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Efficacy of a Polyphenolic, Standardized Green Tea Extract for the Treatment of COVID-19 Syndrome: A Proof-of-Principle Study

Saverio Bettuzzi *, Luigi Gabba and Simona Cataldo

Istituto Nazionale Biostrutture e Biosistemi (INBB), 00136 Rome, Italy; Ggabba@gmail.com (L.G.); cataldo.simona@gmail.com (S.C.)

* Correspondence: saverio.bettuzzi@unipr.it

Abstract: The lack of therapies for moderate COVID-19 syndrome prompted us to use a standardized polyphenolic green tea extract rich in catechins during the lockdown due to the pandemic in Italy (Autumn 2020). Catechins are powerful antioxidant, anti-inflammatory and antiviral agents that are safe for human use. While awaiting hospitalization, 10 swab-positive patients, symptomatic for SARS-COV-2, were treated for 15 days at home with two sessions of inhalation plus three capsules per day (total catechins: 840 mg; total EGCG: 595 mg). All patients recovered fully and had no symptoms at a median of 9 days, with a range of 7–15 days. Seven switched to a negative SARS-COV-2 nasopharyngeal swab test at a median of 9 days, with a range of 6–13 days. Among the 3 patients still swab-positive, one had a strong decrease of infection down to a "very low" SARS-COV-2 nucleic acid load at 5 days. All patients exited quarantine at the end of therapy because they were free of symptoms. Inflammation markers α-1 anti-trypsin, C-reactive protein and eosinophils had significantly decreased. The IL-6 and erythrocyte sedimentation rate decreased in 7 out of 10 patients. To the best of our knowledge, this is the first report of the efficacy of green tea catechin against COVID-19 syndrome. These results may open new perspectives in the fight against the disease.

Keywords: antioxidant; antiviral; catechin; COVID-19; cytokine storm; EGCG; green tea; IL-6; inflammation; polyphenols; SARS-COV-2; theaphenon E

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1. Introduction

The COVID-19 pandemic is worsening globally [1]. No proven therapies are available for mild or moderate cases other than supportive care. Attempts to find an active therapy are being pursued in many countries. Several of these trials have been off-label, under compassionate care, and many lack appropriate controls.

Green tea is a good source of catechins, which are polyphenolic compounds [2]. In a green tea infusion, the most abundant catechin is epigallocatechin-3-gallate, EGCG (~60%), followed by epigallocatechin, EGC (~20%), epicatechin-3-gallate, ECG (~14%) and epicatechin, EC (~6%) [3,4]. Green tea catechins (GTC) exhibit powerful antioxidant, antiviral and anti-inflammatory properties [5]. They also bind proteins [6] and intercalate into plasma membranes, affecting lipid rafts, as extensively reviewed by Patra et al. [7]. EGCG binds to 67-kDa laminin receptor (LamR: OMIM, 150370), which use rafts as a platform [8,9].

Patients infected with SARS-COV-2 who develop pneumonia often die of a severe acute respiratory syndrome induced by a cytokine-mediated inflammatory response ("cytokine storm") [10] that is very similar to SARS caused by SARS-COV. Several studies have identified IL-6 levels as prognostic for severe disease in COVID-19 positive patients at presentation [11]. SARS is very similar to acute respiratory distress syndrome (ARDS). ARDS has been studied extensively and exhibits about the same death rate of 20% in patients with intubation [12,13]. GTC have demonstrated strong positive effects on the innate immune

system in animal models of ARDS, including lethal influenza [14], lipopolysaccharide injection [6,15,16], seawater aspiration [17], zymosan injection [18], carrageenan injection [19], obliterative bronchiolitis [20] and fluorescein isothiocyanate instillation [21]. In these studies, significantly more animals treated with either GTC or EGCG alone survived than did the control animals. Morphological lung damage was far less, and several parameters of inflammatory damage were lessened. GTC or EGCG inhibit neutrophil [22] or monocyte [23] chemotaxis in the air pouch animal model by greater than 90%. In vitro, GTC or EGCG reduce cytokine production from activated immune cells such as macrophages [24–26] and neutrophils [21,27–29].

In addition to anti-inflammatory properties, the antiviral action of catechins has been well known since the 1990s, as reviewed by Xu et al. in 2017 [30]. Recently, Ohgitani et al. demonstrated 3 logs of inhibition of infectivity of SARS-COV-2 when the inoculant is exposed to 500 μM EGCG, and a greater effect from theasinensin A, the product of EGCG dimerization [31,32].

