Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Efficacy of a multiple-indication antiviral herbal drug (Saliravira®) for COVID-19 outpatients: A pre-clinical and randomized clinical trial study

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ABSTRACT

Background: The scientific researches on COVID-19 pandemic topics are headed to an explosion of scientific literature. Despite these global efforts, the efficient treatment of patients is an in-progress challenge. Based on a meta-study of published shreds of evidence about compounds and their botanic sources in the last six decades, a novel multiple-indication herbal compound (Saliravira®) has been developed. Based on the antiviral, anti-inflammatory, and immune-enhancing properties of its ingredients, we hypothesized that Saliravira® has the potential to act as an antiviral agent, accelerate treatment, and reduce undesirable effects of COVID-19.

Methods: In this randomized, controlled, open-label clinical trial, COVID-19 outpatients were included by RT-PCR test or diagnosis of physicians according to the symptoms. Participants were randomly divided into intervention and control groups to receive Saliravira® package plus routine treatments of COVID-19 or routine treatments of COVID-19 alone, respectively. Saliravira® package includes tablets, nasal-sinuses spray, oral-pharynx spray, and inhaler drops. The treatment was for 10 days and followed up till 23 days after admission.

Results: On the 8th day, the "mean reduction rates" of viral load of the patients in the intervention group was 50% lower compared to the control group with a p-value < 0.05. The improvement of 10 out of 14 COVID-19 symptoms in the intervention group was significantly accelerated. The mean treatment duration of patients in the intervention group was 4.9 days less than the control group. In addition, no patients in the intervention group were hospitalized compared to 28% of the control group needed to be hospitalized.

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1. Introduction

The morbidity and mortality of COVID-19 increases daily. The confirmed and probable cases exceed 340 million patients and 5.5 million deaths [1]. Acute COVID-19 is a multi-organ disease with a broad range of manifestations, Patients suffer from prolonged and persistent post-COVID impacts that can be harmful and devastating [2–5]. It is expected that more than half of the world will have been infected with the omicron variant by the end of March 2022 [6]. It seems that COVID-19 as a pandemic will come to an end and it will become another recurrent disease. Societies and health systems will have to live with and manage it [7].

Extensive studies on the human coronavirus diseases such as SARS, MERS, and COVID-2 have been carried out in the last six decades [8–10]. The published scientific documents on topics related to the COVID-19 pandemic have increased rapidly in the last two years and resulted in an explosion of scientific literature [11–13]. By February 2020, there were more than 337,000 citations related to coronavirus in the Google search. Also, many individual compounds, herbs, and compositions have been studied in the literature as remedies for the coronavirus, mostly in labs and micro-level research studies. Despite these extensive global efforts, the efficient treatment of patients is an unfinished and ongoing challenge [9,14–18].

After the outbreak of COVID-19 in 2020 we started working on an R&D project based on a "systems biology" approach [19,20]. A thorough meta-study of the existing researches and studies related to coronavirus in the last six-decade, i.e., from the first outbreak of human coronavirus in 1961–2020 was carried out. A total of 737 scholarly papers, studies, monographs, and reports were reviewed. This effort resulted in identifying 786 compounds with the ability to prevent ailment, cope with coronavirus, and/or remedy its effects.

These efforts resulted in developing a novel herbal composition (Saliravira®). The specific ingredients in this herbal compound are *Echinacea purpurea, Glycyrrhiza glabra, Rheum palmatum, Hyssopus officinalis, Rosmarinus officinalis, and Panax ginseng*. Based on published evidence of the ingredients' substances, such as governmental monographs, scientific researches and reports, and also databases of medicinal substances, such as the EMA database, we assumed that the new composition can act as an antiviral, anti-inflammatory, and/or immune-enhancing agent for COVID-19 [21].

The dosage forms of Saliravira® are designed to treat COVID-19 outpatient stages (i.e., positive "polymerase chain reaction" (PCR) test without symptoms as Stage 2, Positive PCR with mild symptoms as Stage 3, and Positive PCR with severe symptoms without shortness of breath, as a part of Stage 4). To cope with these stages, Saliravira® is designed as a multiple-indication product, including a nasal-sinuses spray, an oral-pharynx spray, tablets, and inhaler drops. The sprays are to inhibit virus proliferation in the focal points, i.e., throat and sinuses. The tablets and drops are to inhibit binding in body organs and respiratory systems, respectively [22]. To meet the EMA (European Medicines Agency) standardization of botanical sources, the suppliers of all herbal ingredients are either certified organic producers or qualified active providers in the European pharmaceutical market. This prevents risks due to the change of plant species, warrants the reproducibility of the ingredients, and finally ensures that the safety and efficacy regulations required by the EMA are met [23].

2. Material and methods

2.1. Preclinical studies

2.1.1. Physicochemical analyses

Extracts with various alcohol contents were used for the nasal-sinuses spray, oral-pharynx spray, and aromatherapy drops. Based on FDA Guidance for Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products Section IV.L, the content of ethanol alcohol as solvent or as preservative is variable between 10% and 95%. The content of alcohol in aromatherapy drops is 94% v/v, where the aromatherapy process is inhaled in 500 ml hot water. The alcohol content of nasal-sinuses and oral-pharynx sprays are less than 40%. The total alcohol content in the SaliraVira® package is below the permitted daily exposure (PDE) level based on APPENDIX 6. TOXICOLOGICAL DATA FOR CLASS 3 SOLVENTS of the FDA Guidance [24]. Also, the SaliraVira® extract has been granulized in a "fluid bed processor" to be used in the tablet dosage. The liquid and the solid composition were submitted for Physicochemical analysis.

