ejpmr, 2022,9(1), 517-527

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

A CLINICAL STUDY TO EVALUATE THE EFFICACY AND SAFETY OF RECOVEREEZ FORTE TM AS AN ADJUNCT TO COVID-19 TREATMENT

Prashanth Varkey M. S M. Ch[1]; Amita Ajit M. Tech PhD[1]*, Aimy Hynse M.Sc[1], Aparajita Kumar[1] M. Sc and Matthan Tharakan[2]

[1]Research and Development, Zum Heilen Diagnostic and Therapeutics Pvt. Ltd, Science &Technology Park, Savitribai Phule Pune University Campus, Pune University Road, Ganeshkhind, Pune, Maharashtra 411007, India.
[2]College of Engineering and Natural Sciences, the University of Tulsa, 800 S. Tucker Drive, Tulsa, Oklahoma 74104, USA.

***Corresponding Author: Dr. Amita Ajit M. Tech PhD**
Research and Development, Zum Heilen Diagnostic and Therapeutics Pvt. Ltd, Science &Technology Park, Savitribai Phule Pune University Campus, Pune University Road, Ganeshkhind, Pune, Maharashtra 411007, India.

Article Received on 21/10/2021 **Article Revised on 11/11/2021** **Article Accepted on 01/01/2022**

ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) provokes demanding immune and inflammatory events. Despite numerous reports on the use and testing of several potent therapeutic options against coronavirus disease 2019 (COVID-19), satisfactory treatment has not so far been determined. RECOVEREEZ FORTE ™, consisting of a standardized cardamom extract is a natural product with substantial indications of immunomodulatory and anti-inflammatory actions along with inhibitory capacity averse to viral targets. In this context, we speculated that RECOVEREEZ FORTE ™ may ameliorate adverse effects in COVID-19 patients. Accordingly, in a multicenter prospective, open label, randomized trial, adult COVID-19 patients having mild to moderate symptoms were treated with RECOVEREEZ FORTE ™ as an adjunct therapy. Patients were assigned to obtain standard of care along with a three times per day oral dose of 500 mg of RECOVEREEZ FORTE ™ for ten days, or standard of care alone. Standard of care comprised all essential interventions, as per the discretion of the attending physician. Time to clinical improvement in terms of biochemical parameters such as IL6, CRP, D-DIMER, LDH and an RTPCR COVID-19 test negativity in the treated patients was considered to be the primary end point. We enrolled 60 patients; of which 30 were allocated to RECOVEREEZ FORTE ™ and 30 to the control group. The duration of COVID-19 positivity post-intervention was significantly shorter in RECOVEREEZ FORTE ™ treated group than in the control group ($p<0.001$). RECOVEREEZ FORTE ™ group also showed significant decrease in the levels of IL6, LDH, D-dimer along with increased lymphocytes confirming its immunomodulatory and anti-inflammatory action. We conclude that RECOVEREEZ FORTE ™ can reduce the impact of COVID-19 manifestations and could serve as an alternative to oral steroids. We also suggest that RECOVEREEZ FORTE ™ may aid faster recovery mediated by its immunomodulatory and anti-inflammatory action in diseases other than COVID-19. The trial was registered in Clinical Trials Registry India (CTRI - CTRI/2021/04/033143) in compliance with the International Council on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use – Good Clinical Practice (ICH–GCP) guidelines.

KEYWORDS: RECOVEREEZ FORTE ™, COVID-19, Anti-inflammatory action, Immunomodulatory action, Anti-viral Activity, Cardamom extracts.

1. INTRODUCTION

COVID-19 has presented unrivalled adverse bearings on health care increasing the global economic burden. Identifying appropriate therapeutic drugs that can prevent, treat or reduce the COVID-19 manifestation still remains on demand. Our research team's investigations on the potential of cardamom extract to curb inflammation and render antimicrobial effects in topical wound application came handy at the time of COVID-19 pandemic. We thus redirected our investigations on whether the bioactive compounds of cardamom extract could be exploited in bringing about clinical benefits in COVID-19 patients. In the context of COVID-19, various studies report the cytokine storm as an exaggerated immune response, closely linked with its progression and associated severity. Interventions that could possibly discourage or extinguish the cytokine storm have thus gained attention.[1] The utility of cardamom extracts in ameliorating the inflammatory cytokine storm has been reported demonstrating regulation of oxidative stress by scavenging reactive oxygen species (ROS),[2] attenuation of inflammation by downregulating NF-κB, the key regulator of inflammatory disease pathogenesis.[3] and by regulation of NO (nitric oxide) synthesis.[1] These therapeutic effects especially in respiratory ailments like asthma and

COPD have been accounted to the predominant bioactive compounds in cardamom such as alpha terpineol,[4] and 1,8-cineole.[5,6] Reports also show the inhibitory action of 1,8-cineole against pro-inflammatory agents such as cyclooxygenase (COX-1, COX-2) and lipoxygenase.[7] which further substantiates the anti-inflammatory action of cardamom.[8] In addition to targeting the anti-inflammatory and immunomodulatory effects, medicinal interventions that could possibly block the SARS-CoV-2 viral spike protein interaction with cellular angiotensin-converting enzyme 2 (ACE2) that permits SARS-CoV-2 to attach and enter the host cells are looked upon.[9,10] Molecular docking reports have demonstrated that alpha-terpinyl acetate can bind with the ACE2 receptor while 1,8-cineole can bind with Mpro, a SARC-CoV-2 proteinase and thus can inhibit viral reproduction.[11,12] Therefore, based on thorough scientific and critical evaluation of previously published reports promising the potentialities of cardamom extract as an anti-viral, anti-oxidant, anti-inflammatory and immunomodulatory agent, we developed a standardized cardamom extract-based food supplement product (RECOVEREEZ FORTE ™) that merits testing.

1.1. Pre-clinical Evidence

Preclinical studies using molecular docking, *in vitro* cytotoxicity and anti-inflammatory assays have demonstrated the antiviral and anti-inflammatory properties of RECOVEREEZ FORTE ™. The molecular docking *in silico* study evaluated the effect of alpha-terpinyl acetate on the ACE-2 receptor indicating effective binding of the ligands representing its ant-viral potential [**Supplementary Fig 1**]. Molecular docking was conducted using AutoDock Vina 4.2 and Desmond 2020.1 tools. Protein interaction mode was calculated using the PRODIGY-LIG tool. *In vitro cytotoxicity* was performed as per standard protocol on L929 (Fibroblast) cell lines procured from National Centre for Cell Sciences (NCCS), Pune, India and the cell viability was evaluated by MTT assay.[13] **[Supplementary Fig 2].** The LC50 value was determined as 328.999 µg/mL using ED50 PLUS V1.0 software). *In vitro* anti-inflammatory assays were carried out on RAW 264.7 cells procured from National Centre for Cell Sciences (NCCS), Pune, India. Cells were grown to 60% confluency followed by activation with 1 µL lipopolysaccharide (LPS: 1µg/mL). LPS-stimulated RAW cells were exposed to different concentrations (25, 50, 100 µg/mL) of RECOVEREEZ FORTE ™ and diclofenac sodium, a standard anti-inflammatory drug for 24h. Anti-inflammatory assays targeting COX, LOX, MPO, iNOS and nitrite levels were performed using the cell lysate post 24h as per published protocols.[14,15] RECOVEREEZ FORTE ™ showed significant dose dependent effect in inhibition of COX, LOX, MPO, iNOS and nitrite levels **[Supplementary Fig 3].**

Due to the therapeutic evidences of the bioactive compounds in cardamom, based on *in vitro* research, we hypothesized that RECOVEREEZ FORTE ™ could ameliorate the clinical effect of COVID-19 through anti-inflammatory and immunomodulatory effects, without interfering with other treatment options. Thus, to evaluate the efficacy and safety of RECOVEREEZ FORTE ™ for SARS-CoV-2 infection, we conducted a multicenter prospective, open label, randomized trial in Vishwanand Kendra and Shree Sai Hospital Pune, Maharashtra, India, in mild to moderately affected COVID-19 patients.

