia, CelWel has the potential to improve the symptoms of Long COVID. The purpose of this study was to assess the efficacy and safety of CelWel in patients with Long COVID.

Methods: This was a non-randomized, open-label pilot study with 15 COVID-19 infected male and female subjects who had Long COVID symptoms. Subjects were given 0.4 mL of the CelWel supplement 4-6 times per day for 14 days. The severity of Long COVID symptoms was assessed using the Fatigue Severity Scale Questionnaire (FSSQ) and the Yorkshire COVID-19 Rehabilitation Screening Test (C19-YRS) before and after treatment. In addition, plasma levels of pro inflammatory cytokines and chemokines and the post-acute sequelae score of COVID-19 (PASC) were assessed. Safety parameters such as adverse events, haematology, and serum biochemistry were evaluated.

Results: Results showed that all COVID-19 survivors had higher FSSQ, C19-YRS, and PASC scores a Long with elevated plasma levels of pro inflammatory cytokines and chemokines before treatment. CelWel supplementation for 14 days significantly reduced FSSQ and C19-YRS scores and plasma cytokine and chemokine levels. Furthermore, with CelWel treatment, PASC scores showed a decreasing trend in 11 (73%) subjects, while 4 subjects showed a reverse trend. All laboratory safety parameters were within the normal range, and no adverse events were reported during the study period.

Conclusion: These findings suggest that the CelWel supplement is a viable and safe option for reducing the severity of symptoms in patients with Long COVID.

Key Words: CelWel; Fatigue; Long COVID; Cytokines; Clinical trial

INTRODUCTION

The SARS-CoV-2 coronavirus (COVID-19) has affected more than 750 million people around the world to date [1]. Acute manifestations of COVID-19 widely affect the pulmonary, cardiovascular, neurological, hematological, and gastrointestinal systems [2]. Its Long impacts are not well known, but the large numbers of COVID-19 survivors represent post-COVID-19 sequelae [3]. The etiology of post-COVID symptoms is unknown; however, several factors have been proposed to be involved, including comorbidities, post-critical illness, and immune and inflammatory damage [4]. The most common signs and symptoms reported by COVID survivors include fatigue, cognitive decline, dyspnea, anxiety, weakness, shortness of breath, depression, palpitations, arthralgia, chest and muscle pain which impair their mobility, daily activities, and health related quality of life (HR-QoL) [5-8]. Fatigue is one of the most common and severe post-COVID symptoms among 52-58% of COVID-19 sur-

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vivors and the symptom has been reported to persist for more than a year after the onset of illness [9,10]. Fatigue is characterized by an ongoing experience of weakness, exhaustion, and increased difficulty doing physical (e.g., walking, climbing stairs, or lifting) or mental work [11]. In post-COVID syndrome, fatigue is frequently accompanied by muscle fatigue and fatiguability, which coincides with markers of inflammation and hypo-perfusion [12].

ProLonged fatigue after COVID-19 shares clinical manifestations with other post-infectious fatigue syndromes as well as myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) [13]. In both situations, there are higher levels of circulating pro-inflammatory cytokines (such as IL-1, IL-4, GM-CSF, IFN-γ, TNF-α, etc.) and CD8+ T cells, which are presumed to be involved in the pathophysiology of these illnesses. In such chronic inflammatory conditions, as well as possibly in proLonged COVID, inflammatory cytokines reduce basal ganglia function and dopamine while increasing neurotransmitter release in the brain, including serotonin, which causes persistent fatigue [14,15]. In patients recovering from West Nile virus infections, pro-inflammatory (e.g., IL-2, IL-6) and antiviral (e.g., IFN-γ) cytokines were found to be elevated [16]. In contrast, the administration of IL-6 caused fatigue and sleep disturbances in healthy patients [17]. A similar mechanism could be at work in Long-COVID fatigue. Considering the prominent role of inflammatory cytokines in Longstanding COVID-19 symptoms, it was hypothesised that blocking pro-inflammatory cascades might alleviate COVID-19 symptoms, particularly fatigue.

