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\*Corresponding author: Chih-Cheng Lin, Professor, Department of Biotechnology and Pharmaceutical Technology, Yuanpei University of Medical Technology, Hsin-Chu, Taiwan, Tel: 886-935298400; E-mail: [lcc@mail.ypu.edu.tw](mailto:lcc@mail.ypu.edu.tw)

ORCID: <https://orcid.org/0000-0003-1842-666X>

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## Research Article

# Effects of a nutraceutical combining green tea extract, vitamin C, D, and zinc in patients with post-COVID conditions

Chian-Yi Liu<sup>1</sup>, Chih-Yu Hsieh<sup>2</sup>, Shih-Heng Chang<sup>3</sup>, Shu-Chuan Wang<sup>3</sup> and Chih-Cheng Lin<sup>2,4\*</sup>

<sup>1</sup>Nature Nurture Farmacy, Chehalis, WA, USA

<sup>2</sup>Department of Biotechnology and Pharmaceutical Technology, Yuanpei University of Medical Technology, Hsin-Chu, Taiwan

<sup>3</sup>Lohasiinfra Holistic Medical Clinic, Taipei, Taiwan

<sup>4</sup>Department of Food Science, Yuanpei University of Medical Technology, Taiwan

## Abstract

Tea and ascorbic acid have antioxidative and anti-inflammatory effects, and vitamin D and zinc have immunomodulatory effects. This study investigated the effect of a nutraceutical prescription combining these four nutrients on the blood biochemical markers and the modified C19-YRS questionnaires in patients who had recovered from COVID-19 disease for four months. Analysis of six markers associated with long COVID symptoms, i.e., INR, d-dimer, hs-CRP, NT-ProBNP, IL-6 and IgA, indicated that the nutraceutical could positively affect the values, especially for the inflammatory markers. The physician's clinical observation and questionnaire analysis reported significant improvements in post-exertional malaise, sleep quality and overall health of the patients. These results suggested that formulated nutraceutical could not only reduces or eliminates the symptom of long COVID, but potentially also prevents reinfection.

## Introduction

Teas have attracted much attention for their potential use to prevent and treat diseases. Studies have shown that tea has prominent antiviral effects and can treat various diseases by regulating multiple pathways [1]. Tea can hinder the process of viral infection and inhibit RNA replication of the virus. In coronavirus disease 2019 (COVID-19), tea can alleviate its sequelae by reducing cytokine levels through multiple signaling pathways and by modulating the immune system [2]. Catechins are the main active ingredient in green tea. They can act synergistically with other components to help prevent and treat COVID-19 and its sequelae [2,3]. Our previous research has demonstrated that tea extracts rich in catechins can function as natural antioxidants and anti-inflammatory agents to prevent cytokine storms in the presence of superimposed

LPS-mediated inflammation [4,5]. By scavenging free radicals, green tea exerts robust prophylactic effects and modulates the expression of cytokines such as monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- $\alpha$ ) [5].

Certain vitamins and minerals have antioxidant and immunomodulatory functions that could aid the immune response against viral infection [6]. Vitamin C, or ascorbic acid, plays a major role as a cofactor and modulator in various immune system pathways. Some evidence supports the therapeutic effect of vitamin C in patients with COVID-19 [7]. Vitamin D has been associated with the prevention of influenza and COVID-19 infections, as well as the reduction in COVID-19 severity [8]. Vitamins C and D have been extensively used for immune support at various levels, including prevention



of infection, alleviation of symptoms during infections, and attenuating disease sequelae (such as long COVID) [9]. Zinc can suppress the expression of ACE2, reduce viral replication, and boost immunity, rendering it a potentially useful prophylactic and therapeutic element in COVID-19 patients [10]. Given their prominent activity in preventing infection and supporting immunity during the COVID-19 pandemic [11,12], vitamins C, D and zinc are integral parts of the immune system that exhibit synergistic functions in maintaining biological barrier integrity and cellular functions [13].

The COVID-19 disease displays multifaceted symptoms due to various organ manifestations. Its detrimental effects on health are not limited to the acute period but also extend to the sequelae. Post-COVID conditions, also known as long COVID, are characterized by a range of symptoms, including fatigue but also cardiac, pulmonary, neuropsychiatric, and other organ system manifestations [14]. Although epidemiological and experimental evidence suggests that green tea or vitamin C, D, and zinc may potentially alleviate symptoms and reduce over-inflammation, there is limited clinical evidence on Post COVID conditions and related laboratory findings. Therefore, the study aims to investigate the clinical efficacy of a nutraceutical formulated with green tea extract, vitamin C, D, and zinc in patients with post-COVID conditions.