GTC as Polyphenon 60, an extract by Mitsui Norin whose total catechin and EGCG content is lower than ThE, was safely administered through inhalation to a total of 48 elderly, disabled and hospitalized patients with methicillin-resistant Staphylococcus aureus infection in two independent studies [33,34]. There were no adverse effects from a week of nebulizer treatment three times per day, for a total of 10 mg/day of EGCG [33,34].

Recently, an in silico molecular docking study showed that EGCG, GCG, and CG (catechin-gallate) are among the molecules with the highest binding affinity to the active site of the SARS-COV-2 3CLpro protease and, therefore, possess the theoretical ability to inhibit its activity [35]. The authors urged clinical trials based on these molecules. A review of the in silico studies of SARS-COV-2 proteins showed that EGCG and GTC scored high in several such studies [36]. Menegazzi et al. studied green tea extract and recommended it be tried in COVID-19 treatment [37].

Green tea extracts (GTE) rich in catechins are safe for human use [38,39]. In the past, we used GTE in both animal studies [40] and clinical trials [41] with only mild side effects. Our previous experience prompted us to investigate a standardized GTE, ThE [42] in a real clinical setting.

To this end, we treated 10 COVID-19 patients, symptomatic and with positive nasopharyngeal swab tests for the SARS-COV-2 virus, for 15 days while they were awaiting hospitalization and receiving standard of care therapy at home. The study was performed by family doctors in accordance with the rules in force during the lockdown in the Emilia-Romagna region of Italy. We report here the results of our pilot, compassionate, proof-of-principle study. To the best of our knowledge, this is the first report documenting the efficacy of a highly purified GTE against COVID-19 syndrome.

2. Materials and Methods

ThE is an improved version of Polyphenon E (PoE), which has been used orally in 34 clinical trials in the U.S. [43]. PoE was approved by the USFDA in 2006 as an anti-viral for Veregen, a topical drug for external genital warts caused by the papilloma virus [44]. The same team that developed PoE when it was manufactured by Mitsui Norin Co., Ltd., Shizuoka 426-0133 Japan, developed ThE as a substitute for PoE when Mitsui Norin decided to stop making PoE for corporate reasons [42]. ThE is composed of GTC and EGCG (for this batch, total catechins were 85–95% and total EGCG was 65–70%), and is virtually free of caffeine (< 0.5%). It is currently in use in our laboratory for experiments in animal models [40] and freely sold on the market as an oral supplement in Japan. The chemical composition of the ThE powder batch for inhalation and the ThE capsules used in this trial are shown in Appendices A and B, respectively. ThE was kindly provided for the study for free by Yukihiko Hara (Tea Solutions, Tokyo, Japan), the chief developer of both ThE and PoE, who retired as Director of the Food Research Institute of Mitsui Norin in 2008.

Before testing the efficacy of ThE on COVID-19 patients, we tested the safety and tolerability of inhalation and capsule administration in two healthy volunteers (S.B. and his

wife, G.M.). Both were negative for COVID-19, but affected by symptoms of influenza. The treatment was well tolerated and the flu symptoms disappeared rapidly. One volunteer with COVID-19 symptoms was treated at home by the study doctor SC early in the pandemic. Later, S.C. was badly exposed to the virus when her personal protective equipment failed while treating a violently-ill COVID-19 patient in the ward. She used the treatment as described here, had no adverse effects, and never got sick.

We recruited 9 more patients in the fall surge, all symptomatic and with positive nasopharyngeal swabs for COVID-19, while they were awaiting hospitalization. The hospitals were crowded, and triage kept those with mild or moderate symptoms at home; they were visited and treated there by family doctors, including SC.

2.1. Description of the Compassionate Clinical Trial

Inclusion criteria: symptomatic adults over 18 years of age with a positive nasopharyngeal swab for COVID-19. Symptoms: fever > 38 °C, loss of taste, smell, and respiratory or gastrointestinal symptoms. Exclusion criteria: none.

Primary objective: patient recovery. Secondary objectives: reduction of markers of inflammation and time until negative SARS-COV-2 swab.

According to the rules in force during the lockdown, patients had to be symptomatic to receive a nasopharyngeal swab test for SARS-COV-2. Following the positive result, they were visited and treated by their family doctors at home (L.G. and S.C.) within a few days (median 5 days) of their symptoms worsening.