2. METHODOLOGY

2.1. Trial Design

This study was a multicenter, open-label, randomized, controlled trial conducted from May 2021 through July 2021. Due to the urgent nature of the trial, placebos were not prepared. The protocol was approved by the Orchid Specialty Hospital Ethics committee, and the trial was registered in Clinical Trials Registry India (CTRI - CTRI/2021/04/033143) in compliance with the International Council on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use – Good Clinical Practice (ICH–GCP) guidelines. All participating patients were informed about the objectives and risks of participation and gave written informed consent. Eligible patients were randomly assigned in a 1:1 ratio to receive RECOVEREEZ FORTE ™ capsules produced for ten days at 500 mg, thrice a day plus standard care or standard of care alone (control group). RECOVEREEZ FORTE ™ comprises cardamom extract at 200mg, rosemary extract at 200mg; and pepper extract at 10mg accounting to a total 1,8 cineole content of 97 mg per capsule. The total sample size for the study was 60 with 30 subjects enrolled in each group. The decisions on standard supportive treatment were made by the attending physicians, who were not involved in the study design or in the randomization process. Standard of care in the treatment group and control group was as per government public health standards defined from time to time. The control group patients had the same with addition of oral steroids in a tapering manner for 10 days of the study period. Patients were assessed daily for a period of 10 days. Patients who become COVID-19 negative but were not available for follow up tests till the 10th day were not considered in the data analysis.

2.2. Randomization

Computer generated randomization using case record forms was performed. The data analysis was carried out with external statistical support and in an impartial manner.

2.3. Outcome Measures

The major primary endpoint was to observe negativity in COVID-19 RTPCR test to evaluate the effectiveness of RECOVEREEZ FORTE ™ in treating SARS CoV-2 patients. Additionally, the serum levels of IL6, CRP, D-DIMER and LDH were assessed to observe the inflammatory changes in relation to the primary endpoint.

Secondary endpoints were the adverse events (AEs), frequency and severity at day 5 and day 10, number of subjects who discontinued study due to adverse events at day 5, changes in vital parameters and safety laboratory parameters (liver function test, renal function test, serum electrolyte) from baseline to day 10. Safety outcomes included adverse events that occurred during treatment, serious adverse events, and premature or temporary discontinuation of treatment.

2.4. Patients

Patients over 18 years to 75 years of age of either sex, diagnosed with SARS-CoV-2 infection, confirmed by polymerase chain reaction–reverse transcriptase testing, having mild to moderate disease (NEWS score less than/equal to 8) who can take oral medicines were considered eligible for randomization. Exclusion criteria included patients of age less than 18 years and more than 75 years, patients with a COVID-19 positive test done more than 24 hours prior to enrolment in study, pregnancy and lactation, severe or complicated course of COVID-19 disease, presence of acute hypoxic respiratory failure/ need for intensive care unit (ICU) stay/ patients who need mechanical ventilation, any uncontrolled systemic disease/infection, those with serious cardiovascular, cerebrovascular, respiratory, liver or renal disease or any other disorder /conditions, which in the opinion of the investigators made the patient unsuitable for enrolment or could interfere in adherence of the study protocol such as impossibility of using the medication orally.

2.5. Statistical Analysis

The statistical analysis was performed by IBM SPSS 26.0 software. Categorical variables are expressed using frequency and percentage. Continuous variables are presented by mean and standard deviation. Statistical significance of difference in proportion of categorical factors between groups (treatment and control) was performed using chi square χ^2 test along with Pearson χ^2 testto assess association between the treatment arms and COVID-19 negativity. To find the probability of overall survival time of the event negativity in COVID-19 between days (D0-D5 and D0-D10), Kaplan -Meier (KM) analysis was used and comparison was done by log rank test.To test statistical significance in the reduction percentage between groups, chi square χ^2 test along with Fishers Exact test was used for parameters i.e IL6, CRP, D-DIMER and LDH. To test the statistical significance of the changes in the mean of continuous variables between pre and post findings, a paired t test was applied in the case of lymophycyte and total White Blood Cell count analysis. ANOVA along with Dunnet Multiple Comparison Test was performed to analyze *in vitro* cytotoxicity and *in vitro* anti-inflammatory assays.

3. RESULTS

3.1. Patients

Of the 80 patients who were screened for eligibility, 60 met inclusion criteria and underwent enrolment with randomization. 30 patients were allocated to get RECOVEREEZ FORTE ™ (500 mg, three times per day) and 30 to get standard of care alone (control group). The mean (± standard deviation) age of patients in this trial was 39.67± 12.62 years while 53.3% of the patients were men. Follow-up information at day 10 after admission for the primary outcome was complete for all 60 patients. However, all patients in the control group had received oral Prednisolone 10mg three times daily for the first three days which was tapered to 10 mg twice daily for the next three days and 10mg once a day for the subsequent three days.

3.2. Primary Outcomes

Patients who turned COVID-19 negative were significantly higher in groups receiving RECOVEREEZ FORTE ™ than in the control group. On 5th Day, 18 (60%) patients in the treatment group tested COVID-19 negative while only 7(23.37%) patients in the control group tested negative (p-value= 0.004), which is statistically significant. Subsequently, on the 10th day, only 13(43.3%) patients became negative in the control group, whereas 29 (96.7%) patients in the treatment group were tested negative confirming a statistically significant reduction in test positivity (p-value = < 0.001) [**Table 2**], supporting the efficacy of RECOVEREEZ FORTE ™ to hasten recovery. **Figure 1 (A) shows that t**he mean overall survival time in days for D0 to D5 was 6.857 ± 0.172, with a 95% confidence interval of 6.5 and 7.2 days. Here, the mean survival time of Recovereez Forte ™ group was 6.182 ± 0.168 days, with a 95% confidence interval of 5.9 and 6.5 days while the mean survival time of Control was 7.374 ± 0.209 days with a 95% confidence interval of 6.9 and 7.8 days, which is statistically significant (p-value = 0.010). In terms of D0 to D10 [**Figure 1(B)],** the mean overall survival time in days was 11.999 ± 0.286, with a 95% confidence interval of 11.4 and 12.6 days. Here, the mean survival time of Recovereez Forte™ group was 10.267 ± 0.205 days with a 95% confidence interval of 9.9 and 10.7 days while the mean survival time of the Control group was 13.735 ± 0.275 days, with a 95% confidence interval of 13.2 and 14.3 days, showing statistical significance (p-value = <0.001).