## Materials and methods

### Nutraceutical formulation

The Indian green tea extract used in this study was obtained from HealthTake Corporation (Taiwan). The total phenol content is 924.4 mg GAE/g and the total catechin is 812.2 mg. Moreover, Epigallocatechin-3-Gallate (EGCG), the most significant catechin in green tea, was present at a concentration of 540.8 mg/g. Ascorbic acid (92.9%) was purchased from One Innovation LABS (USA), while vitamin D<sub>3</sub> (0.25%) and zinc yeast (54.8 mg zinc/g) were purchased from Material World Industrial Co. (Taiwan). The nutraceutical, which contained 150 mg of ascorbic acid, 75 mg of green tea extract, 4 mg of zinc, and 4.17 mcg (417 IU) of vitamin D<sub>3</sub>, was formulated by Lohasiinfra Holistic Medical Clinic (Taipei, Taiwan) and prepared into 454 mg capsules. The participants were instructed to take three capsules twice daily.

### Human Study

Participants who recovered from COVID-19 were recruited from Lohasiinfra Holistic Medical Clinic through advertisements. Inclusion criteria were: age 18 or older, non-smoker, no history of cardiovascular disease or diabetes, not taking any medications and not pregnant. Participants were diagnosed and instructed by a medical doctor to refrain from consuming alcohol or engaging in strenuous physical activity prior to blood sampling. This study was carried out in accordance with the recommendations of the Yuanpei University of Medical Technology Human Research Ethics Committee with written informed consent obtained from all participants. The protocol was approved by the IRB (YPU-IRB-1110719). The participants received the nutraceutical treatments for four months, and blood tests were analyzed

for INR, d-dimer, hs-CRP, NT-ProBNP, IgA, and IL-6 before and after treatment. The modified C19-YRS questionnaires (COVID-19 Yorkshire Rehabilitation Scales) were completed monthly during the treatment period [15].

### Statistics

Unless stated otherwise, all data are presented as means  $\pm$  Standard Deviation (SD). Our analysis used the multiple paired *t*-test to compare pre- and post-treatment data within the same patient. This method allowed us to assess the changes in individual scores before and after the nutraceutical treatment. We employed a two-way ANOVA with Dunnett's multiple comparison tests to further verify these findings and calculate differences between groups. The consistency of the results obtained from both methods strengthens our conclusion about the significant differences observed in specific items. All statistical analyses were performed using GraphPad Prism 9 version 9.5.1 (GraphPad Software, Boston, MA, USA).

## Results and discussion

Due to the specific medical treatment for confirmed patients in Taiwan during the COVID-19 epidemic, it was not easy to recruit patients with long COVID, and the high variability of this group of patients resulted in a small number of subjects in this study. This study is actually more of a multiple case report study. The participants of this study were 10 patients (four males and six females) who had previously been diagnosed with COVID-19 and were in the process of recovery, as determined by the physician. They were prescribed a four-month course of nutraceutical therapy designed for this study, and the blood was analyzed before and after the treatment period. Additionally, they were asked to complete a quality-of-life questionnaire on a monthly basis. One participant, who was the youngest among all at 29 years old, withdrew from the study after three months due to feeling fully recovered with no symptoms. Therefore, this study includes a case series of nine patients (mean age  $49.7 \pm 17.2$  years; male: female = 5:4) who received oral nutraceutical treatment for persistent symptoms following COVID-19.

The sequelae of COVID-19 are mainly in the respiratory, cardiovascular and other systems related to chronic inflammation. The main respiratory sequelae of COVID-19 are pulmonary embolism or fibrosis [16] resulting from abnormal immune mechanisms and cytokine storms [17,18]. Six blood biochemical markers were selected to investigate the effects of combining green tea extract, vitamins C, D, and zinc on patients with long COVID-19. Both d-dimer elevations and international normalized ratio (INR) prolongation are useful in identifying COVID-19-associated coagulopathy and COVID-19 severity [19]. The INR results in Table 1 showed that all data are within the normal range (0.85 - 1.1) and no significant change was found. However, we found that almost all patients who originally had higher d-dimer were reduced to normal or close to normal values (less than 0.5 U<sub>g</sub>/mL FEU) after four months of nutraceutical treatment except for case No. 5. Elevations of d-dimer, indicating a pro-thrombotic state and coagulopathy, predict adverse symptoms in COVID-19 [19]. This nutraceutical



formulation could be effective in improving symptoms associated with thrombosis and coagulopathy. Inflammatory markers like high sensitivity C-reactive protein (hs-CRP), N-terminal pro-brain natriuretic peptide (NT-proBNP), and interleukin 6 (IL-6) may be associated with post-COVID conditions [20]. As shown in Table 2, except for case No. 3 who was having a common cold on post-test, those who had exceeded the reference range were reduced back to normal or close to normal values after treatment. The effect was most pronounced in case No. 4, whose hs-CRP value decreased from 2.511 to 0.056 mg/dL and IL-6 decreased from 102.6 to 12.7 pg/mL. The IgA level associated with immunity and persistent symptoms [21, 22] also improved from 92 to 121 mg/dL.