All ten patients received, discussed, and signed the informed consent form with S.C. during her first visit (T0). She took a blood sample and initiated the treatment, staying with the subject for one-half hour following the nebulizer treatment to ensure that there were no negative reactions. Table 1 shows their clinical condition at the doctor's first visit, when the first nasopharyngeal swab test was performed and before ThE administration. Table 1 also shows patient demographics, co-morbidities and other medications. Only one patient received anti-viral medication, hydroxychloroquine (HCQ) for 5 days, during the study trial.

On recruitment day (T0), the doctor (S.C.) took the first blood sample. Blood samples were sent to different laboratories in the territory for standard analyses. Later, when patients' symptoms were gone (a median of 9 days later with a range of 7–15), S.C. took a second blood sample (T1). Analytes included: blood count, hemoglobin, interleukin 6 (IL-6), and other indicators, as shown in Table 2. At recruitment (T0), ThE therapy began (capsules plus inhalation) and lasted for 15 days. Patients received a second nasopharyngeal swab for SARS-COV-2 independently from the trial, as required under the rules in force during the lockdown, to permit them to leave quarantine.

Patients were monitored closely for adverse events; there were no adverse effects, and all patients completed the full treatment. They were then followed for a month and all were free of symptoms or signs, recovering completely, except Patients 1 and 2, who felt fatigue (see Table 1). These two were also the oldest patients, at 74 and 73 years respectively, and had co-morbidities and, therefore, were regarded as very high risk.

2.2. Dose and Method of Administration

1. Nebulization of 5 mL of a 0.3% ThE solution, made fresh daily in phosphate saline buffer at pH 5.8, with a breathing mask, twice a day for up to 15 days (total catechins: 27 mg/day; EGCG: 19 mg/day). For the ThE powder composition, see Appendix A.

2. Oral administration: 3 cps/day for up to 15 days. Each capsule contained 300 mg of ThE (813 mg total catechins/day and 576 mg EGCG/day). For ThE capsules composition, see Appendix B;

This total daily dose of 595 mg was considered safe because it was "below 800 mg EGCG", as suggested by the European Food Safety Agency [38]. Considering the average content of catechin found in brewed green tea, this total daily dose corresponded to about 850 mL of a tea infusion [38].

Table 1. Patients of the trial at the time of the doctor's first visit, with demographics, co-morbidities, and other medications including duration and dosage.

Patient number	1	2	3	4	5	6	7	8	9	10
Symptoms:										
Difficulty breathing	yes	yes		yes					yes	
Cough	yes	yes		yes	yes	yes	yes	yes	yes	yes
Tiredness 30 d after	yes	yes								
No sense of smell						yes		yes		
No sense of taste						yes		yes		
Signs:										
Fever (Temperature)	>38 °C	>38 °C		>38 °C	>38 °C	>38 °C	>38 °C	>38 °C	>38 °C	>38 °C
Pneumonia CT score	10–15%	10–15%		10–15%					40%	
Hospitalized	no	no	no	no	no	no	no	no	yes	no
Patient demographics:										
Age	74	73	50	53	47	27	55	28	66	38
Gender	M	F	M	M	F	F	F	F	F	F
Comorbidities	yes	yes							yes	
Other treatments:										
Steroids	yes 10 days	yes 10 days								
Azithromycin 500 mg	Yes 10 days	yes 10 days		yes 4 days					yes 4 days	
Low Mol Weight Heparin	yes 10 days	yes 10 days		yes 10 days					yes 10 days	
Hydroxychloroquine 400 mg once, plus 200 mg later									yes 5 days	
Methylprednisolone 32 mg, one shot				yes 2 days						

Table 2. Laboratory data at recruitment (T0, first blood withdrawal, beginning of ThE treatment) and at second blood withdrawal (T1, full recovery of patients).