Patients assigned to the RECOVEREEZ FORTE ™ treatment showed regulated expression of IL6 in the plasma. On account of percentage of patients who had reduction from the pool randomized to test and control treatment, a 56.7% reduction in IL-6 is seen in the treatment group from D0 to D5, compared to the 43% reduction in the control group. Whereas from D0 to D10 in the treatment group, a 56.7% reduction is seen compared to the 50% reduction in the control group, indicating potential superiority of RECOVEREEZ FORTE ™ in regulating IL-6 as compared to steroids

[**Figure 2 (A)**]. The initial CRP levels of both control and treatment groups on Day 0 were observed to be elevated from the normal reference range. During the course of treatment, a comparable reduction pattern in patients were seen in D0 to D10 further indicating the action of RECOVEREEZ FORTE ™ to be non-inferior to orally administered steroids [**Figure 2(B)**].

In terms of regulating LDH, 63.3% reduction in D0 to D5 in treatment group compared to the control group that showed only a 23.3% reduction (p value = 0.003), 53.3% reduction from D5 to D10 in treatment group compared to the control group showed only a 36.7% reduction. In the D0 to D10 period, 53.3% the treatment group showed reduction as compared to 23.3% in the control group (p value = 0.032) [**Figure 2(C)**]. This may propose that RECOVEREEZ FORTE ™ aided regulation of the LDH levels indicating a reduced risk in developing lung damage. **Figure 2(D)** depicts the percentage of patients that showed reduction in the D-Dimer levels in both groups. A visible reduction in the D-dimer levels of the RECOVEREEZ FORTE ™ treated group was observed with 56.7% at D0 to D5, 46.7% at D5 to D10 and 46.7% from D0 to D10 in comparison to the control group that showed 33.3% from D0 to D5, 36.6% from D5 to D10 and 33.3% from D0 to D10.

3.3. Secondary Outcomes

Figure 3(A) shows the total WBC count over the period of the treatment course. It was observed that the RECOVEREEZ FORTE ™ treatment group had an initial total WBC count of 5384.33 ± 2204.29 cells/cmm on Day 0, to 6976.33 ± 2444.58 cells/cmm on Day 5, to 7169.0 ± 2335.37 cells/cmm on Day 10 which remained within the normal range. On the other hand, an elevation in the total WBC count in the control group was observed from 5770.67 ± 1642.27 cells /cmm on Day 0 to 9010.79 ± 3728.32 cells /cmm on Day 5 to 10692.41 ± 4048.45 cells/cmm on Day 10 crossing the normal range. Furthermore, a statistically significant elevation in the WBC count was observed on Day 5 and Day 10 in the control group when compared to the treatment group (p value = <0.001). In terms of lymphocyte count [Figure 3(B)], the control group showed a lower percentage of lymphocytes throughout the course of treatment ranging from 25.38 ± 10.52 % on Day 0 to 24.90 ± 11.16 % on Day 5 and 24.85 ± 10.42 % on Day 10. In the RECOVEREEZ FORTE ™ treatment group, the lymphocyte percentage ranged from 38.52 ± 12.04 % on Day 0 to 37.30 ± 10.89 % on Day 5 to 34.62 ± 6.48 % on Day 10.

3.4. Safety Outcomes

No patient enrolled in the RECOVEREEZ FORTE ™ group discontinued due to side effects. Gastrointestinal adverse events like gastroesophageal reflux were observed in patients treated with RECOVEREEZ FORTE ™.

4. DISCUSSION

Through this randomized clinical trial, we found that oral administration of RECOVEREEZ FORTE ™ for 10 days was safe and beneficial. As mentioned earlier, the ACE-2 receptor present on human lung epithelial cells is a major target for novel antiviral drugs against SARS-CoV-2.[16] Molecular docking experiments carried on alpha-terpinyl acetate, a major compound present in cardamom extract against the ACE-2 receptor demonstrated hydrophobic alkyl bond interactions with a binding energy of -7.2 kcal/mol depicting potent antiviral action. We presume the molecular docking inference to justify the clinical benefits in the RECOVEREEZ FORTE ™ treated group which showed that 60% of patients tested COVID-19 negative by the 5th day of treatment and a 96.7% testing negative by the 10th day accounting to a much faster and higher percentage of recovery as compared to the control group. In comparison with published reports on randomized trials using other antiviral drugs such as Oseltamivir, Ribavirin, and Remdesivir, which took 7 days,[17] 12.8 ± 4.1 days.[18] and 10 days.[19] respectively for patients to test SARS-CoV-2 negative, the potential of RECOVEREEZ FORTE ™ stands superior in terms of a shortened negative test time. Moreover, evidence on the negative effects of pro-inflammatory molecules in COVID-19 through stimulated cytokine storms and oxidative stress are often linked to poor outcomes in patients.[20,21,22,23] Inhibition activity of these molecules is thus looked into as promising therapeutic effects in COVID-19 prognosis. The *in vitro* anti-inflammatory potential established by RECOVEREEZ FORTE ™ through its inhibitory action against COX, LOX, MPO and iNOS was further validated in the clinical perspective. Although the individual components of cytokine storm are varied, IL-6 has become one of the major cytokines in the context of SARS-CoV-2 infection after being found as the most important predictor of mortality in COVID-19 patient survival studies.[24] There is also evidence that the severity of COVID-19 infection is connected to circulating IL-6 levels.[25] Similarly, IL-6 levels in the blood have been linked to the onset of respiratory failure in several studies.[26] Patients treated with RECOVEREEZ FORTE ™ showed regulated IL-6 levels that were maintained within the normal range of 0.00 – 7.00 pg/mL and a significant number of patients showed reduction in IL-6 on D5 in comparison to the control group, indicating its potential superiority to orally administered Prednisolone. Additionally, serum lactate dehydrogenase (LDH) and lymphocyte count are accessible biomarkers that correlate with the severity of COVID-19, the need for hospitalization and mortality, reflecting the host immune response's contribution to the seriousness of SARS-CoV-2 infection.[27,28] LDH is often looked at as a marker of lung damage.[29] It was observed that the serum LDH levels in the RECOVEREEZ FORTE™ treated group showed significant reduction in patients throughout the course of treatment in comparison to the control group that received oral Prednisolone. Moreover, on considering the pivotal role

that lymphocytes play in maintaining immune homeostasis and the inflammatory response, the observation that RECOVEREEZ FORTE ™ treated patients showed elevated numbers of lymphocytes compared to the control group, correlates to a milder disease state. Thus, RECOVEREEZ FORTE ™ could be a potent candidate to prevent the ill-effects that lymphopenia brings about in COVID-19. Therefore, a decreased LDH and an elevated lymphocyte count in the RECOVEREEZ FORTE ™ treatment group are hallmarks of recovery. Similarly, plasma c-reactive protein (CRP) and D-dimer levels are positively correlated to the severity of COVID-19 and are used as an effective prognostic marker and prompt indicator for severe illness.[30,31] CRP enhances inflammation by elevating TNF-α and IL-6 levels,[32] while elevated D-Dimer levels are related to the inflammation in COVID-19 infected patients and their limited prediction to thrombosis.[33] The observations in this study correlates to the synchronized relation of D-Dimer with CRP levels in the control and treatment groups. The RECOVEREEZ FORTE ™ treatment group showed a better synchronized pattern in the percentage of patients with reduction in CRP levels on Day 5 and Day 10 to their reduction in D-dimer levels as compared to the control group, demonstrating good clinical prognosis. Additionally, in COVID-19 infection, studies have reported a drastic increase in white blood cell count, especially in critical groups compared to severely affected patients.[34] A significant increase in WBC count is associated with mortality and must be given more attention during treatment.[35] Complying with existing literature, patients in both groups recorded an increase in their total white blood cell count on Day 5. However, the average WBC counts of the patients in each group showed that, at the peak of infection on Day 5, the control group (9010.79 ± 3728.32 cells/cmm) showed a higher cell count compared to the RECOVEREEZ FORTE ™ treated group (6967.33 ± 2444.58 cells/cmm). Our study has numerous limitations. The trial had only 30 patients in the treatment group, demanding careful interpretation and generalization regarding the findings. Likewise, the patients were followed only for a short period, restraining the prospect of assessing long-term benefits.