All patients were surveyed using the modified C19-YRS questionnaires to evaluate symptoms of long COVID. We used a multiple-paired sample t-test to evaluate the scores' changes in all items, as depicted in the volcano plots in Figure 1. Although most questionnaire items did not demonstrate statistically significant differences in response scores, items on post-exertional malaise (10a) and sleep quality (8a) showed potential positive results. A two-way ANOVA was performed to verify these findings further, consistently demonstrating significant differences between these items. The congruence of the results across both tests strongly supports the conclusion that there is a significant difference between items 8a and 10a. Figure 2 highlights the significant improvements in post-exertional malaise and sleep quality after one to two months of nutraceutical treatment. As for overall health in the survey, where a higher score represented greater satisfaction (with

**Table 1:** Changes in INR and d-dimer values before and after taking the nutraceutical for four months.

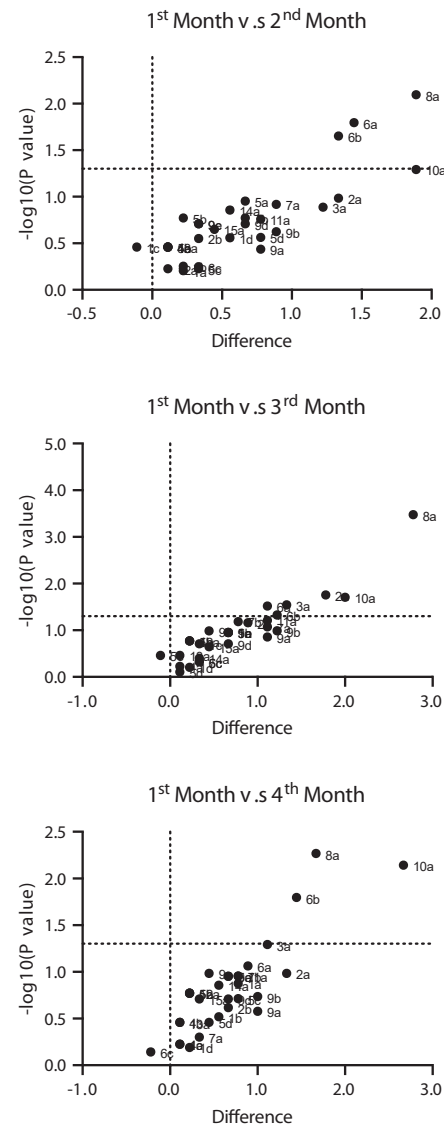
Case No.	INR		d-dimer	
	before	after	before	after
1	0.99	1.04	0.359	0.441
2	0.94	0.86	0.779	0.682
3	0.97	0.98	0.582	0.31
4	0.96	0.88	0.38	0.324
5	0.96	1	0.891	0.943
6	0.86	0.92	2.056	0.48
7	0.97	0.97	<0.215	<0.215
8	0.93	0.93	<0.215	<0.215
9	1.03	0.98	0.234	0.415

The normal range of INR is between 0.85 and 1.10, and the d-dimer is less than 0.5 Ug/mL FEU.

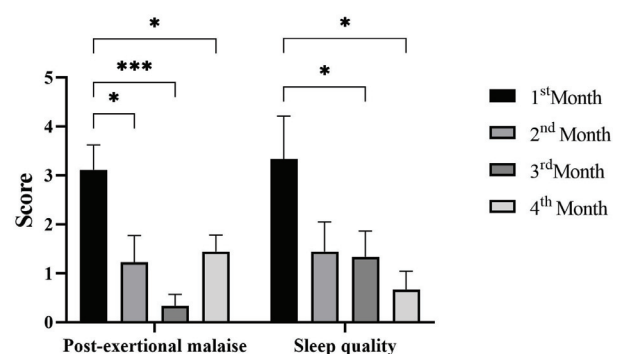
**Table 2:** Changes in inflammation-related markers before and after taking the nutraceutical for four months.