CelWel is an oral supplement containing extracts of Tinospora cordifolia and Piper nigrum, as well as a very low concentration of thimerosal. T. cordifolia, one of the constituents of CelWel, has demonstrated anti-inflammatory activity by lowering pro-inflammatory cytokine levels (IL-1, TNF-α, IL-6, and IL-17), the frequency of IL-17-producing T cells, and the production of chemokines (RANTES) [18]. This study aimed to evaluate the efficacy and safety of the CelWel supplement in reducing Long COVID symptoms, specifically fatigue, and levels of various inflammatory biomarkers.

METHODS

Study Design, and Setting

This single-arm, non-randomized, open-label prospective study on the CelWel supplement was conducted from December 2022 to January 2023, by the Government Medical College and Government General Hospital in Srikakulam, Andhra Pradesh, India. The efficacy and safety of the CelWel supplement were evaluated as adjunctive therapy for a maximum of 14 days for COVID-19 survivors with persistent COVID-19 symptoms. The study was carried out in accordance with the Declaration of Helsinki, the Indian Council of Medical Research (ICMR) ethical guidelines for biomedical research, and ICH guidelines for good clinical practice (GCP). This study protocol was approved by the institutional medical ethical committee (Protocol no.: CW/C-19/1122, Version 1.0) and was prospectively registered with the Clinical Trials Registry-India (ID: CTRI/2022/12/048144) dated 15 December, 2022. All potential participants were voluntarily recruited, and informed consent was obtained prior to the initiation of any study-related procedures.

Participants

The electronic database of the Government Medical College and Government General Hospital, Srikakulam, Andhra Pradesh, was used to screen potential participants infected with SARS-CoV-2. Male and female patients over the age of 18 who had been diagnosed with COVID-19 by RT-PCR and had persistent mild to moderate post-COVID symptoms lasting 4 to 12 weeks since disease onset were recruited. Patients were excluded if they were asymptomatic, had severe systemic diseases, uncontrolled comorbid conditions, vascular disorders, and cancers, were hospitalized or required oxygen support, or had received antiviral medication in the last 36 hours. Potential participants were screened for eligibility, and demographic data was collected at the outset.

Interventions

CelWel is an oral liquid supplement, and each 100 mL contains T. cordifolia extract (200 mg), P. nigrum extract (2.5 mg), and thimerosol (0.05 mg) as active ingredients. Subjects were instructed to take 0.4 mL of the CelWel supplement 4-6 times daily, 5 minutes before eating or drinking. After 14 days of treatment, the efficacy and safety were evaluated.

Primary Outcomes

The primary outcomes were improvements in chronic fatigue and other symptoms associated with Long COVID patients as measured by fatigue severity scale questionnaires (FSSQ) [19] and a modified version of the COVID-19 Yorkshire Rehabilitation Screening (C19-YRS) checklist [20]. Fatigue severity was determined using self-administered FSSQ questionnaires (9 items). Each item was scored on a 7-point scale, with 1 representing strongly disagree and 7 representing strongly agree. Higher FSS scores for each item indicate more severe fatigue, and if an FSSQ score ≥ 4 indicates clinically significant fatigue [19]. The final score was the average of the 9 items. ProLonged COVID symptoms were assessed using a modified C19-YRS tool consisting of 18 items rated on an 11-point rating scale as 3=mild symptom, 3-5=moderate symptom, and 6-10=severe symptom. The C19-YRS is well validated tool that correlates well with exercise tolerance, lower limb strength and mobility, anxiety, depression and HR-QoL [8]. The C19-YRS checklist analyzed symptoms related to body functions and structures (12 items), activity limitations (5 items), and personal factors (1 item). After 14 days of treatment, the FSSQ and C19-YRS scores were evaluated.