In conclusion, RECOVEREEZ FORTE ™ has yielded promising anti-viral, immunomodulatory and anti-inflammatory effects, suggesting its ability to combat the cytokine storm that is elicited in the COVID-19 infection. Additionally, the comparative analysis of the treatment and control outcomes do indicate the promising use of RECOVEREEZ FORTE ™ as an alternative to oral short course Prednisolone. The inclusion of oral RECOVEREEZ FORTE ™ to the standard of care treatment regimen was also established to be safe with clinical benefits for the COVID-19 patients, evidenced by a shortened recovery time. Based on our findings in D-dimer reduction, the intake of RECOVEREEZ FORTE ™ post recovery seems encouraging to manage post Covid-19 complications such as pulmonary thrombosis. Future studies can further assess the impact of RECOVEREEZ FORTE ™ on other systemic diseases where inflammation is profound and where immunomodulation at onset of the disease is demanded. Based on our findings, the use of RECOVEREEZ FORTE ™ can be considered as an adjuvant in the treatment of COVID-19 patients and may be explored for therapeutic benefits in diseases other than COVID-19.

Ethics approval and consent to participate (Human Ethics, Animal Ethics or Plant Ethics)

Prior to the study, the protocol was approved by the Orchid Specialty Hospital Ethics committee, and the trial was registered in Clinical Trials Registry India (CTRI - CTRI/2021/04/033143) in compliance with the International Council on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use – Good Clinical Practice (ICH–GCP) guidelines. All participating patients were informed about the objectives and risks of participation and gave written informed consent.

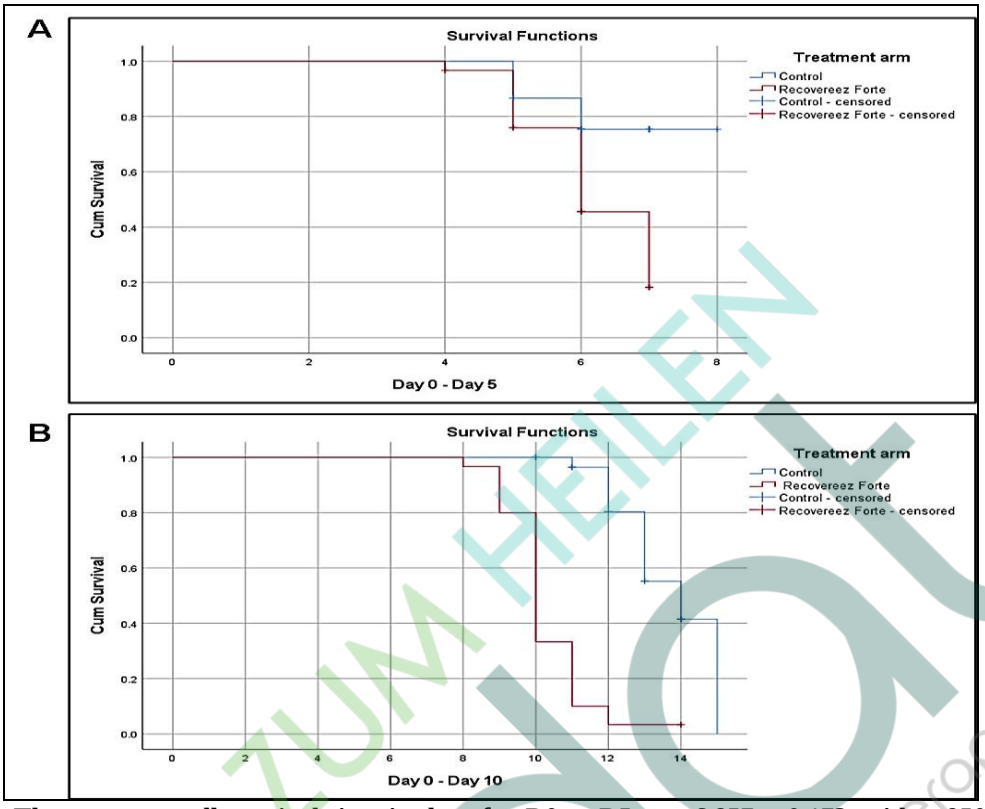


Figure 1: (A) The mean overall survival time in days for D0 to D5 was 6.857 ± 0.172, with a 95% confidence interval of 6.5 and 7.2 days. Here, the mean survival time of Recovereez Forte ™ group was 6.182 ± 0.168 days, with a 95% confidence interval of 5.9 and 6.5 days while the mean survival time of Control was 7.374 ± 0.209 days with a 95% confidence interval of 6.9 and 7.8 days, which is statistically significant (p-value = 0.010).(B) In terms of D0 to D10 , the mean overall survival time in days was 11.999 ± 0.286, with a 95% confidence interval of 11.4 and 12.6 days. Here, the mean survival time of Recovereez Forte™ group was 10.267 ± 0.205 days with a 95% confidence interval of 9.9 and 10.7 days while the mean survival time of the Control group was 13.735 ± 0.275 days, with a 95% confidence interval of 13.2 and 14.3 days, showing statistical significance (p-value = <0.001).

