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Research article

An oral formula of vanillin and wheat germ oil for treatment of mild and moderate COVID-19 viral disease: a randomized controlled trial

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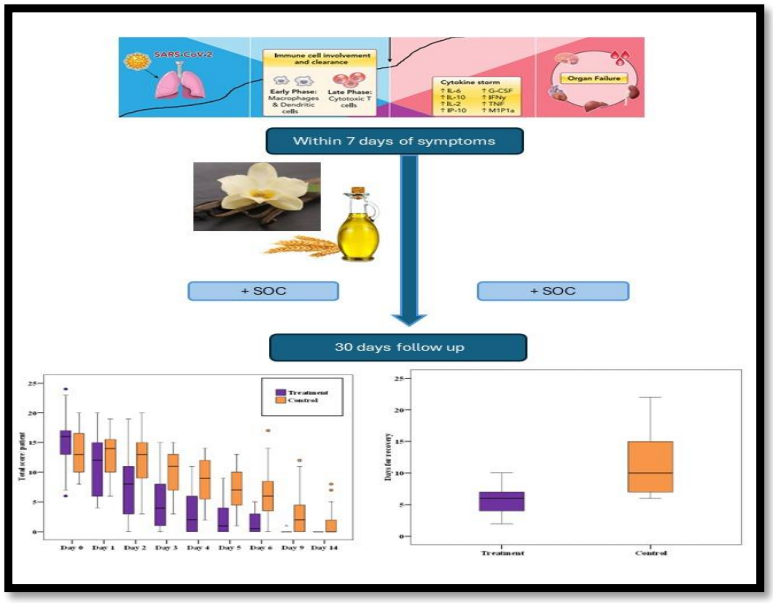
Abstract:

The world has been suffering from the consequences of COVID-19. Our study aimed to determine the efficacy of 5-day treatment course of wheat germ oil-vanillin mix for outpatients with mild to moderate COVID-19 symptoms.

Positive COVID-19 patients presented with symptoms within 7 days, were randomized into control and treatment arms. The treatment arm received the capsule and standard-of-care therapy (SOC) while the control arm received SOC only. All patients were followed up for 30 days. Symptoms were assessed based on the FDA-assessment of COVID-19 symptoms. The scoring system was used to assess the severity of symptoms. Blood samples were obtained for CBC and CRP analysis. A total of 61 patients were enrolled in the study. Patients in the treatment arm showed a significant improvement in disease severity when compared to the control arm (68.32% vs 30% reduction in symptoms at day 3, $p<0.001$). Time for recovery was significantly shorter in patients receiving treatment ($p<0.001$). No significant side effects were reported in either arm.

The herbal mix showed potential activity against COVID-19 and further assessment is recommended as a safe and affordable add-on therapy for inflammatory conditions.

The protocol was approved by the ethics committee of Alexandria University, protocol-ID:0106922. Trial registration: ClinicalTrials.gov.ID (NCT05157139).



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1. Introduction

The world has been suffering from the consequences of Coronavirus disease 2019 (COVID-19) for more than two years now [(1)]. As of 24 October 2022, according to the World Health Organization (WHO), there are more than 600 million confirmed COVID-19 cases and more than 6 million deaths globally. In Egypt, more than 500,000 cases were confirmed and more than 24,000 died during the pandemic. Actual numbers are likely higher than the reported ones [(2)]. According to WHO, COVID-19 is a public health emergency. Coronaviruses are significant pathogens that can harm the lower respiratory tract causing symptoms as mild as a simple cold to severe infections with up to 50% mortality. One infected individual can spread the COVID-19 infection to an average of three other people, indicating that SARS-CoV-2 has a high potential for outbreaks. Clinical signs and symptoms of COVID-19 can vary from asymptomatic infection, flu-like symptoms such as fever, cough, and tiredness to more advanced symptoms such as pneumonia, acute respiratory distress syndrome, and potentially multi-organ failure with substantial morbidity and mortality. It is still unclear how symptoms can greatly vary from one person to another, but it can be related to the patient's age, medical history, comorbidities, and immunity status [(3)].

Although the exact pathophysiology of COVID-19 disease is still unclear, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is known to be transmitted through respiratory droplets invading upper and lower respiratory tract cells through Angiotensin-Converting Enzyme 2 (ACE2) receptor which is also found in other human tissues including the small intestine, the kidneys, the heart, and the thyroid tissue. After entering the alveolar epithelial cells, viral-induced cell injury and death take place causing chemokine and cytokine release and thus recruiting dendritic cells and macrophages. More proinflammatory cytokines are then recruited and virus phagocytosis occurs activating antigen-specific T-cells. T-cells attack the lungs and destroy infected alveolar cells causing lung injury [(4)]. The mentioned hyper-inflammatory condition is known as “cytokine storm”. Extrapulmonary involvement in COVID-19 has also been linked to uncontrolled inflammation (**Fig. 1**) [(4)].