Patient Number	Blood Withdr.	White	Hb	PLT	Neutro	Limph	Mono	Eosin	ESR	AAT	CRP	IL-6	Swab Result	Time T0–2nd Swab	Time T0–T1
		$\times 10^3/\mu L$	gr/dL	$\times 10^3/\mu L$	%	%	%	%	mm	mg/dL	mg/L	pg/L			
1	T0	7.35	13.5	242	82.4	10.6	6.9	0	55	165	5.65	26.03	pos		
	T1	6.77	13.3	179	86.1	9.4	4.1	0.2	18	139	0.41	1.5	NEG	6	9
2	T0	6.06	13.7	295	62.4	27.1	9.2	0.8	71	235	27.9	55.5	pos		
	T1	9.27	14.3	325	83.7	11.5	4	0.3	20	158	0.76	5.21	NEG	6	9
3	T0	4.99	15.3	315	71.6	22.4	5.6	0	29	114	0.4	6	pos		
	T1	5.03	14.3	254	51.4	38.4	7.8	1.4	16	97	0.61	24.19	POS	6	9
4	T0	8.33	14.9	269	73.8	19.6	4.7	1.7	38	170	23	69.08	pos		
	T1	8.65	15.5	460	59.6	28.4	7.6	3.7	55	155	6.33	9.9	NEG	10	7
5	T0	4.7	10.9	275	49.4	35.5	12.6	2.3	74	166	15	8.25	pos		

Table 2. *Cont.*

Patient Number	Blood Withdr.	White	Hb	PLT	Neutro	Limph	Mono	Eosin	ESR	AAT	CRP	IL-6	Swab Result	Time T0–2nd Swab	Time T0–T1
		$\times 10^3/\mu L$	gr/dL	$\times 10^3/\mu L$	%	%	%	%	mm	mg/dL	mg/L	pg/L			
	T1	9.3	10.4	395	54.7	33.9	8.6	2.6	38	114	0.61	2.87	NEG	13	15
6	T0	6.63	13.8	197	41.8	48	9.4	0.3	22	207	3.72	7.82	pos		
	T1	5.91	13.4	261	59.9	33.2	4.5	1.8	19	230	6.54	4.34	NEG	10	12
7	T0	7.27	13.1	205	58.8	32.3	8	0.8	6	120	3.72	184.95	pos		
	T1	8.13	13.6	265	63.3	29.4	6.2	0.7	10	116	0.42	3	POS	(*) 4 (*)	7
8	T0	7.12	12.3	277	61.5	25.6	9.6	2.7	6	127	1	12.75	pos		
	T1	7.41	11.7	258	61.9	23.9	10.5	3.2	2	81	0.39	12.75	NEG	6	9
9	T0	4.16	14.2	142	59.2	35.3	5	0	78	254	18	124.42	pos		
	T1	7.63	12.5	ND	66.1	23.3	7.6	2.5	ND	ND	13.3	14.76	NEG	10	8
10	T0	5.13	13.4	192	61.2	30.6	7.6	0.4	34	151	0.53	17.37	pos		
	T1	6.26	13	210	65.3	28.1	5.9	0.5	21	136	0.29	194.05	POS	6	7
	Normal range	4–10	13.5–18.0	150–400	40–75	15–45	3–12	1–8	2–25	90–200	< 5.00	0–10			
Mean T0		6.174	13.51	240.9	62.21	28.7	7.86	0.9	41.3	170.9	9.89	51.22		8.7 (**)	9.2
Mean T1		7.414	13.28	289.667	65.2	25.95	6.68	1.69	22.11	136.22	2.97	7.26		6–13 (days)	7–15 (days)
SD T0		1.377	1.256	54.836	11.705	10.226	2.448	1.68	23.56	137.89	2.99	9.59			
SD T1		1.53	1.5	89.202	11.326	9.353	2.125	0.9	41.3	170.9	9.89	51.22			
	T-test for paired data, bilateral, 2 tails							<0.05		<0.05	<0.05				

Bolded values are outside of normal range. SWAB result: result of first nasopharyngeal swab test (before treatment) and second test (during ThE treatment): positive/negative; (*) PT 7 was positive at second test but with a very low load of virus nucleic acid. T0–2nd swab: elapsed time (days) from T0 to second swab, i.e., (**) time to first negative swab during ThE treatment for 7/10 patients; T0–T1: elapsed time from start of ThE therapy to full recovery of patients (days). Statistical analysis by paired T-test (2-tails) shows statistical significance ($p < 0.05$) for Eosin, AAT and CRP. Seven patients showed a decrease of IL-6 and ESR following treatment. Abbreviations: PT, patient; white, white blood cell count; Hb, hemoglobin; Neutro, neutrophils; Lymph, lymphocytes; Mono, mononuclear cells; Eosin, eosinophils; ESR, erythrocyte sedimentation rate; AAT, α-1 antitrypsin; CRP, C-reactive protein; IL-6, interleukin-6 cytokine; ND = no data available.