Secondary Outcomes

Secondary outcomes of this study included changes in plasma levels of inflammatory cytokines (IL-2 and IFN-γ) and chemokines (CCL4 (MIP-1β). IL-2, IFN-γ, and CCL4 (MIP-1β) levels in plasma were measured using multiplex bead-based flow cytometry (IncellKINE, IncellDx, Inc.) [21]. The assay was carried out in a 96-well microplate, and samples were analysed using a CytoFlex LX 3-laser flow cytometer (Beckman Coulter Life Sciences, USA) and Kaluza Analysis Software (Beckman-Coulter, Miami, FL). Changes in laboratory parameters, such as the complete blood count (CBC), ESR, renal function, and liver function parameters from baseline, were evaluated as part of the safety study.

Statistical Analysis

A per-protocol (PP) population analysis was used to assess efficacy. Demographic data were presented as mean, percentage, and standard deviation (SD). Efficacy parameters, such as FSSQ and C19-YRS scores, were compared between baseline data and final follow-up data using the Wilcoxon signed-rank test. To compare the mean change in plasma IL-2, IFN-γ, and CCL4 (MIP-1β) levels from baseline to the final visit, the Wilcoxon signed-rank test was used. Unless otherwise stated, all hypotheses were tested at a significance level of 0.05. All statistical analysis was carried out using SSPS software version 10.0.

RESULTS

Demographic and Baseline Characteristics

A total of 15 participants were included in the study, of whom 9 (60%) were men and 6 (40%) were women. The subjects' average age was 47.0 ± 10.58 years. The mean height, weight, and BMI of the participants were 161 ± 8.16 cm, 61.60 ± 7.49 kg, and 23.71 ± 1.17 kg/m2, respectively.

Primary Outcomes

Assessment of fatigue: The fatigue severity in COVID-19 survivors was assessed using the FSSQ on days 1 and 14. The results revealed that patients consistently had higher FSSQ scores (i.e.,>4) for each item as well as a higher mean FSSQ score at day 1. The FSSQ score of each item was significantly reduced after 14 days of CelWel treatment, and the changes were statistically significant (**Table 1**). The mean FSSQ score also decreased significantly (p<0.001) from day 1 (43.30 ± 7.77) to day 14 (32.30 ± 5.35).

Table 1: FSSQ scores after 14 days of treatment with CelWel supplement.

FSSQ	Day 1, N=15 FSSQ score (mean ± SD)	Day 14, N=15 FSSQ score (mean ± SD)	Z-Score	P-value
Motivation lower when fatigued	4.80 ± 1.61	4.00 ± 0.76	-2.164	0.030*
Exercise brings on my fatigue	5.00 ± 1.07	3.93 ± 0.83	-2.739	-2.739
I am easily fatigued	4.67 ± 1.35	3.80 ± 0.86	-2.124	0.034*
Fatigue interferes with my physical functioning	4.80 ± 1.01	3.73 ± 0.80	-3.066	0.002**
Fatigue causes frequent problems for me	4.80 ± 0.78	3.33 ± 0.82	-3.115	0.002**
My fatigue prevents sustained physical functioning	4.87 ± 0.92	3.60 ± 0.83	-2.994	0.003**
Fatigue interferes with carrying certain out certain duties and responsibilities	4.93 ± 0.96	3.67 ± 0.98	-3.079	0.002**
Fatigue is among my 3 most disabling symptoms	4.60 ± 0.83	3.27 ± 0.88	-3.115	0.002**
Fatigue interferes with work, family & social life	4.87 ± 0.92	3.27 ± 1.03	-3.247	0.001***
Total fatigue scores	43.30 ± 7.77	32.30 ± 5.35	-3.295	0.001***

Assessment of Long COVID symptoms by C19-YRS checklist: The severity of Long COVID symptoms was assessed by the C19-YRS checklist at days 1 and 14. Among the Body Functions and Structures items, the intensity of the breathlessness on climbing stairs, fatigue, continence, cognition, anxiety, and depression at day 1 were the prominent symptoms compared to other symptoms, with C19-YRS reporting intensities of 5.43 ± 1.22, 6.08 ± 1.04, 6.0 ± 0.78, 5.17 ± 1.27, 6.00 ± 0.78, 5.40 ± 0.91 and 5.27 ± 1.10, respectively (**Table 2**). After 14 days of treatment, all these symptoms were present but significantly reduced. Improvements in fatigue, continence, cognition, anxiety, and depression symptoms were found to be statistically significant. Among the activities, a statistically significant improvement in mobility, personal care, usual activities, and social role were observed after 14 days of treatment compared to day 1. The global health perception was also statistically signifi-

Table 2: Intensity change in Long COVID symptoms assessed by C19-YRS.