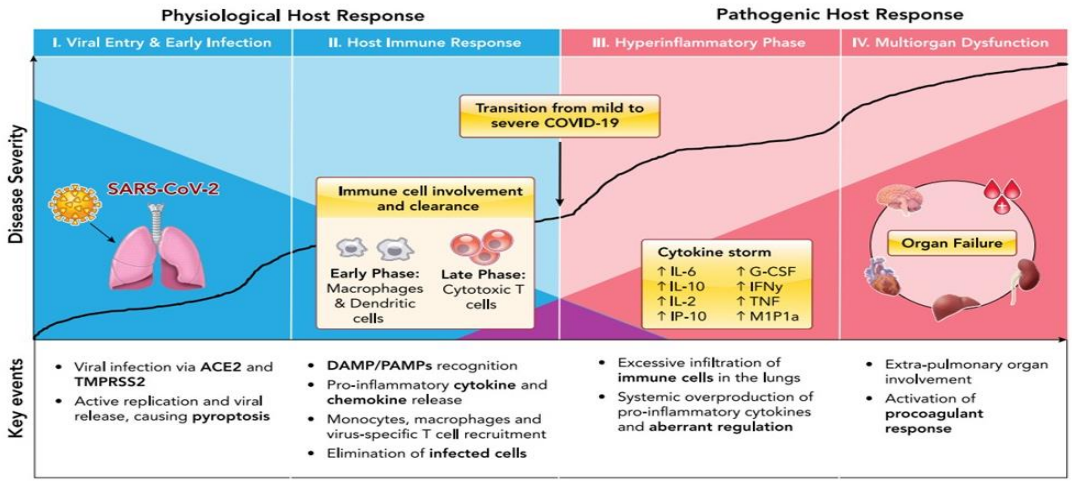

Fig. 1. Pathophysiology of COVID-19 disease [(4)].

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The development of a cytokine storm involves numerous cytokines, including members of the interleukin- 1 (IL-1) family, IL-6, IL-8, IL-10, tumor necrosis factor (TNF), and interferon (IFN), though the main pathogenic cytokines seem to vary depending on the condition [(5)].

Due to the SARS-CoV2 infection's rapid global spread, an urgent need for a vaccine or therapeutic intervention to prevent or treat COVID-19 disease could not be ignored [(6)].

IL-1 receptor antagonist was administered to COVID-19 patients at an early stage of the illness since interleukin-1 (IL-1) inhibits the production of IL-6 and other proinflammatory cytokines. It was concluded that early IL-1 receptor inhibition is beneficial in COVID-19 patients with acute hyperinflammatory respiratory failure [(7)].

Klinger *et al..*, (2021) identified cyclin-dependent kinase 6 (CDK6) as a possible therapeutic target for COVID-19. A benefit that makes CDK6 a desirable pharmacological target is that, unlike vaccinations and antivirals, medication action is unaffected by viral alterations because CDK6 is a human protein [(8)]. The role of CDK6 and CDK6 inhibitors is well acknowledged by the review reported by Ochsner *et al.* (2020) [(9)].

In-silico studies also discovered that the SARS-CoV2 spike protein binds to surface Toll-Like Receptors (TLRs) (TLR1, 4, and 6), particularly TLR4. Therefore, developing competitive TLR4 antagonists that target only the TLR4-spike protein interaction could lead to a novel method of treating COVID-19 [(10)].

Repurposing clinically available medications has been the available option for hindering the ongoing pandemic because new drug development often takes many years. Many Food & Drug Administration (FDA)-approved medications and medications derived from natural products have been tested for anti-SARS-CoV-2 effect, and some of them are potentially effective against SARS-CoV-2 [(11)].

Wheat germ oil (WGO) is a known source of oleic, linoleic, linolenic, and octacosanol [(12)]. Using molecular docking, anti-COVID-19 activity for octacosanol demonstrated comparable RNA-dependent RNA polymerase inhibition to Remdesivir (a nucleoside analog approved by the FDA for the treatment of COVID-19 [(13)]). Additionally, octacosanol showed a strong affinity to the major protease and Spike protein S1 [(14)]. Oleic acid, another component of WGO, interestingly showed antiviral activity. Using the enveloped bacteriophage model, oleic acid decreased the virus titer to less than 1% of the starting titer [(15)]. Comparing bromate (BRO)-intoxicated rats to control rats, a study found a significantly higher level of serum pro-inflammatory cytokines in the BRO-intoxicated rats. Both IL-1 and TNF were dramatically lowered after pretreatment with wheat germ oil (WGO); this effect may be attributable to WGO's antioxidant action [(16)]. In a model of endotoxemia in rats, wheat germ oil pre-treatment was found to significantly reduce blood TNF and IL-6 values [(17)].

Another natural product, vanillin (Van), exhibited a significant binding affinity to CDK6 and suppressed its kinase activity, as shown by molecular docking and fluorescence-based binding assay. Immunoblotting studies for CDK6 in vanillin-treated cells showed that CDK6 cellular expression was reduced in vanillin-treated cells [(18)]. Additionally, *in vivo*, vanillin therapy in lipopolysaccharide (LPS) challenged mice model effectively mitigated LPS-induced kidney injury. Mice treated with vanillin showed considerably lower levels of the inflammatory cytokines TNF, IL-6, and IL-1 [(19)]. A review article by Arya *et al.*, (2021) concluded that the anti-neuraminidase (NA) activity of a novel vanillin derivative was comparable to those

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of oseltamivir and zanamivir. Recent reports on SARSS-CoV-2 also suggest that vanillin has a moderate affinity for spike protein and major protease [20]. Vanilloids have shown molecular interactions with TLR4, and potential inhibition was reported [21].

From the quoted activity both components, wheat germ oil and vanillin, can bear a potential anti-viral activity against COVID-19 as by reviewing current literature, a successful strategy would attempt to target IL-1 IL-6, CDK-6 and TLR4 and hindering "cytokine storm".

The present study aims to perform a clinical trial for an oral herbal mix with a profile that encompasses activity as inhibitors for the inflammatory process. The pharmaceutical formula is composed of vanillin and wheat germ oil in an oral capsule. COVID-19 symptoms and inflammatory markers were assessed as possible indicators of disease deterioration.

2. Patients and methods

2.1. Study population & setting

Suspected COVID-19 outpatients visiting the chest department at Alexandria University's Students Hospital were screened for the virus.

2.2. Randomization

Random allocation was generated using a mobile application that randomly generates the letters C or T. Patients who received the letter C were randomized into the control arm and those who received the letter T received treatment course. Only the clinical pharmacist assigned to recruit patients had access to the mobile application during recruitment, other healthcare providers and outcomes assessors were blinded.