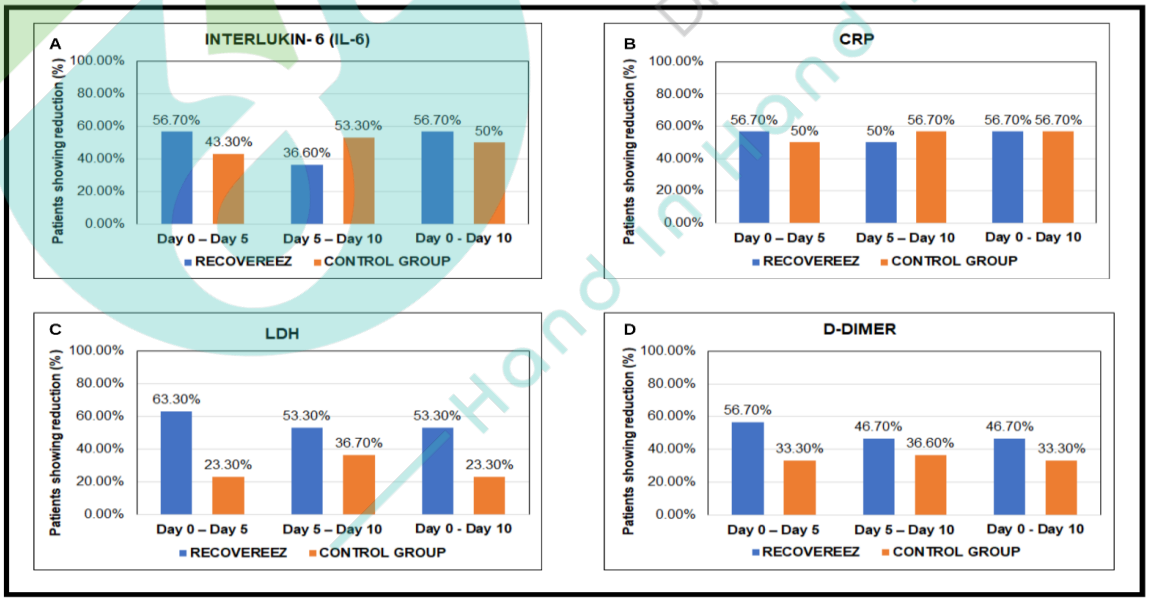


Figure 2: *Anti-inflammatory and Immunomodulatory Effect of RECOVEREEZ FORTE ™:* (A) Percentage of patients showing reduction in IL-6 Levels (B) Percentage of patients showing reduction in CRP Levels (C) Percentage of patients showing reduction LDH Levels (D) Percentage of patients showing reduction in D-Dimer levels.

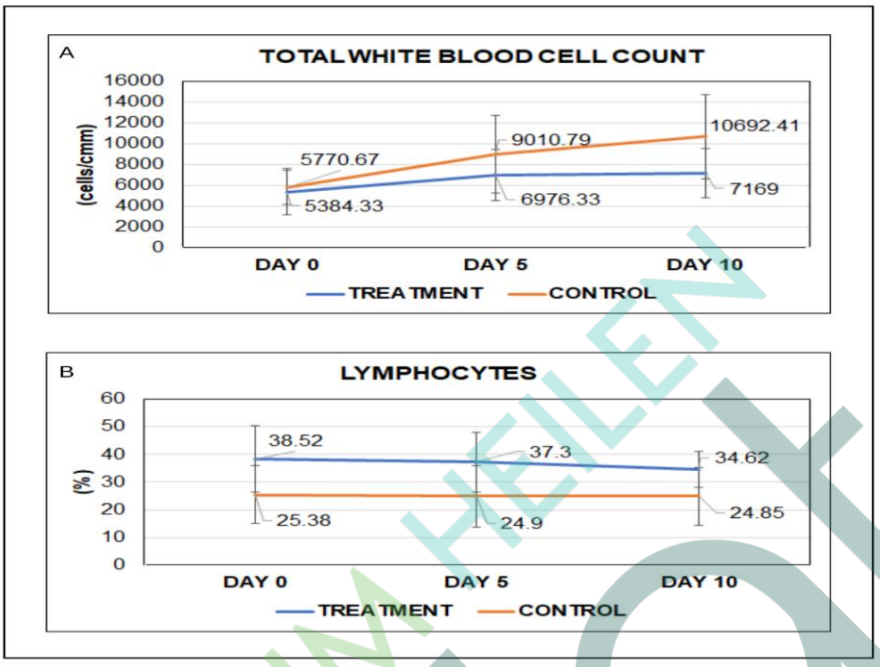
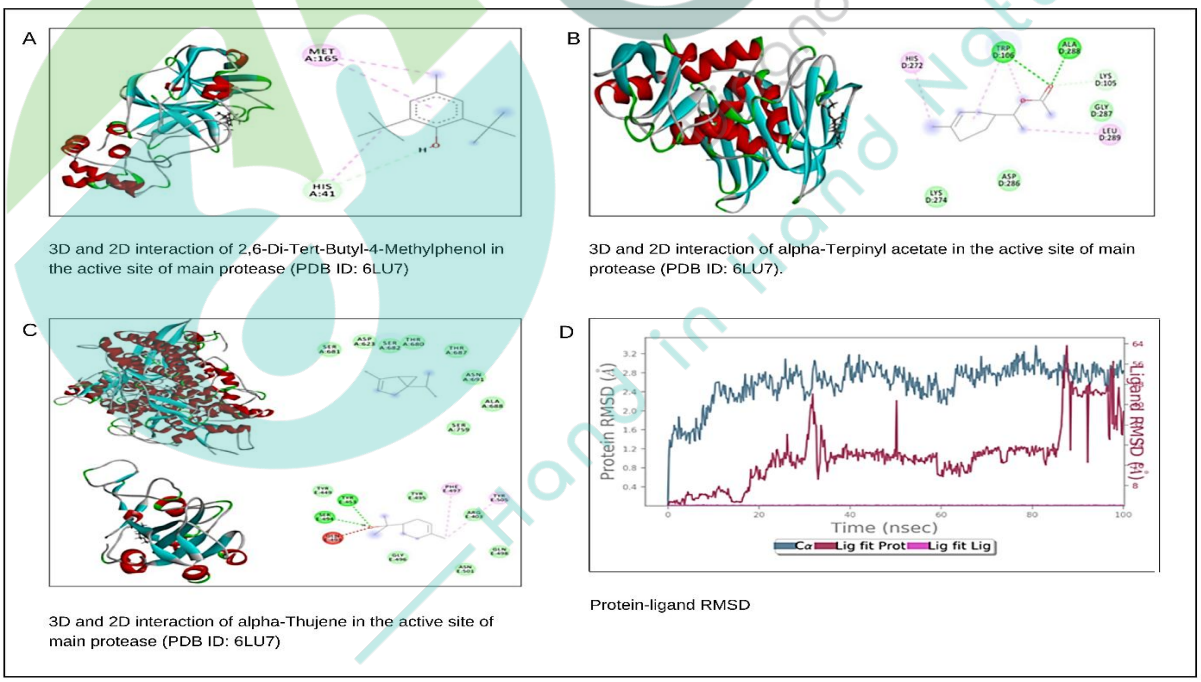
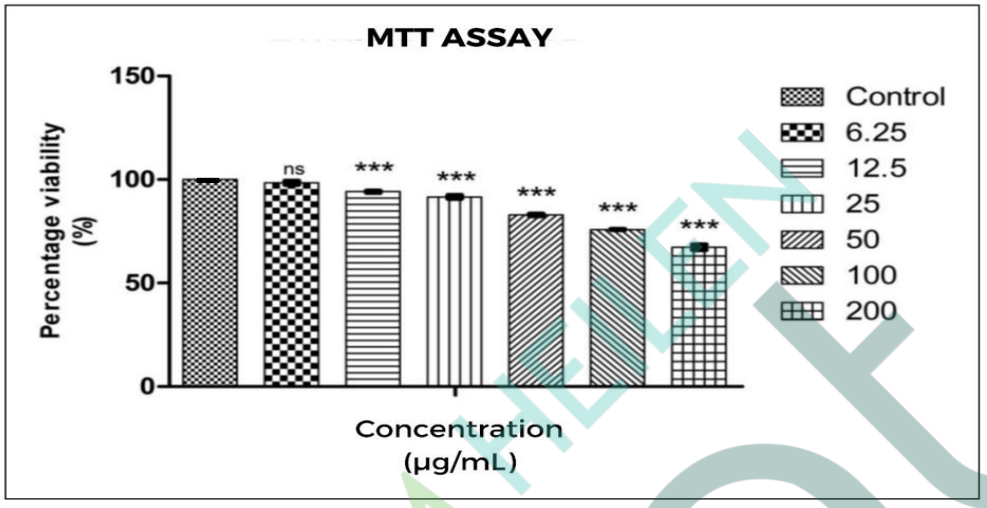