3. Results

Clinical signs and symptoms were typical for all patients, such as cough and fever > 38 °C (Table 1).

All patients received a nasopharyngeal swab test and were positive before starting the ThE therapy. They were visited by S.C. and enrolled in the study a median of 5 days following their first swab test, within a range of 3–6 days. Only PT 3 never had any clear symptoms (see comments below); nevertheless, PT 3 was swab-positive. PT 1, 2, 5 and 8 were nested in familial clusters. Family members of the clusters who refused to sign the informed consent form did not receive the ThE treatment, only standard of care therapy ($n = 4$). All 4 of them took longer until their first negative swab (<1 month) and recovery (from 2 to 6 months). These subjects may be considered as internal controls for the study.

All 10 patients recovered fully within treatment time with a median of 9 days and a range of 7–15 days. Seven out of 10 patients had a negative SARS-COV-2 swab at a median of 9 days from starting ThE therapy, with a range of 6–13 days. Only PT 3, 7 and 10 had a positive second swab at days 6, 5 and 6, respectively, but all three were free of symptoms and fully recovered a few days later, exiting quarantine at the end of ThE therapy. They did not infect anyone afterwards, including cohabiting persons. The results of laboratory tests from the blood draws are shown in Table 2.

Seven patients showed a decrease of IL-6, one did not change, and only two increased. Among these two patients was PT 3: IL-6 increased in the blood test at 9 days; the earlier test results showed no eosinophils and high ESR, both markers for inflammation due to COVID-19. These values had improved to the normal range at 9 days S.C. commented: "PT 3 is a mystery. Although swab-positive, he never had symptoms. PT 3 lives with his/her

old mother in a small flat, but she never had COVID. This patient may be a false positive case. In any event, both eosinophils and ESR decreased in response to ThE therapy. PT 3 is still healthy, without symptoms, just as though he never had the disease."

PT 7 had a high IL-6 of 185 pg/mL in the first lab test, which returned to a normal range at the second lab test 7 days later. The only other initial lab result was marginally low eosinophils. PT 7 had a very low viral load on the second swab and was declared free of disease a few days later, thus exiting quarantine.

Regarding PT 10, S.C. commented: "There is no explanation for the high IL-6 of PT 10 on day 7 (T1) or positive swab on day 6. At that time, PT 10 already had no symptoms and had recovered completely, upon exiting quarantine."

IL-6 level was the most informative in the blood tests. Seven of 10 patients had an IL-6 greater than 10 pg/mL and 3/10 had greater than 100 pg/mL. The four oldest (PT 1, 2, 7, and 9) had high IL-6 values of 26, 55, 185 and 124 pg/mL, respectively. A statistical analysis performed on the patients with age > 52 years (n = 5; PT 1, 2, 4, 7, and 9), who were at high risk as demonstrated by high IL-6 values, showed that IL-6 significantly decreased after treatment (t-test, $p < 0.03$). These high values would normally indicate that these patients were developing SARS, therefore, their rapid recovery was even more surprising.

There are many published studies on IL-6 as a prognosis for death or severe disease in COVID 19 patients. Values over 100 pg/mL have high relative risk or odds ratios for severe, critical disease or death. Del Valle et al. [45] measured cytokine values in 1484 patients immediately upon admission to Mount Sinai Health System, New York City. They determined a cutoff value of 70 pg/mL for IL-6 and tested it for prognosis of death and severity. Eight percent of patients below the cutoff had died within 30 days, vs 15% of those above, with a hazard ratio (HR) of 2.0, $p < 0.002$. For disease severity, 76% of those below the cutoff sustained O_2 saturation on room air >95%, vs. 46% below the cutoff, HR 3.4, $p < 0.0021$ (n = 1112) [45]. In this report, PT 4, 7, and 9 had IL-6 values of 69, 185, and 194 pg/mL, yet they recovered in 7, 7, and 8 days, respectively.

Considering other clinical parameters, 7/10 patients had elevated ESR and normal rates by the second test. Seven out of 10 patients had reduced eosinophils, but 4 were only marginally low; of these 7, 4 patients did not return to normal levels by the second blood test. Other lab values were normal for most patients.

Statistical analyses (paired t-test, two tails) showed a significant decrease of eosinophils, AAT, and CRP in the patients following treatment.