2.3. Eligibility criteria

Inclusion criteria*:* Only Polymerase Chain Reaction (PCR)-confirmed COVID-19 patients with mild to moderate symptoms manifested within 7 days (ideally within 72 hours) were included. Mild, moderate, and severe illness was determined in accordance with The Egyptian Ministry Of Health and Population (MOHP) criteria [22] as follows:

- *Mild Illness*: Individuals who have any of the various signs and symptoms of COVID-19 (e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell) but who do not have shortness of breath, dyspnea, or abnormal chest imaging.
- *Moderate Illness:* Individuals who show evidence of lower respiratory infection during clinical assessment or imaging and who have an oxygen saturation (SpO_2) ≥92% on room air at sea level.
- *Severe Illness:* Individuals who have SpO_2 <92% on room air at sea level, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) <300 mmHg, respiratory rate >30 breaths/min, or lung infiltrates >50%.

Exclusion criteria: Patients with severe illness, advanced kidney, liver or cardiovascular disease, active cancer, allergy to vanillin or wheat germ oil, pregnant and lactating females and patients receiving other COVID-19 investigational drugs or steroids were excluded.

2.4. Study design

Recruited patients with mild and moderate disease were randomized in a near 1:1 ratio into control and treatment arms using computer-generated random numbers. All patients went through face-to-face or telephone interviews where the study was explained, and detailed patient history was taken. Blood samples were obtained from patients on day zero for complete blood count (CBC) and C-reactive protein (CRP) analysis and again on day 3.

Control arm: Patients received standard of care (SOC) therapy. SOC therapy included paracetamol 1gm tablets given when needed, azithromycin 500mg given once daily for 5 days, vitamin C 500 mg and zinc 23.9 mg

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tablet given twice daily according to the Egyptian MOHP protocol.

Treatment arm: Patients received SOC therapy + a 5 day-course of vanillin and wheat germ oil capsules (2 capsules twice daily for 3 days followed by 1 capsule twice daily for 2 days).

2.5. Capsule of vanillin and wheat germ oil specification:

The formula of vanillin and wheat germ oil is in the form of a soft gelatin capsule, intended to be marketed as food supplement. Each capsule is composed of 250mg vanillin and 150mg wheat germ oil.

The source, specification, and analysis methods are included in **Annex 1**.

2.6. Assessment

Based on the FDA-suggested assessment of key COVID-19 related symptoms [23] presented in **Fig. 2**; symptoms from 1 to 9 were graded on a scale ranging from 0 to 3 each, where 0= no manifestation and 3= severe manifestation. Regarding fever, 1 point was given to patients with body temperatures ranging from 37.5 to 38, 2 points for temperatures from 38.1 to 38.5 and 3 points for those with temperatures above 38.5.

Taste and smell along with other manifestations were also traced. Patients with a total score below 8 points were excluded. Follow-up took place daily for the first week, twice weekly for the second week and once weekly thereafter for a total duration of 30 days. Patients were followed up through telephone interviews, each symptom was graded according to the mentioned scale and any side effect was recorded.

2.7. Parameters Measured

2.7.1. Complete Blood Count (CBC)

For CBC, on day zero blood samples were collected from patients on EDTA tubes, serum was separated and then analyzed within 12 hours. CBC was repeated on day 3. Samples were analyzed by Sysmex XE- 2100 (TOA Medical Electronics, Kobe, Japan) using fluorescent flow cytometry [24].

Example items *For items 1–10, sample item wording could be: "What was the severity of your **[insert symptom]** at its worst over the last 24 hours?"*	**Example response options and scoring***
1. Stuffy or runny nose	None = 0 Mild = 1 Moderate = 2 Severe = 3
2. Sore throat	
3. Shortness of breath (difficulty breathing)	
4. Cough	
5. Low energy or tiredness	
6. Muscle or body aches	
7. Headache	
8. Chills or shivering	
9. Feeling hot or feverish	
10. Nausea (feeling like you wanted to throw up)	
11. How many times did you **vomit (throw up)** in the last **24 hours**?**	I did not vomit at all = 0 1–2 times = 1 3–4 times = 2 5 or more times = 3
12. How many times did you have **diarrhea (loose or watery stools)** in the last **24 hours**?**	I did not have diarrhea at all = 0 1–2 times = 1 3–4 times = 2 5 or more times = 3
13. Rate your sense of **smell** in the last **24 hours**	My sense of smell is THE SAME AS usual = 0 My sense of smell is LESS THAN usual = 1 I have NO sense of smell = 2
14. Rate your sense of **taste** in the last **24 hours**	My sense of taste is THE SAME AS usual = 0 My sense of taste is LESS THAN usual = 1 I have NO sense of taste = 2

Fig. 2: FDA suggested assessment of key COVID-19 related symptoms [23].

2.7.2. C-Reactive Protein (CRP)

For CRP, on day zero samples were also collected from patients on EDTA tubes, and serum was separated and then analyzed within 12 hours. On day 3, CRP analysis was repeated. Samples were analyzed using

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Cobas® (Roche, Manheim, Germany). CRP concentration is calculated as the change in absorbance measured at 525 nm and 625 nm which is in relation to the amount of agglutination [25].

2.8. Outcome

The primary outcomes included the clinical assessment of the mean change in disease severity based on the previously mentioned FDA-suggested assessment criteria, rate of disease remission and survival rate along the 30-day follow-up period.

The secondary outcomes included one or more of the following:

- Mean change in body temperature.
- Mean change in the complete blood picture, specifically lymphocytic count.