Figure 3: ***Trends in WBC and Lymphocyte count over the duration of treatment*****: (A) Patients treated with RECOVEREEZ FORTE ™ showed a regulation in WBC count from 5384.33 ± 2204.29 cells/cmm on Day 0, to 7169.0 ± 2335.37cells/cmm on Day 10 falling within the normal range. Control subjects showed an elevation in cell counts from 5770.67 ± 1642.27 cells/cmm on Day 0 to 10692.41 ± 4048.45 cells/cmm on Day 10 crossing the normal range. (B) The RECOVEREEZ FORTE ™ treated group maintained a higher percentage of lymphocytes compared to the control subjects, throughout the course of treatment. The control values ranged from 25.38 ± 10.52 % on Day 0 to 24.90 ± 11.16 % on Day 5 and 24.85 ± 10.42 % on Day 10 while the treatment subjects recorded a lymphocyte percentage from 38.52 ± 12.04 % on Day 0 to 37.30 ± 10.89 % on Day 5 to 34.62 ± 6.48 % on Day 10.**

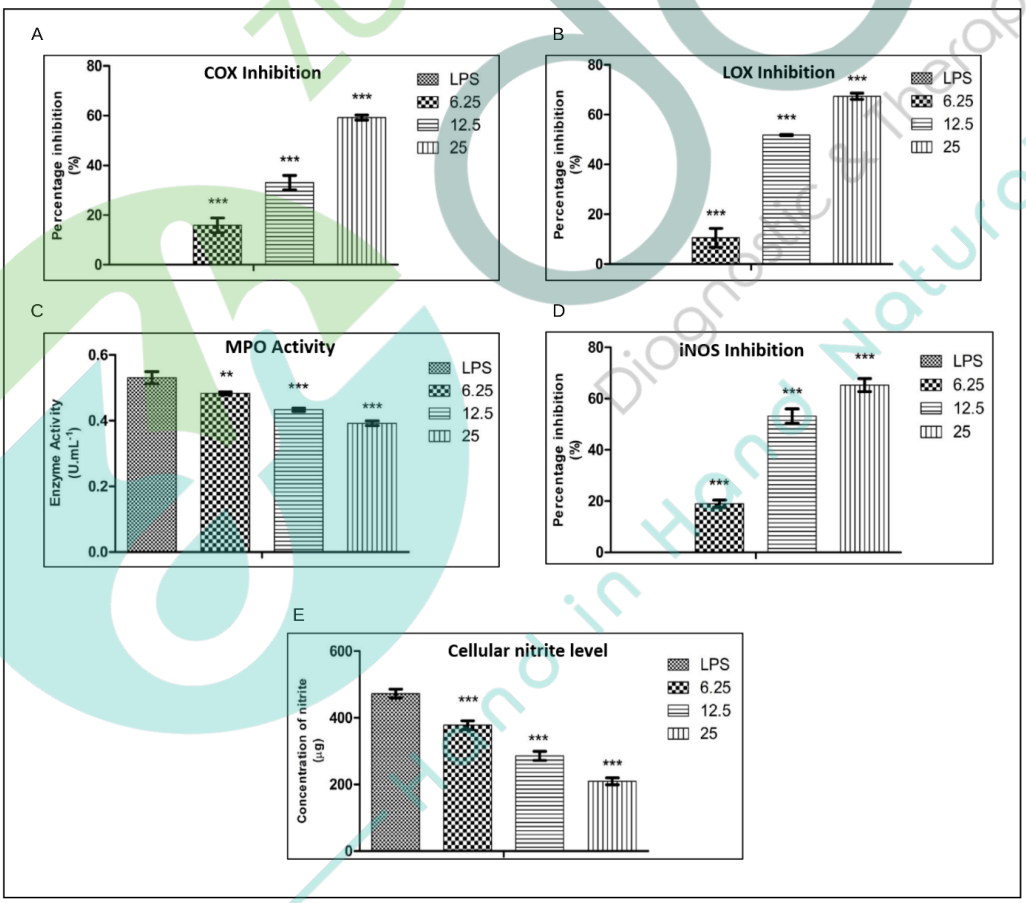


Supplementary Figure 1: ***Molecular Docking:*** **The energy minimized structure of alpha-Terpinyl acetate obtained was used for docking with the receptor binding domain of SARS-CoV-2. (A) 3D and 2D interaction of 2,6-Di-tert-butyl-4-methylphenol in the active site of main protease (PDB ID: 6LU7).(B) 3D and 2D interaction of alpha-Terpinyl acetate in the active site of the main protease (PDB ID: 6LU7). (C) 3D and 2D interaction of**

alpha-Thujene in the active site of main protease (PDB ID: 6LU7). (D) The plot shows the RMSD evolution of a protein (left *Y*-axis).



Supplementary Figure 2: In vitro Cytotoxic assay: Graphical representation of cytotoxicity of cardamom extract at concentrations of 6.25 µg/mL, 12.5 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL. (P value <0.0001).



Supplementary Figure 3: In vitro assessment of anti-inflammatory activity of cardamom extract in RAW 264.7 cells :(A) COX inhibition (B) LOX inhibition (C) MPO activity (D) iNOS inhibition (D) cellular nitrite level. (P value <0.0001).

Table 1: trial enrollment fraction according to age and sex (a) average and standard deviation of participants' ages.

	N	Minimum	Maximum	Mean	Std. Deviation
Age	60	19	74	39.67	12.616

(B) Participant Distribution Based On Gender.

VARIABLES	N (60)	Percentage (%)	
Gender	**Female**	28	46.7
	Male	32	53.3

Table 2: RT-PCR Positivity In Patients With Covid-19.

Study Trial Groups	DAY 5	DAY 10		
	COVID-19 Positivity	COVID-19 Negativity	COVID-19 Positivity	COVID-19 Negativity
Control	23 (76.7%)	7 (23.37%)	17 (56.7%)	13 (43.3%)
RECOVEREEZ FORTE™	12 (40.0%)	18 (60.0%)	1(3.3%)	29 (96.7%)
P-value	0.004	<0.001		

Table 1: Percentage Of Covid-19 Patients Showing Clinical Parameters In The Normal Range During The Course Of Treatment.