4. Discussion

All patients were free of symptoms and fully recovered by the time of the second blood test, which was the primary objective. Median time to recovery was 9 days (range 7–15) and 70% had a negative swab. These results were fully documented with official lab records.

PT 1, 2, 4, and 9 received standard of care drug treatment (see Table 1) for a time shorter than ThE therapy. Only one (PT 9) was treated with HCQ. The latest and largest international study for COVID-19 treatments, launched by the World Health Organization (WHO), concluded that HCQ had little or no effect on COVID-19 and, therefore, discouraged its use [46]. In a recent study on the effect of HCQ in hospitalized patients with Covid-19, Horby et al. concluded that patients in the HCQ group were less likely to be discharged from the hospital alive within 28 days as compared to the controls [47].

Methylprednisolone was given for a very short time (2 days) to PT 4. Steroids were given to PT 1 and PT 2. It is well known that these treatments against inflammation cause a delay of the first negative swab. Azithromycin is usually given only due to its protective effect against sepsis. Low molecular weight heparin is known to be effective only for the prevention of thrombus and embolism, but does not affect time to first negative swab or recovery. In the final analysis, the other treatments administered to 4/10 patients cannot explain the result obtained with ThE in this study.

Such rapid recovery from COVID-19 is rare. Mancuso et al. [48] followed 4480 patients with a positive swab and date data in Reggio Emilia province, Italy, from 26 February to 22 March; the province of Reggio Emilia is next to the province of Parma. Under local regulations, only symptomatic patients were tested. In their study, the median time to a first negative swab was 31 days from the first positive swab (IQR 24–41), estimated using the Kaplan-Meier method. At 10 days after the first swab, only 0.7% of these patients had a first negative swab, while at 20 days 19.0% had a first negative swab. In our study, 20% of patients had a first negative swab within 10 days of the first swab (in fact, the total was 70%, with a median of 8 days, when considered from T0 when ThE therapy started: see Table 2). By 20 days, 70% had a first negative swab. The null hypothesis was that the rate of negative testing would be unaffected by GTC and would follow the same distribution as the population of Reggio Emilia. A statistical comparison between Mancuso's data and ours showed the one-tailed probability by the Fisher-Yates test that this would happen by chance after 10 days was $p < 0.0023$ and $p < 0.005$ at 20 days.

The mechanism of action of GTC is unknown. The anti-inflammatory effects of GTC have been studied and demonstrated repeatedly, as discussed above. The results of Ohgitani et al. suggest direct anti-viral action in the lungs [31,32]. The concentration of EGCG in the nebulizer solution used for the trial (see Dose and Methods of Administration Section) was 5 mM, ten times 500 μM (Ohgitani et al. [31]), and the exposure time was much longer. Direct inactivation of fresh virus in the lung may be part of the effectiveness of this therapy. Other mechanisms may be present.

We recently searched the scientific literature, but did not find any study or evidence showing that drinking green tea by itself prevents infection with SARS-COV-2 or is curative for COVID-19 syndrome. Besides having a stronger effect due to purity and dosage, we may speculate that delivery to the lungs may be important for efficacy and avoidance of the "cytokine storm", but further clinical studies, with more patients, will be necessary to properly address this issue.

5. Conclusions

To the best of our knowledge, this is the first report on the efficacy of green tea catechin against COVID-19 syndrome in a real clinical setting. The major limitation of our study was the small number of patients; nevertheless, the very high rate of positive response that we observed may open new perspectives in the fight against COVID-19 syndrome.

Author Contributions: Conceptualization, S.B.; methodology, S.B. and S.C.; validation, L.G. and S.C.; investigation, L.G. and S.C.; data curation, S.B., S.C., and L.G.; writing—original draft preparation, S.B.; writing—review and editing, S.B. and S.C.; supervision, S.B. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki. Ethical review and approval were waived for this study due to the fact that it was a compassionate study performed under strict lockdown rules at the end of 2020. We administered a food supplement, freely available on the market.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data supporting reported results are available (S.B.).

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

AAT: alpha-1 anti-trypsin;CRP, C-reactive protein; EC: (-)-Epicatechin; ECG, Epicatechin-3-gallate; EGC: (-)-Epigallocatechin; EGCG, epigallocatechin-3-gallate; ESR, erythrocyte sedimentation rate; GTC, green tea catechins; GTE, green tea extract; Hydroxy-chloroquine (HCQ); IL-6, interleukin 6; PoE, Polyphenon E; ThE, Theaphenon E.