(Patients were considered lymphopenic if the lymphocytic count was less than 20% of the total white blood cell count)

- Mean change in C-reactive protein.
- Mean change in interleukin- 6.
- Nature and severity of adverse drug events.

2.9. Ethical Considerations

Informed consents were provided by patients. The trial was conducted in accordance with the declaration of Helsinki [26] and good clinical practice guidelines. The protocol was also approved by the ethics committee of Alexandria University, protocol ID:0106922 The study was registered on ClinicalTrials.gov ID (NCT05157139)

2.10. Statistical Analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0**.** **(**Armonk, NY: IBM Corp**)** Qualitative data were described using number and percent. The Shapiro-Wilk test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). The significance of the obtained results was judged at the 5% level.

The used tests were the Chi-square test for categorical variables, to compare between different groups, Fisher's Exact or Monte Carlo correction for correction for chi-square when more than 20% of the cells have expected count less than 5, student t-test: for normally distributed quantitative variables, to compare between two studied groups, and Mann Whitney test: for abnormally distributed quantitative variables, to compare between two studied groups.

3. Results

153 non-hospitalized patients were screened for COVID-19 virus. All patients who tested positive for COVID-19 during the period from the 6th of November 2021 to the 17th of January 2022 were offered to join our study. A total of 64 patients were recruited based on the inclusion criteria listed below and were randomized into two groups (intervention and control), 3 patients withdrew leaving a total of 61 patients who were randomized into two arms. **Fig. 3.**

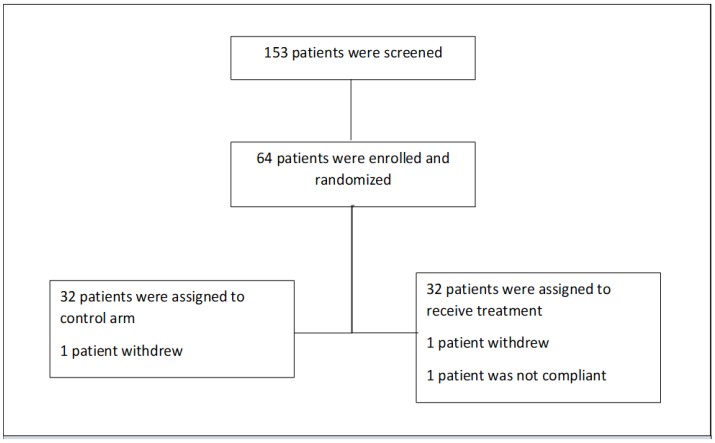

Fig. 3: Flow chart of the study adherence from day 0 to day 30.

3.1.Participants

A total of 61 patients were randomized into two arms: control and treatment arms. 30 patients received treatment + SOC therapy while the remaining 31 received only SOC therapy. Baseline demographics are

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presented in **Table 1**. More female patients were enrolled in the study however there was no statistically significant difference in gender distribution between the two arms (p=1.00). Signs and symptoms were tracked among different age groups as the youngest participant was 19 years old while the oldest was 60 years old, with more elderly patients included in the treatment group. There was no statistically significant difference found between baseline BMI and in the two arms. **(Table 1).**

Most enrolled patients were vaccinated. No statistically significant difference was found between the two arms in the percentage of vaccinated participants (p=0.806). When assessing the total score of signs and symptoms at day zero (D_0), treatment group patients showed slightly higher median score when compared to the control group, although the difference was not statistically significant (p=0.112). The score ranged from 6 to 24 points. All patients in both arms were enrolled within 6 days from the onset of symptoms. **Table 1.**

Table 1: Baseline demographics of treatment and control arms**.**

Demographic data	Treatment (n = 30)		Control (n = 31)		p
	No.	%	No.	%	
Sex					
Male	5	16.7	5	16.1	^{FE}p=
Female	25	83.3	26	83.9	1.000
Age (years)					
Min. – Max.	20.0 – 60.0		19.0 – 59.0		
Mean ± SD.	41.20 ± 11.57		34.65 ± 11.44		0.030*
BMI (kg/m^2)					
Min. – Max.	18.94 – 35.16		16.36 – 36.21		0.471
Mean ± SD.	26.57 ± 4.31		25.72 ± 4.85		
Vaccine					
No	6		20.0		0.806
Yes	24		80.0		
Total score at D_0					
Min. – Max.	6.0 – 24.0		8.0 – 20.0		0.112
Mean ± SD.	15.40 ± 3.96		13.77 ± 3.90		

*: Statistically significant at p ≤ 0.05
FE: Fisher Exact

3.2.Outcomes

Primary outcomes included the clinical assessment of the mean change in disease severity which was assessed using the FDA-suggested criteria presented in **Fig. 2**. Median score of symptoms was compared daily over 14 days between treatment and control arms. Daily median FDA scores for both treatment and control arms were recorded. The median FDA score for patients in the treatment arm was significantly lower than those in the control arms since day 2 and all along the 14 days of follow-up. On day 2 the median score for the treatment arm was 8 and for the control arm was 13 (p = 0.001) as presented in **Fig. 4**.

Median scores on days 3 and 6 were analyzed. At day 3 the median score of patients assigned to receive treatment was 4 while those assigned to the control arm was 11 which was a statistically significant difference (p <0.001). Also, at day 6 there was a statistically significant difference between treatment and control arms, scores 0.5 and 6, respectively (p <0.001).