Activities	Day 1, N=15 FSSQ score (mean ± SD)	Day 14, N=15 FSSQ score (mean ± SD)	Z-Score	P-value
Body Functions and Structures				
Breathlessness at rest	4.13 ± 1.96	4.27 ± 2.02	-0.816	0.414
Breathlessness dressing	2.80 ± 1.01	2.87 ± 1.06	-1.0	0.317
Breathlessness stairs	5.43 ± 1.22	5.25 ± 1.67	-1.0	0.317
Cough/throat sensitivity/ voice	3.40 ± 1.34	3.33 ± 0.58	-0.447	0.655
Swallowing	2.50 ± 0.71	2.80 ± 2.39	na	na
Fatigue	6.08 ± 1.04	4.85 ± 1.41	-2.858	0.004**
Continence	6.00 ± 0.78	4.43 ± 1.22	-3.236	0.001***

Cognition	5.17 ± 1.27	4.25 ± 1.71	-2.373	0.018*
Pain/discomfort	1.50 ± 0.58	2.50 ± 1.69	-0.447	0.655
Anxiety	5.40 ± 0.91	3.87 ± 1.55	-3.108	0.002**
Depression	5.27 ± 1.10	3.29 ± 1.64	-3.108	0.002**
PTSD screen	1.50 ± 0.71	0.50 ± 0.71	-1.0	0.317
		Activities		
Communication	1.80 ± 1.30	1.80 ± 1.30	-1.414	0.157
Mobility	5.60 ± 1.50	5.60 ± 1.50	-3.354	0.001***
Personal care	5.14 ± 1.99	5.14 ± 1.99	-2.831	0.005**
Usual activities	5.20 ± 1.78	5.20 ± 1.78	-3.105	0.002**
Social role	5.13 ± 1.46	5.13 ± 1.46	-3.471	0.001***
		Personal Factors		
Global health	3.47 ± 1.51	2.33 ± 1.50	-2.358	0.018**

cantly reduced from 3.47 ± 1.51 (day 1) to 2.33 ± 1.50 (day 14).

Secondary Outcomes

Assessment of inflammatory cytokines and chemokines: Summarizes the plasma levels of IL-2, IFN-γ, and CCL4 (MIP-1β). Plasma levels of IL-2, IFN-γ, and CCL4 (MIP-1β) were measured on days 1 and 14. **Table 3** shows that IL-2 levels increased slightly from 4.74 ± 2.45 (day 1) to 5.01 ± 2.90 (day 14), whereas IFN-γ and CCL4 (MIP-1β) levels decreased from 37.58 ± 86.27 to 18.23 ± 22.61 and 1527.07 ± 2185.50 to 1883.81 ± 3834.16, respectively. However, the changes were not statistically significant. We calculated the post-acute sequelae of COVID-19 (PASC) disease score based on the findings using the Patterson et al. [21] proposed formula: S1=(IFN-γ+IL-2)/CCL4 (MIP-1β) with optimized threshold for S1=0.5. At day 1, all patients had a higher PASC score (>0.5), and after 14 days of treatment, there was a trend in the reduction of PASC score among the 11 participants, but 4 patients showed a reversal of the trend in PASC score (**Table 4**).

Safety: The safety parameters included CBC, ESR, and liver and kidney function parameters that were evaluated before and after 14 days of treatment. On day 14, analysis of safety parameters showed no significant differences compared to day 1, and all parameters were within the normal range (**Tables 5 and 6**).