VARIABLES		CONTROL (30)			TREATMENT (30)		
		DAY 0	DAY 5	DAY 10	DAY 0	DAY 5	DAY 10
D-Dimer (ng/ml)	Normal (0 – 500)	28 (93.33%)	27 (90%)	25 (83.33%)	26 (86.67%)	25 (83.33%)	26(86.67%)
CRP (mg/L)	Normal (0 – 6)	21 (70%)	21 (70%)	24 (80%)	20 (66.67%)	23 (76.67%)	28 (93.33%)
LDH (U/L)	Normal (230 – 460)	17 (56.67%)	19 (63.33%)	17 (56.67%)	14 (46.67%)	17 (56.67%)	20 (66.67%)
Interleukin-6 (IL-6)	Normal (0.00 – 7.00)	17 (56.67%)	17 (56.67%)	19 (63.33%)	25 (83.33%)	26 (86.67%)	29 (96.67%)
Total White Blood Cell Count (/cmm)	Normal (4000 – 10000)	26 (86.67%)	19 (63.33%)	16 (53.33%)	20 (66.67%)	25 (83.33%)	25 (83.33%)
Lymphocytes (%)	Normal (20.0 - 40.0)	22 (73.33%)	19 (63.33%)	18 (60%)	15 (50%)	15 (50%)	23 (76.67%)

Competing interests

Dr. Prashanth is the Director of Zum Heilen Diagnostic & Therapeutics Pvt. Ltd.

Funding

This research was funded and supported by Zum Heilen Diagnostic & Therapeutics Pvt. Ltd.

Author Contributions

Prashanth Varkey designed the trial and was the principal investigator, with overall responsibility for conducting the trial and for medical oversight of trial implementation. Amita Ajit wrote the final report, contributed to the trial design, coordination of participants' data and reviewed the interpreted data. Aimy Hynse organized and performed the data analysis. Aparajita Kumar contributed in drafting the manuscript and interpretation of the data. All authors reviewed the final report. Matthan Tharakan verified the statistics data, its interpretation and representation.

ACKNOWLEDGMENTS

The authors thank the patients for their willingness to be part of this trial and also the treating clinicians; Dr. Ajit Mandlecha, Dr. Gous Mujawar and Dr. Aslam for their valuable contributions. The authors thank Zum Heilen Diagnostic & Therapeutics Pvt. Ltd. for the financial support in conducting this trial and for providing RECOVEREEZ FORTE ™. The authors also thank the staff of Vishwanand Kendra and Shree Sai Hospital, Pune, Maharashtra India for their assistance in conducting the study.

REFERENCES

1. A.B. Kunnumakkara, V. Rana, D. Parama, K. Banik, S. Girisa, H. Sahu, K.K. Thakur, U. Dutta, P. Garodia, S.C. Gupta, B.B. Aggarwal, COVID-19, cytokines, inflammation, and spices: How are they related?, Life Sci. (2021). https://doi.org/10.1016/j.lfs.2021.119201.
2. S. Ryu, H. Park, G.H. Seol, I.-Y. Choi, 1,8-Cineole ameliorates oxygen-glucose deprivation/reoxygenation-induced ischaemic injury by reducing oxidative stress in rat cortical neuron/glia, J Pharm Pharmacol. 66 (2014) 1818–1826. https://doi.org/10.1111/jphp.12295.
3. L.J. Juergens, K. Racké, I. Tuleta, M. Stoeber, U.R. Juergens, Anti-inflammatory effects of 1,8-cineole

(eucalyptol) improve glucocorticoid effects in vitro: A novel approach of steroid-sparing add-on therapy for COPD and asthma?, Synergy. 5 (2017) 1–8. https://doi.org/10.1016/j.synres.2017.08.001.
4. G. Horváth, K. Ács, Essential oils in the treatment of respiratory tract diseases highlighting their role in bacterial infections and their anti-inflammatory action: a review, Flavour and Fragrance Journal. 30 (2015) 331–341. https://doi.org/10.1002/ffj.3252.
5. H. Worth, C. Schacher, U. Dethlefsen, Concomitant therapy with Cineole (Eucalyptole) reduces exacerbations in COPD: a placebo-controlled double-blind trial, Respir Res. 10 (2009) 69. https://doi.org/10.1186/1465-9921-10-69.
6. U.R. Juergens, Anti-inflammatory properties of the monoterpene 1.8-cineole: current evidence for co-medication in inflammatory airway diseases, Drug Res (Stuttg). 64 (2014) 638–646. https://doi.org/10.1055/s-0034-1372609.
7. A.-M. Beer, P. Zagorchev, M. Draganova-Filipova, J. Lukanov, Effects of 1,8-Cineole on the Activity of Cyclooxygenase and Cyclooxygenase 1 and Cyclooxygenase 2 Isoforms, Natural Products Chemistry & Research. 05 (2017). https://doi.org/10.4172/2329-6836.1000253.
8. A. Sengupta, S. Ghosh, S. Bhattacharjee, Dietary cardamom inhibits the formation of azoxymethane-induced aberrant crypt foci in mice and reduces COX-2 and iNOS expression in the colon, Asian Pac J Cancer Prev. 6 (2005) 118–122.
9. M. Hoffmann, H. Kleine-Weber, S. Schroeder, N. Krüger, T. Herrler, S. Erichsen, T.S. Schiergens, G. Herrler, N.-H. Wu, A. Nitsche, M.A. Müller, C. Drosten, S. Pöhlmann, 2020. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor, Cell. 181, 271-280.e8. https://doi.org/10.1016/j.cell.2020.02.052.
10. J. Lan, J. Ge, J. Yu, S. Shan, H. Zhou, S. Fan, Q. Zhang, X. Shi, Q. Wang, L. Zhang, X. Wang, Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor, Nature. 581 (2020) 215–220. https://doi.org/10.1038/s41586-020-2180-5.
11. M. Asif, M. Saleem, M. Saadullah, H.S. Yaseen, R. Al Zarzour, COVID-19 and therapy with essential oils having antiviral, anti-inflammatory, and immunomodulatory properties, Inflammopharmacology. (2020) 1–9. https://doi.org/10.1007/s10787-020-00744-0.
12. S. Kumar, P. Kashyap, S. Chowdhury, S. Kumar, A. Panwar, A. Kumar, Identification of phytochemicals as potential therapeutic agents that binds to Nsp15 protein target of coronavirus (SARS-CoV-2) that are capable of inhibiting virus replication, Phytomedicine. (2020). https://doi.org/10.1016/j.phymed.2020.153317.
13. L.B. Talarico, R.G.M. Zibetti, P.C.S. Faria, L.A. Scolaro, M.E.R. Duarte, M.D. Noseda, C.A. Pujol, E.B. Damonte, Anti-herpes simplex virus activity of sulfated galactans from the red seaweeds Gymnogongrus griffithsiae and Cryptonemia crenulata, International Journal of Biological Macromolecules. 34 (2004) 63–71. https://doi.org/10.1016/j.ijbiomac.2004.03.002.
14. B. Axelrod, T.M. Cheesbrough, S. Laakso, Lipoxygenase from soybeans: EC 1.13.11.12 Linoleate:oxygen oxidoreductase, in: Methods in Enzymology, Academic Press, (1981): pp. 441–451. https://doi.org/10.1016/0076-6879(81): 71055-3.
15. M. Lepoivre, B. Chenais, A. Yapo, G. Lemaire, L. Thelander, J.P. Tenu, Alterations of ribonucleotide reductase activity following induction of the nitrite-generating pathway in adenocarcinoma cells, J Biol Chem. 265 (1990) 14143–14149.
16. G. Thomson, 2020. COVID-19: Social distancing, ACE 2 receptors, protease inhibitors and beyond?, Int J Clin Pract., e13503. https://doi.org/10.1111/ijcp.13503.
17. J. Akram, S. Azhar, M. Shahzad, W. Latif, K.S. Khan, Pakistan Randomized and Observational Trial to Evaluate Coronavirus Treatment (PROTECT) of Hydroxychloroquine, Oseltamivir and Azithromycin to treat newly diagnosed patients with COVID-19 infection who have no comorbidities like diabetes mellitus: A structured summary of a study protocol for a randomized controlled trial, Trials. 21 (2020) 1–3. https://doi.org/10.1186/s13063-020-04616-4.
18. S. Tong, Y. Su, Y. Yu, C. Wu, J. Chen, S. Wang, J. Jiang, Ribavirin therapy for severe COVID-19: a retrospective cohort study, International Journal of Antimicrobial Agents. 56 (2020) 106114. https://doi.org/10.1016/j.ijantimicag.2020.106114.
19. J.H. Beigel, K.M. Tomashek, L.E. Dodd, A.K. Mehta, B.S. Zingman, A.C. Kalil, E. Hohmann, H.Y. Chu, A. Luetkemeyer, S. Kline, D. Lopez de Castilla, R.W. Finberg, K. Dierberg, V. Tapson, L. Hsieh, T.F. Patterson, R. Paredes, D.A. Sweeney, W.R. Short, G. Touloumi, D.C. Lye, N. Ohmagari, M. Oh, G.M. Ruiz-Palacios, T. Benfield, G. Fätkenheuer, M.G. Kortepeter, R.L. Atmar, C.B. Creech, J. Lundgren, A.G. Babiker, S. Pett, J.D. Neaton, T.H. Burgess, T. Bonnett, M. Green, M. Makowski, A. Osinusi, S. Nayak, H.C. Lane, Remdesivir for the Treatment of Covid-19 — Final Report, New England Journal of Medicine. 383 (2020) 1813–1826. https://doi.org/10.1056/NEJMoa2007764.
20. B.V. Chernyak, E.N. Popova, A.S. Prikhodko, O.A. Grebenchikov, L.A. Zinovkina, R.A. Zinovkin, COVID-19 and Oxidative Stress, Biochemistry (Mosc). 85 (2020) 1543–1553. https://doi.org/10.1134/S0006297920120068.
21. L.M.F. Guimarães, C.V.T. Rossini, C. Lameu, Implications of SARS-Cov-2 infection on eNOS and iNOS activity: Consequences for the respiratory and vascular systems, Nitric Oxide. 111–112 (2021) 64–71. https://doi.org/10.1016/j.niox.2021.04.003.
22. P.T. Goud, D. Bai, H.M. Abu-Soud, A Multiple-Hit Hypothesis Involving Reactive Oxygen Species and