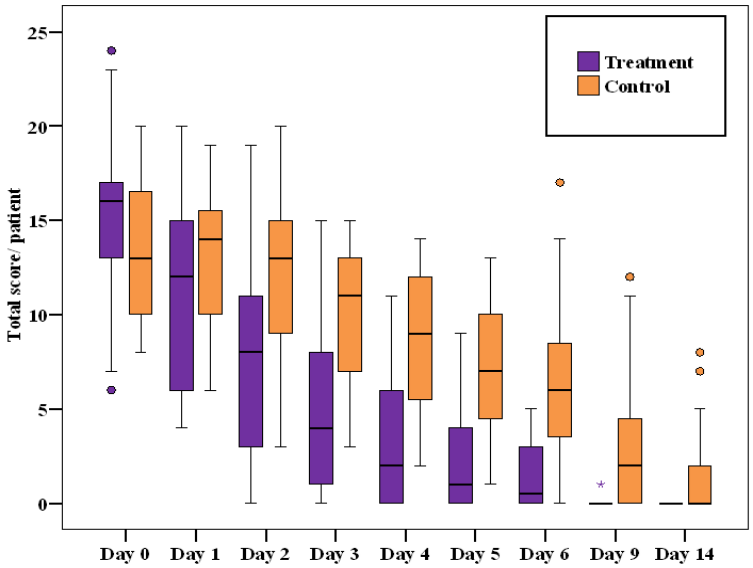

Fig. 4: Box plot comparing median score of symptoms between Van-WGO + SOC and SOC only arms for 14 days.

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Patients in the treatment arm had a higher percentage reduction of symptoms throughout the 14 days. At day 3 the percentage reduction of symptoms for treatment and control arms was 68.32% and 30% (p <0.001), respectively. At day 6 the percentage reduction of symptoms was also significantly higher in the treatment arm reaching 97.83% compared to 56.25% (p < 0.001) in the control arm. **Table 2.**

Table 2: Percentage reduction of symptoms for treatment and control arms at days 3 & 6.

Percentage reduction of symptoms	Treatment (n = 30)	Control (n = 31)	p
Day 3			
Min. – Max.	23.53 – 100.0	-66.67 – 66.67	<0.001*
Median (IQR)	68.32 (45.0 – 90.91)	30.0 (15.63 – 38.75)	
Day 6			
Min. – Max.	61.54 – 100.0	-40.0 – 100.0	<0.001*
Median (IQR)	97.83 (82.35 – 100.0)	56.25 (48.33 – 70.29)	

The comparison between the severity of respiratory & constitutional symptoms in treatment and control arms was performed.

Each symptom of the nine symptoms was compared between the two arms over 14 days. Starting with runny nose, on day 3 the percentage of patients in the treatment arm suffering from severe runny nose was zero compared to 12.9% in the control arm (p=0.008) **Table 3**. By day 5, no patient in the treatment arm reported having a sore throat compared to 25.8% of patients in the control arm (p=0.006). When comparing shortness of breath, a statistically significant difference was found by day 4 as 96.7% of patients in the treatment arm reported no problem with breathing compared to 67.7% of patients in the control arm (p=0.007). Considering cough, by day 4 only 3.3% of patients in the treatment group suffered from cough compared to 9.7% in the control arm (p=0.044). Tiredness was one of the significant symptoms in COVID-19 carriers and when compared between both arms, at day 0, 73.3% of patients in the treatment arm reported severe signs of tiredness compared to 61.3% in the control arm. By day 2 it was noted that the percentage had dropped to 20% in the treatment arm while there was no change in the percentage of the control arm (p=0.004). An improvement in muscle aches was also noted in the treatment arm compared to the control arm by day 2 (p<0.001) and for 14 days. On day 14, 29% of patients in the control arm still reported muscle aches, while by day 9 all patients in the treatment arm reported having no muscle aches. The severity of headaches has also decreased in patients assigned to the treatment arm by day 3, as only 6.7% of patients still had severe headaches compared to 22.6% in the control arm **Table 3**. The chill pattern was variable throughout the 14 days and no significant difference was noted between the two arms. Unlike fever, a change in severity was noted since day one. On day one, fever was absent in 86.7% of patients in the treatment arm although 43.3% reported having no fever on day zero showing that the number of patients with no fever was doubled in 24 hours (p=0.021). In the control arm fever was absent in only 58.1% of patients and on day zero 38.7% of patients were not feverish (p=0.021).

Among the nine symptoms, constitutional symptoms were significantly reduced from day 2, this includes tiredness (p<0.004), and muscle aches (<0.001). Besides the nine main symptoms, taste and smell profiles was analyzed in both groups. The percentage of smell and taste loss in patients who received treatment was 26.67% and in those who did not receive treatment was 41.94%. The median duration of smell loss in the treatment arm was 6 days and 9 days in the control arm. The median duration of taste loss was also lower in the treatment arm when compared to the control arm; 5.5 (3.5 to 7) and 9 (6 to 11) days, respectively, **Table 4.**

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Table 3: Comparison between treatment and control arms regarding the severity of symptoms on D0, D3, and D6 of follow-up in percentage.