Table 3: Plasma levels of cytokines/chemokines before and after treatment

Cytokine/ Chemokine	Day 1, N=15 Concentration (mean ± SD)	Day 1, N=15 Concentration (mean ± SD)	P-value
IL-2	4.74 ± 2.45	5.01 ± 2.90	0.865
IFN-γ	37.58 ± 86.27	18.23 ± 22.61	0.191
CCL4 (MIP-1β)	1883.81 ± 3834.16	1527.07 ± 2185.50	0.955

There were no adverse or serious reactions reported during the treatment period.

Table 4: PASC Score among the participants before and after treatment

Patients	PASC Score (Day 1)	PASC Score (Day 14)	Score diff.
P01	8.80	2.79	-6.01
P02	7.40	9.31	1.91
P03	4.31	2.77	-1.54
P04	3.82	23.63	19.81
P05	345.85	72.36	-273.49
P06	2.69	2.04	-0.66
P07	11.22	3.83	-7.38
P08	25.95	3.16	3.16
P09	11.24	48.82	48.82
P10	23.94	17.77	17.77
P11	19.50	11.09	11.09
P12	50.38	4.32	-46.06
P13	3.03	55.90	52.87
P14	29.46	2.32	-27.14
P15	18.75	16.78	-1.97

Table 5: CBC parameters and ESR before and after treatment

Cytokine/ Chemokine	Day 1, N=15 Concentration (mean ± SD	Day 14, N=15 Concentration (mean ± SD)	P-value
RBC (Million/c.mm)	3.76 ± 0.3	3.81 ± 0.27	1.0
Hb (gm/dl)	12.27 ± 1.59	12.39 ± 1.51	0.102
WBC (cells/c.mm)	8340 ± 1000.67	8373.33 ± 1230.86	0.683
Neutrophils (%)	60.47 ± 2.33	60.73 ± 1.53	0.509
Lymphocytes (%)	28.53 ± 1.6	29.73 ± 0.8	0.017
Monocytes (%)	2.0 ± 1.41	4.27 ± 1.79	0.006
Eosinophils (%)	8.67 ± 2.09	5.07 ± 1.87	0.001
Basophils (%)	0.4 ± 0.83	0.2 ± 0.41	0.527
Platelet count (lakhs)	2.32 ± 0.35	2.35 ± 0.32	0.317
ESR (mm 1st hour)	8.67 ± 4.37	4.87 ± 3.27	0.001

Table 6: Serum biochemical parameters before and after treatment

Cytokine/ Chemokine	Day 1, N=15 Concentration (mean ± SD)	Day 14, N=15 Concentration (mean ± SD)	P-value
RBS (mg/dl)	110.53 ± 14.6	107.47 ± 10.91	0.317
BUN (mg/dl)	9.07 ± 2.09	9.13 ± 2.0	0.785
Sr. uric acid (mg/dl)	2.47 ± 1.06	2.47 ± 0.99	1.0
Sr. creatinine (mg/dl)	0.72 ± 0.12	0.72 ± 0.11	1.0
Sr. albumin (gm%)	3.71 ± 0.46	3.68 ± 0.51	1.0
Bilirubin (mg/dl)	0.63 ± 0.13	0.62 ± 0.14	0.317
ALT (IU/L)	39.53 ± 9.53	38.07 ± 8.46	0.012
AST (IU/L)	37.2 ± 11.07	36.47 ± 9.85	0.235

DISCUSSION

Long COVID syndrome (the post-acute sequelae of SARS-CoV2 infection) is a growing public health problem. There is increasing evidence that a significant proportion of COVID-19 survivors develop a chronic and debilitating set of multisystem signs and symptoms [22]. It has a negative impact on society, the economy, and patients' quality of life, physical functioning, work participation, care dependency, and activities of daily living [23]. Long COVID has been associated with over 60 physical and psychological symptoms [24]. Profound fatigue is one of the most commonly reported symptoms in post-acute COVID-19 patients, with a prevalence of 52% within 3 weeks of hospital discharge [25]. The persistence of fatigue following an acute COVID infection is also associated with episodes of overwhelming exhaustion, depressive symptoms, poor sleep quality, memory impairment and low energy levels, all of which influence quality of life [26]. There is currently no specific pharmacotherapy available for the treatment of Long COVID. Recently, efforts have been made to understand the cause, epidemiological burden, and potential biomarkers of Long COVID [27].