Case No.	hs-CRP		NT-ProBNP		Interlukin-6	
	before	after	before	after	before	after
1	0.126	0.087	51.8	29.7	<1.5	<1.5
2	0.022	0.023	25.2	5.26	<1.5	<1.5
3	0.04	0.486	8.53	33	<1.5	4.2
4	2.511	0.056	115	66.2	102.6	12.7
5	0.1	0.044	33	21.8	<1.5	1.6
6	0.21	0.193	59.2	25.8	5.2	5.5
7	0.13	0.195	44.2	23.7	<1.5	2.1
8	0.021	0.023	20.6	24.7	<1.5	<1.5
9	0.053	0.042	65	131	2.8	2.6

The normal range of hs-CRP is less than 0.1 mg/dL, NT-ProBNP is less than 125 pg/mL, and Interlukin-6 is less than 7 pg/mL.



**Figure 1:** Volcano plots of multiple paired sample t-tests for pre- and post-treatment between the first and other months. The x-axis displays the log2-fold change in expression between pre-and post-treatment conditions, while the y-axis shows the negative log10-transformed p-values for each comparison. The data show 8a and 10a have a significant difference in each 1st month compared with others ( $p < 0.05$ ).



**Figure 2:** Changes in questionnaire score of post-exertional malaise and sleep quality over the four-month treatment period. The post-exertional malaise item is defined as a crash or relapse that occurs hours or days after physical, cognitive, or emotional exertion. On the other hand, the sleep quality item refers to sleep problems such as difficulty falling asleep, staying asleep, or oversleeping. Lower scores denote better outcomes. The symbols \* and \*\*\* represent statistically significant differences at the level of  $p < 0.05$  and  $p < 0.001$ , respectively.



a maximum score of 10), the nutraceutical was observed to enhance their health from an average score of 4.86 to 6 after one month of use, 7.57 after two months, and 8.43 after four months.

Overall, the case reports by the physician indicated that most patients who received the nutraceutical had positive outcomes in terms of their symptoms, blood biochemical markers, and questionnaire responses. Case No. 4 particularly benefited from the treatment and although case No. 3 experienced an increase in hs-CRP and IL-6 (Table 2) due to upper respiratory tract infection during the fourth month, the results of all other indicators and questionnaires related to long COVID showed improved results. Examination of the questionnaire revealed that cases No. 5 and 9 did not show improvement in their quality of life and overall health. Case No. 5 was diagnosed by a physician as having a poor quality of life due to unresolved tinnitus as a probable adverse reaction to COVID vaccination. As for case No. 9, the physician judged the case as having improved symptoms related to long COVID, and all blood biochemical markers were within the normal range. However, despite these positive indicators, the patient's quality of life and overall health score continued to decline. Upon investigation, it was found that this was due to strain from caregiving responsibilities.

During the four-month treatment period, all patients were free of COVID re-infection. When devising treatment strategies for COVID-19 or other respiratory infections, it is important to take into account the influence of nutrients on viral replication and internalization, as well as methods to mitigate the effects of cytokine storms on the body. With this in mind, the combination of green tea extract and nutrients utilized in this study holds promise for mitigating the risk of viral reinfection and prolonged inflammation in its sequelae. Catechins from green tea extract have been shown for their ability to inhibit influenza virus [2,23] and COVID-19 virus replication [24,25]. Tea catechins and ascorbic acid possess both antiviral and antioxidant activities for severe influenza-associated complications [26]. Ascorbic acid with reducing ability inhibited the proliferation of the influenza virus [27] and inhibited inflammation caused by COVID-19 viruses [28]. Our nutraceutical is designed with green tea extract and zinc yeast as the main ingredients, synergized with vitamins C and D to support the immune system. Green tea extract and vitamin C have anti-viral and anti-inflammatory effects, and vitamin D and zinc have immunomodulatory effects. There is evidence to suggest that green tea extract, with its major constituent EGCG, holds great potential in the prevention and treatment of COVID-19 and its sequelae [29]. EGCG has also been shown to act as a zinc ionophore and could improve the cellular utilization of zinc [30]. Symptoms of long COVID may resemble those seen in zinc and vitamin D deficiency [31].

## Conclusion

This study showed that oral administration of green tea extract in combination with vitamin C, D and zinc may reduce or eliminate inflammation and symptoms related to long COVID, as well as improve quality of life. Although more robust

evidence is needed, the fundamental role of these nutrients on immune function is evident. This nutraceutical appears to be a safe and effective tool to add as part of the long COVID treatment strategy. In addition, it can be used to improve immunity and prevent COVID re-infection and potentially other infections.

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