Symptom	D0		D3		D6	
Runny Nose	Treatment	Control	Treatment	Control	Treatment	Control
No	23.3	41.9	73.3	38.7	93.3	64.5
Mild	16.7	19.4	10.0	35.5	6.7	22.6
Moderate	40.0	29.0	16.7	12.9	0.0	12.9
Severe	20.0	9.7	0.0	12.9	0.0	0.0
	p=0.359		p=0.008		p=0.012	
Sore Throat						
No	33.3	41.9	80.0	51.6	100.0	83.9
Mild	13.3	19.4	13.3	29.0	0.0	12.9
Moderate	26.7	3.2	6.7	12.9	0.0	3.2
Severe	26.7	35.5	0.0	6.5	0.0	0.0
	p=0.081		p=0.097		p=0.082	
Shortness of breath						
No	40	45.2	63.3	48.4	90	61.3
Mild	6.7	3.2	20	12.9	10	16.1
Moderate	20	25.8	13.3	29	0	16.1
Severe	33.3	25.8	3.3	9.7	0	6.5
	p=0.814		p=0.303		p=0.02	
Cough						
No	16.7	16.1	16.7	19.4	70.0	25.8
Mild	13.3	41.9	43.3	22.6	26.7	45.2
Moderate	23.3	19.4	33.3	41.9	3.3	19.4
Severe	46.7	22.6	6.7	16.1	0.0	9.7
	p=0.067		p=0.331		p=0.001	
Tiredness						
No	3.3	3.2	30.0	3.2	76.7	16.1
Mild	3.3	3.2	30.0	12.9	10.0	35.5
Moderate	20.0	32.3	30.0	48.4	13.3	29.0
Severe	73.3	61.3	10.0	35.5	0.0	19.4
	p=0.837		p= 0.002		p <0.001	
Muscle aches						
No	3.3	9.7	53.3	6.5	26	29.0
Mild	3.3	9.7	16.7	19.4	1	32.3
Moderate	20.0	9.7	23.3	32.3	3	16.1
Severe	73.3	71.0	6.7	41.9	0	22.6
	p= 0.410		p<0.001		p<0.001	
Headaches						
No	16.7	22.6	20	29.0	26	51.6
Mild	13.3	19.4	4	29.0	2	12.9
Moderate	13.3	12.9	4	19.4	2	19.4
Severe	56.7	45.2	2	22.6	0	16.1
	p=0.008		p=0.025		p=0.013	
Chills						
No	80.0	64.5	96.7	87.1	100.0	96.8
Mild	3.3	19.4	0.0	6.5	0.0	3.2
Moderate	10.0	9.7	3.3	6.5	0.0	0.0
Severe	6.7	6.5	0.0	0.0	0.0	0.0

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	p= 0.294		p=0.512		p=1	
Fever						
No	43.3	38.7	100.0	90.3	100.0	93.5
Mild	26.7	19.4	0.0	6.5	0.0	3.2
Moderate	23.3	19.4	0.0	3.2	0.0	0.0
Severe	6.7	22.6	0.0	0.0	0.0	3.2
	p=0.417		p=0.362		p=1	

Table 4: Comparison between treatment and control arms according to recovery time.

Days of:	Treatment	Control	p
Runny nose recovery	**(n = 23)**	**(n = 23)**	
Min. – Max.	1.0 – 6.0	1.0 – 21.0	0.001*
Median (IQR)	2.0 (1.0 – 5.0)	6.0 (4.0 – 6.0)	
Sore throat	**(n = 19)**	**(n = 20)**	
Min. – Max.	1.0 – 4.0	1.0 – 9.0	<0.001*
Median (IQR)	2.0 (1.0 – 3.0)	3.50 (3.0 – 5.0)	
SOB	**(n = 18)**	**(n = 21)**	
Min. – Max.	1.0 – 6.0	1.0 – 21.0	0.006*
Median (IQR)	3.0 (1.0 – 4.0)	6.0 (4.0 – 9.0)	
Cough	**(n = 29)**	**(n = 28)**	
Min. – Max.	1.0 – 9.0	1.0 – 14.0	<0.001*
Median (IQR)	5.0 (3.0 – 6.0)	6.0 (6.0 – 9.0)	
Tiredness	**(n = 30)**	**(n = 31)**	
Min. – Max.	1.0 – 9.0	1.0 – 21.0	<0.001*
Median (IQR)	4.0 (2.0 – 5.0)	6.0 (6.0 – 9.0)	
Muscle aches	**(n = 28)**	**(n = 30)**	
Min. – Max.	1.0 – 6.0	2.0 – 21.0	<0.001*
Median (IQR)	2.50 (1.0 – 4.50)	6.0 (6.0 – 14.0)	
Headache	**(n = 25)**	**(n = 26)**	
Min. – Max.	1.0 – 6.0	1.0 – 21.0	<0.001*
Median (IQR)	2.0 (1.0 – 3.0)	6.0 (4.0 – 9.0)	
Chills	**(n = 6)**	**(n = 13)**	
Min. – Max.	1.0 – 4.0	1.0 – 9.0	0.722
Median (IQR)	1.0 (1.0 – 2.0)	1.0 (1.0 – 3.0)	
Fever	**(n = 15)**	**(n = 20)**	
Min. – Max.	1.0 – 1.0	1.0 – 3.0	0.006*
Median (IQR)	1.0	1.0 (1.0 – 2.0)	
Days for recovery	**(n = 30)**	**(n = 31)**	
Min. – Max.	2.0 – 10.0	6.0 – 22.0	<0.001*
Median (IQR)	6.0 (4.0 – 7.0)	10.0 (7.0 – 15.0)	
Taste recovery	**(n = 8)**	**(n = 13)**	
Min. – Max.	1.0 – 8.0	4.0 – 24.0	0.013*
Median (IQR)	5.50 (3.50 – 7.0)	9.0 (6.0 – 11.0)	
Smell recovery	**(n = 9)**	**(n = 13)**	
Min. – Max.	1.0 – 8.0	4.0 – 17.0	0.009*
Median (IQR)	6.0 (3.0 – 7.0)	9.0 (7.0 – 11.0)	

Regarding recovery time, the median time in days for the complete recovery of all symptoms in the treatment arm was 6 days vs 10 days in the control arm (p<0.001). **Table 4, Fig. 5**

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When each symptom was analyzed separately, recovery time was shorter for all the nine symptoms in the treatment arm when compared to the control arm. **Table 4**

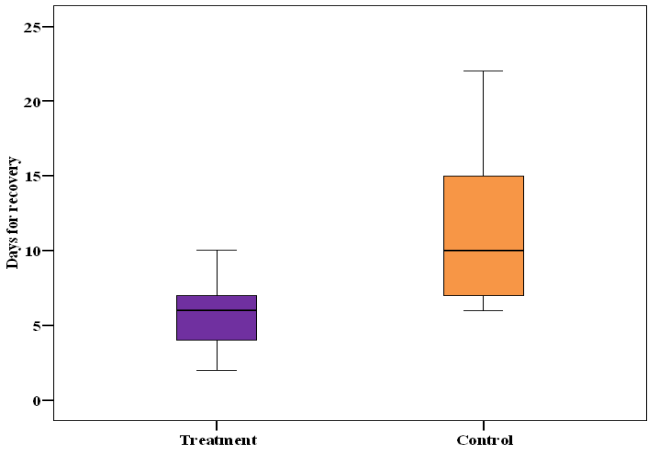

Fig.5: Comparison between the Van-WGO + SOC and SOC only arms according to days for recovery.