This study aimed to evaluate the efficacy of CelWel in improving Long COVID symptoms, particularly persistent fatigue in COVID-19 survivors. The findings revealed that all patients experienced clinically significant fatigue prior to CelWel treatment, as evidenced by a higher FSSQ score (≥ 4) for each question as well as an overall FSSQ score (>40). After 14 days of treatment, CelWel significantly reduced FSSQ scores. The C19-YRS test was also used in this study to assess the effect of CelWel on post-COVID symptoms. Results showed that CelWel significantly improved symptoms related to the body's functions and structures, including fatigue and limitations in activities. Therefore, CelWel was found to be an effective health supplement to ameliorate Long COVID symptoms, including persistent fatigue in COVID-19 survivors. However, its exact mechanism for reducing persistent fatigue and other Long COVID symptoms is unknown.

It has recently been suggested that an ongoing low-grade pro-inflammatory response may be linked to the development of post-COVID symptoms [28]. As mentioned in the introduction, Long COVID and ME/CFS share some clinical manifestations. ME/CFS pathophysiology has been linked to immune function dysregulation, hyper inflammation, oxidative stress, and autoimmunity [29]. Furthermore, ME/CFS-associated post-infectious fatigue syndrome has been linked to other acute viral and bacterial infections such as severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), Epstein-Barr virus (EBV), human parvovirus (HPV)-B19, and Q fever [23,30]. Moreover, neuro immunological involvement is a crucial precondition for Long fatigue [31,32]. IFN-α and other cytokines of the innate immune response have been shown to promote behavioural changes in medically ill patients, including fatigue-related symptoms, by acting on the basal ganglia nuclei [33]. Evidence from studies with neuro immunological disorders suggests strong connections between structural brain abnormalities and fatigue severity, such as changes in the basal ganglia [34,35] and from to parietal white matter [36] associated with fatigue in multiple sclerosis. New structural images of the brain in patients with post-COVID syndrome revealed abnormalities in the thalamus and basal ganglia, which are associated with post-COVID fatigue [26].

With these similarities between Long COVID and ME/CFS, as well as the neuro-immunological disorders associated with fatigue, it is plausible that SARS-CoV-2 infection may promote Long COVID-19 symptoms, particularly fatigue, by inducing an inflammatory state and a dysregulated immune response. We measured plasma levels of IL-2, IFN-γ, and CCL4 (MIP-1β) to see if CelWel treatment affected patients' immunogenic profiles. CelWel had no effect on the pro-inflammatory cytokine IL-2, but it did down-regulate IFN-γ and CCL4 (MIP-1β). In addition to that, after CelWel treatment, the PASC score decreased in 11 patients, indicating a reduction of the inflammatory state by preventing the production of pro-inflammatory cytokines in patients with proLonged COVID-19. However, four patients had the opposite trend in PASC score and would have required a large or very proLonged dose of CelWel to improve their PASC score. During the study period, no patients reported adverse events or abnormal clinical findings in laboratory parameters.

The limitations of the present study are the inability to measure other inflammatory cytokines, the non-randomized, small sample size, and the fact that the study was conducted in a single center. A future study should require comparing the effects of CelWel with placebos or standard NSAIDs on a large cohort for an extended period.

CONCLUSION

The results of this study indicate that the CelWel supplement has the potential to be an effective and safe treatment for pro-Long COVID. CelWel may be able to overcome proLonged COVID symptoms by decreasing the inflammatory state of COVID survivors. However, the underlying mechanisms of action of CelWel need to be established through a large-scale trial.

AUTHOR CONTRIBUTIONS

Dr. Alben Sigamani, study design and manuscript editing; Dr. K. Sunil Naik, clinical investigation and manuscript editing; Sangeetha Sampath Kumar, manuscript preparation and literature support. Janardhana PB, performed blinded analysis of patient samples and supported statistical interpretation of values and scores.

DATA AVAILABILITY

The corresponding author can provide derived data to support the findings of this study upon request.

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