Appendix A

Tea Solutions, Hara office Inc.
1-18-15-510, Taihei, Sumida-Ku, Tokyo, 130-0012 Japan
Tel/Fax: +81-(0)3-3626-8295, Cell: +81-(0)80-3209-0129
Email: ysjrhara@soleil.ocn.ne.jp
www. Theaphenon.jp

Specification Sheet

		Manufactured Date	11-apr-17
		Test Date	13-apr-17
		Report Date	15-apr-17
Product Name	**Theaphenon E**	**Batch Number**	TPE-170401
Latin Name	*Camellia sinensis* O. Kunze	**Quantity**	30kg
Plant Part Used	Leaf (Dried, 1 0 0 %Natural)	**Country of Origin**	P.R. China
Type of Products	Standardized Extract	**Type of extraction**	Water
Herbal Strength	Approx. 45-50 parts of leaves to 1 part of Extract	**Active Ingredient Markers**	EGCG, Catechins

Analysis Items		**Specifications**	**Results**	**Test Methods**
Identification		Positive	Conforms	TLC
Appearrance, Color		Powder, light red-dull reddish yellow	Conforms	Visual Inspection
Foreign Matter		No Foreign Matter observed	Conforms	Visual Inspection
Bulk Density		35-45g/100ml	42g/100ml	Density Meter
Mesh Size		NLT95% thru 80Mesh	Conforms	80 Mesh Screen
Solubility		Good in Water, Ethanol	Conforms	Visual
Assay		**Catechins Spec.85-95%**	90,34%	HPLC
		EGC	19,58%	
		DL-C	/	
		EC	1,63%	
		EGCg Spec: 56-72%	64,04%	
		GCg	0,42%	
		ECg	4,67%	
Caffeine		**NMT 1.0%**	**0,14%**	HPLC
Theobromine		**NMT 1.0%**	**Trace**	HPLC
Gallic Acid		**NMT 0.5%**	**Trace**	HPLC
Method of Extraction		Water	Conforms	
Solvents Used		Water & Ethanol	Conforms	
Loss on Drying(5g)		NMT 4.0%	2,91%	5g/105℃/2hrs
Residue on Ignition		NMT 0.2%	0,01%	2g/525℃/3hrs
Extract Exipient		None	Conforms	
Heavy Metals		NMT 10ppm	Conforms	Atomic Absorption
Arsenic(As)		NMT 1.0ppm	Conforms	Atomic Absorption
Lead(Pb)		NMT 1.0ppm	Conforms	Atomic Absorption
Pesticides Residues		Free	Conforms	Gas Chromatography
Solvent Residues:	**EtOAc**	Free	Conforms	Gas Chromatography
	Methanol	NMT 10ppm	Conforms	Gas Chromatography
Sterilization Method		High Temp High Press for short time (5"-10")		
Total Plate Count		NMT 1,000cfu/g	Conforms	
Total Yeast & Mold		NMT 100cfu/g	Conforms	
E.coli		Negative	Conforms	
Coliform		NMT 10cfu	Conforms	
Salmonella & Staphylococcus		Negative	Conforms	
Packing & Storage		Packed in plastic pack in aluminum pack		
Shelf Life		3 years if sealed and stored away from direct sun light		
Expiration Date		10-apr-20		

Manufacturer	**Phyto-Ingredients Inc.**

Appendix B

Sunsho Pharmaceutical Co., Ltd.

1468 Atsuhara, Fuji-shi, Shizuoka 419-0201 JAPAN / TEL:+81 545 73 0610 FAX:+81 545 73 0611

July 28, 2017

To: Tea Solutions, Hara Office Inc.

Product Formula

Product Name: Theaphenon E Hard Capsule (Active)

We hereby certify that the product is containing ingredients as follows.

Fill material (Per capsule):

Ingredients		Quantity per capsule
Theaphenon® E		300 mg
Corn Starch		58 mg
Microcrystalline Cellulose		18 mg
Calcium Stearate		4 mg
	Sub Total	380 mg

Shell material(Per capsule):

Ingredients		Quantity per capsule
Hard Capsule (HPMC, clear, capsule size: No.1)		70 mg
	Sub Total	70 mg
	Total	450 mg

Sunsho Pharmaceutical Co., Ltd.
1468 Atsuhara, Fuji-shi,
Shizuoka JAPAN

N. Ikoma

NORIKAZU IKOMA
Quality Assurance, Manager

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