Difference between CRP, White Blood Cells (WBCs), lymphocytes percentage and IL-6 at day zero and day 3 was compared between treatment and control arms.

Median decrease in WBCs was statistically significant between the treatment -0.48 and the control group 0.58 (p=0.010). CRP reduction & increase in lymphocytes percent was not statistically significant between the two groups (p=0.737, p=0.836), respectively. **(Table 5)**

Table 5: Comparison between lab parameters in treatment and control arms.

Change	Treatment	Control	P
Decrease in CRP			
Min. – Max.	-0.90 – 20.0	0.20 – 62.70	
Median (IQR)	6.90 (4.1 - 16.2)	4.30 (3.2 – 17.1)	0.737
Decrease in WBCs			
Min. – Max.	-1.31 – 0.51	-1.20 – 6.99	
Median (IQR)	-0.48 (-0.70 - -0.07)	0.58 (0.25 – 1.30)	0.010*
Increase of lymphocytes (%)			
Min. – Max.	-13.60 – 32.40	-2.40 – 14.20	
Median (IQR)	2.30 (0.30 – 3.10)	-1.0 (-1.45 – 8.05)	0.836

When comparing adverse events between the two arms, the percentage of participants with nausea and vomiting was 3% in the treatment arm vs 12.9% in the control arm. Another reported side effect was diarrhea, 3% of patients in the treatment arm reported having episodes of diarrhea vs 6.9% in the control arm.

Through the 30 days of follow-up, no patients were hospitalized in any of the two arms and the number of deaths was zero.

4. Discussion

The COVID-19 virus represented a global burden for almost 3 years. With the emergence of this virus and triggering inflammatory cascade leading to a variety of respiratory & constitutional symptoms, we found the use of a new herbal formula that mainly acts on inflammation cascade. Various cohort studies have found that there are significantly higher levels of circulating proinflammatory cytokines and chemokines, which are highly correlated with mortality and the severity of the disease. The mentioned hyper-inflammatory condition is known as “cytokine storm”[4]. The development of a cytokine storm involves numerous cytokines, including members of the interleukin- 1 (IL-1) family, IL-6, IL-8, IL-10, tumor necrosis factor (TNF), and interferon (IFN), though the main pathogenic cytokines seem to vary depending on the condition [5]. Repurposing clinically available medications has been the available option for hindering the ongoing pandemic because new drug development often takes many years [11]. Blocking the cytokines linked to COVID-19's hyper-inflammation is a focused strategy and a possible treatment route [27]. One of the essential mediators of autoimmunity, inflammation, and the cytokine storm is interleukin-6 (IL-6). Tocilizumab, another repurposed candidate, is a recombinant humanized, monoclonal, anti-interleukin (IL) 6 receptor antibody that binds to both membrane-bound and soluble IL-6 receptors,

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preventing the IL-6 receptor from transmitting signal to inflammatory mediators [11]. On June 24, 2021, the FDA issued an Emergency Use Authorization (EUA) for the use of tocilizumab combined with corticosteroids in hospitalized adults and children under the age of two who have COVID-19 and who need additional oxygen, non-invasive ventilation (NIV), mechanical ventilation, or extracorporeal membrane oxygenation. As previously mentioned, several studies have also proven the activity of the suggested formula against several proinflammatory cytokines including IL-6 thus encouraging testing of its efficacy against the COVID-19 virus [28].

Herbal medicines are used as complementary to conventional medicines [29].

Health authorities should grant proof of evidence of the safety, efficacy and quality of herbal medicines before being used by the patients [30, 31].

Based on the Egyptian Drug Authority (EDA), Vanillin and Wheat Germ Oil are considered traditional herbal medicines as they have been used in the Arab Republic of Egypt for a very long period exceeding 15 years.

The requirements of safety for this category include clear identification and characterization of the constituents in the literature with USP standards for Vanillin and European Pharmacopeia for Wheat Germ Oil. In addition, according to regulatory requirements for quality control, purity test was performed indicating acceptable limit of pesticides and heavy metals residues compared to standards.

Finally, method of analysis (gas chromatography) and certificate of raw materials were also provided according tp EDA [32].

Regarding our clinical trial, the formula has remarkedly shown relief of symptoms in a shorter time when added to standard of care treatment in mild to moderate symptoms.

The findings of the recent study found that there was a statistically significant difference between the two arms when comparing the total score of symptoms since day two and along the 14 days of follow up indicating that a 3-day course of treatment was enough to reduce the reported COVID-19 symptoms significantly. All patients were enrolled within 6 days of symptoms onset and treatment was immediately initiated in patients enrolled in the treatment arm. To minimize the factors that would affect the trial's outcome, number of vaccinated patients in the treatment and control arms were almost the same, 80% of patients in the treatment arm were vaccinated compared to 74% in the control arm ($p = 0.806$). Also, there was no difference in symptoms severity represented by the total score between the two arms at day zero.