Myeloperoxidase Explains Clinical Deterioration and Fatality in COVID-19, Int J Biol Sci. 17 (2021) 62–72. https://doi.org/10.7150/ijbs.51811.
23. Md.A.R. Ripon, D.R. Bhowmik, M.T. Amin, M.S. Hossain, Role of arachidonic cascade in COVID-19 infection: A review, Prostaglandins & Other Lipid Mediators. 154 (2021) 106539. https://doi.org/10.1016/j.prostaglandins.2021.106539.
24. F. Zhou, T. Yu, R. Du, G. Fan, Y. Liu, Z. Liu, J. Xiang, Y. Wang, B. Song, X. Gu, L. Guan, Y. Wei, H. Li, X. Wu, J. Xu, S. Tu, Y. Zhang, H. Chen, B. Cao, Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study, Lancet. 395 (2020) 1054–1062. https://doi.org/10.1016/S0140-6736(20)30566-3.
25. Z.S. Ulhaq, G.V. Soraya, Interleukin-6 as a potential biomarker of COVID-19 progression, Med Mal Infect. 50 (2020) 382–383. https://doi.org/10.1016/j.medmal.2020.04.002.
26. T. Herold, V. Jurinovic, C. Arnreich, B.J. Lipworth, J.C. Hellmuth, M. von Bergwelt-Baildon, M. Klein, T. Weinberger, 2020. Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19, J Allergy Clin Immunol. 146, 128-136.e4. https://doi.org/10.1016/j.jaci.2020.05.008.
27. X. Lv, Y. Zhu, A. Cheng, Y. Jin, H. Ding, C.-Y. Wang, S. Zhang, G. Chen, Q. Chen, Q. Liu, High serum lactate dehydrogenase and dyspnea: Positive predictors of adverse outcome in critical COVID-19 patients in Yichang, World Journal of Clinical Cases. (2020). https://doi.org/10.12998/wjcc.v8.i22.5535.
28. I. Huang, R. Pranata, Lymphopenia in severe coronavirus disease-2019 (COVID-19): systematic review and meta-analysis, Journal of Intensive Care. 8 (2020) 36. https://doi.org/10.1186/s40560-020-00453-4.
29. J. Shi, Y. Li, X. Zhou, Q. Zhang, X. Ye, Z. Wu, X. Jiang, H. Yu, L. Shao, J.-W. Ai, H. Zhang, B. Xu, F. Sun, W. Zhang, Lactate dehydrogenase and susceptibility to deterioration of mild COVID-19 patients: a multicenter nested case-control study, BMC Med. 18 (2020) 168. https://doi.org/10.1186/s12916-020-01633-7.
30. W. Chen, K.I. Zheng, S. Liu, Z. Yan, C. Xu, Z. Qiao, Plasma CRP level is positively associated with the severity of COVID-19, Ann Clin Microbiol Antimicrob. 19 (2020) 18. https://doi.org/10.1186/s12941-020-00362-2.
31. Y. Yao, J. Cao, Q. Wang, Q. Shi, K. Liu, Z. Luo, X. Chen, S. Chen, K. Yu, Z. Huang, B. Hu, D-dimer as a biomarker for disease severity and mortality in COVID-19 patients: a case control study, Journal of Intensive Care. 8 (2020) 49. https://doi.org/10.1186/s40560-020-00466-z.
32. A. Bhargava, E.A. Fukushima, M. Levine, W. Zhao, F. Tanveer, S.M. Szpunar, L. Saravolatz, Predictors for Severe COVID-19 Infection, Clin Infect Dis. 71 (2020) 1962–1968. https://doi.org/10.1093/cid/ciaa674.
33. B. Yu, X. Li, J. Chen, M. Ouyang, H. Zhang, X. Zhao, L. Tang, Q. Luo, M. Xu, L. Yang, G. Huang, X. Liu, J. Tang, Evaluation of variation in D-dimer levels among COVID-19 and bacterial pneumonia: a retrospective analysis, J Thromb Thrombolysis (2020) 1–10. https://doi.org/10.1007/s11239-020-02171-y.
34. W. Yan, D. Chen, F.M. Bigambo, H. Wei, X. Wang, Y. Xia, Differences of blood cells, lymphocyte subsets and cytokines in COVID-19 patients with different clinical stages: a network meta-analysis, BMC Infectious Diseases. 21 (2021) 156. https://doi.org/10.1186/s12879-021-05847-9.
35. B. Zhu, X. Feng, C. Jiang, S. Mi, L. Yang, Z. Zhao, Y. Zhang, L. Zhang, Correlation between white blood cell count at admission and mortality in COVID-19 patients: a retrospective study, BMC Infect Dis. 21 (2021) 574. https://doi.org/10.1186/s12879-021-06277-3.