When we compared the percentage reduction of symptoms between the two arms, it was interesting to find that by day 3 symptoms improved by around 68% in the treatment arm and 30% in the control arm, while after 6 days the percentage improvement was around 56% in the control arm. By day 6 and after the completion of the 5-day treatment course, patients in the treatment arm were almost completely cured as percentage improvement of symptoms was 97.83%.

Knowing the anti-inflammatory effect of the herbal formula, it was expected to find an improvement in constitutional symptoms such as fever, muscle ache, and headaches specifically. Fever was one of the most significant symptoms and an important sign for prognosis. Within only 24 hours 86.7% of patients in treatment arm reported having no fever and within 48 hours, number of patients with fever in the treatment arm was zero indicating that the suggested formula has met the expected outcome considering fever alleviation. When comparing these numbers to another trial assessing the efficacy of ivermectin + doxycycline and ivermectin

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alone it was found that 84.2% and 94.1% of patients in both arms respectively were afebrile by day 7, which is almost 1 week later than the Van-WGO herbal formula [33].

When analyzing and comparing the pattern of muscle aches it was found that by day 2 there was a significant difference in muscle aches reduction between the two arms, as 33.3% of patients in the treatment arm reported having no muscle aches compared to only 3.3% in the control arm. By day 9, zero patients reported having muscle aches in the treatment group compared to 61.3% in the control arm and until day 14, 29% of patients in the control arm still had muscle aches and 3.2% of them reported having severe muscle aches.

Regarding headaches, by day 3 there was a statistically significant difference in the number of patients suffering from headaches when comparing both arms and there was also a difference in severity as only 6.7% in the treatment arm reported having severe headaches compared to 22.6% in the control arm. The overall feeling of tiredness improved significantly on the second day of treatment. When comparing the other remaining 5 symptoms including runny nose, sore throat, cough, SOB and chills, there was a significant difference in symptoms alleviation by day 4 regarding sore throat, SOB and cough.

Taste and smell profiles were analyzed as some patients reported losing the senses of taste and smell. 8 patients have reported loss of taste in the treatment arm and 13 in the control arm. The median time needed for patients in the treatment arm to recover their sense of taste was 5.5 days compared to 9 days in the control arm. When analyzing the pattern of smell loss, 9 patients have reported the inability to smell in the treatment arm while 13 patients lost the sense of smell in the control arm. The median time for recovery was also shorter in the treatment arm (6 days) when compared to the control arm (9 days) showing that the herbal formula did not only improve the common COVID-19 symptoms but also was effective in improving taste and smell senses.

Recovery time is an important factor when questioning the efficacy of the herbal formula. The median recovery time was 6 days in the treatment arms and 10 days in the control arm. The minimum number of days patients needed for recovery was only 2 days in the treatment arm and 6 days in the control arm. When comparing the median number of days for complete recovery to another trial assessing ivermectin, patients taking ivermectin recovered 8 days later (after a total number of 14 days) [34]. All patients in the treatment arm completely recovered by day 10 while it took some patients in the control arm 22 days to report complete recovery. During the 30-day follow-up period, no patients were hospitalized, and no death cases were reported.

No safety concerns were noticed in patients taking the herbal formula, only few patients reported gastrointestinal tract (GIT) side effects that might be related to the COVID-19 virus itself [35].

Also, there were no 30-day hospitalization or mortality in both groups. By day 30 we could conclude that the suggested herbal formula has effectively improved COVID-19 related symptoms and shortened the time needed for recovery without any significant side effect providing a safe available alternative for COVID-19 patients.

Limitations of the present study: The present study could not be tested on severe patients' fear of the fast deterioration of these cases, that's why they were excluded from the study.

Not all patients were able to follow up on lab parameters including CRP, CBC and IL-6 and this is due to different factors and obstacles including the difficulty in transferring samples to the lab for analysis within the required time and patients not being able to

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visit the lab for following up two days later. During the enrollment procedure, blood samples were withdrawn from patients and were separated and transferred to the lab for analysis at the end of the day. This process was not suitable for analyzing IL-6 as the test had to be done immediately. We tried overcoming these obstacles by providing home visits for withdrawing samples, but even this solution was not always suitable as some patients lived hours away from the available labs and transferring samples would take time risking sample spoilage. When analyzing available lab parameters at day zero and 3 days after treatment we found no significant difference between lab parameters.

Strength of the study: The present study represents a new hope for inflammatory symptoms associated with viral diseases. We recommend this natural combination to be extended to other viral or inflammatory disorders to relieve annoying symptoms of inflammation.

5. Conclusion

Among patients with mild and moderate COVID-19, those randomized to a 5-day course of the antiviral capsule with SOC treatment showed a statistically significant difference in clinical status compared with those receiving SOC, with a resolution of all symptoms in a shorter time. These outcomes are endorsed with the quoted antiviral and anti-inflammatory activity for the new formula, as addressing key cytokines in COVID-19 induced inflammatory and immune reactions in the host, so wheat germ oil and vanillin possess potential Anti-COVID-19 activity.

Declarations

Ethical approval and consent to participate

Informed consents were provided by patients. The trial was conducted in accordance with the declaration of Helsinki [26] and good clinical practice guidelines. The protocol was also approved by the ethics committee of Alexandria University, protocol ID:0106922

The study was registered on ClinicalTrials.gov ID (NCT05157139)

Competing interests

The authors declare that they have no competing interests.

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The authors declare that no funding was received for the current study.

Authors' contributions

AIE hypothesized the combination of the natural products and the mechanistic approach in COVID-19 infection, AIB & HHS recruited the patients, HHS followed the patients, AIE, HHS & NAH interpreted the results, HHS drafted the manuscript, AIE, AIB & NAH revised & approved the manuscript.

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