A pH test of the liquid extract was performed three times in temperatures of 15 and 25 degrees Celsius with a calibrated digital pH meter. The measured pH of 6 falls within the permissible range of 5–7 for respiratory extracts. Total flavonoids have been determined for the compositions in dosage forms nasal-sinuses spray, oral-pharynx spray and aromatherapy drops. This assay is done according to the Aluminum chloride colorimetric method. Extracts (0.5 ml of 1:10 g/ml) in methanol were mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min. Then, the absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/Visible spectrophotometer. The calibration curve was prepared by preparing Rutin (Standard flavonoid) solutions at concentrations of 1–100 mg/ml in methanol. The result was 3.65 mg in ml for each dosage form. Total flavonoids determined, based on Hyperoside, is 0.048 mg/ml.

Based on BP 2017 and using a standard oven, the dry residue of 100 gr of extract is 2.81 w/w. Using a pycnometer, the density of the solution is 0.82 kg/liter. Based on USP-40, the microbial tests of the extract indicate, the numbers of all bacteria are zero and the amount of yeast in the extract is less than 10.

2.1.2. Acute and chronic toxicity study on mice

To evaluate the effects of the pharmaceutical composition of the SaliraVira® composition, 20 C57BL/6 inbred mice were studied for acute and chronic prescription. The mice were housed in an animal house for one week, provided with proper light, temperature and moisture and fed with standard mouse food pellets and water ad libitum. Seven-week-old mice weighing 23 ± 5 g were used for the experiments.

The mice were randomly divided into four groups, twenty mice were included in control groups and twenty mice were included in the treatment group for evaluating acute and chronic toxicity. SaliraVira® in the dosage form of ethanol extract has been taken by mice via gavage for 48 h at a dose of 1000 mg/kg BW/day. To study the chronic prescription of SaliraVira®, a dose of 100 mg/kg BW/day of the drug was taken by each mouse via gavage, once a day, for 7 days. At the end of the treatment, hematological factors, liver enzymes and histopathology of brain, liver and kidney tissues were examined.

2.1.3. In vivo bone marrow assay

To examine the clastogenic effects of the SaliraVira® composition, femurs dissected from the seven-week-old male NMRI mice were acquired from the Pasteur Institute of Iran. They were housed in an animal house for one week, provided with proper light, temperature and moisture and fed with standard mouse food pellets and water ad libitum. Seven-week-old mice weighted 23 ± 5 g. Five mice were allocated to the control group and 10 mice were allocated for treatment. All animal experiments in this study were carried out with the prior approval of the Institutional Ethics Committee, strictly adhering to the Helsinki guidelines provided for the care and treatment of animals. Mice were treated with an acute dose of 1000 mg/kg BW/day by administering the pharmaceutical tablet of SaliraVira® dissolved in water by gavages. Treated and untreated mice were sacrificed 72 h after drug treatment. The mice were killed by cervical dislocation; their femoral bone marrow was flushed out by means of fetal calf serum, and a cell suspension from both femurs was prepared. The suspension was centrifuged for 5 min at

1000 rpm. After centrifuging, the supernatant was removed and cells were re-suspended in the remaining serum, and a smear was prepared on glass slides, fixed with methanol, and stained in May Grunwald–Giemsa (Merck, Darmstadt, Germany). In this method of staining, polychromatic erythrocytes (PCEs) are stained blue–violet, while normochromatic erythrocytes (NCEs) are stained in yellow– orange. In order to study the cytotoxic effects of the - SaliraVira® composition on the proliferation of the bone marrow cells, the ratio of PCE/ NCE was calculated. This ratio is an indicator of the proliferation rate and turnover of PCE to NCE which should be similar for healthy mice, but decline in the case of cytotoxicity.

2.2. Clinical Trial study

2.2.1. Clinical Trials design

This study was a randomized, controlled, open-label, single-center clinical trial with two parallel intervention and control groups conducted from December 21, 2020, to March 1, 2021. The participants were recruited in the Infectious Diseases Department of the Imam Khomeini Hospital Complex (IKHC), which is affiliated to the Infectious and Tropical Disease Department (ITDP) of Tehran University of Medical Sciences (TUMS). Written informed consent was obtained from all participants and/or their legal representatives.

Before the clinical trial (CT), this study was approved by the Institutional Research Ethics Committee of the School of Pharmacy, Nursing and Midwifery, Shahid Beheshti University of Medical Sciences (SBMU) with an ethic code (IR.SBMU.PHARMACY.REC.1399.276) and received the clinical trial approval, registered at www.irct.ir, as primary registry in the WHO Registry Network, with CT code (IRCT20201220049771N1).

Participants were randomly divided into intervention and control groups that received the Saliravira® package plus routine treatments of COVID-19 (intervention group) or routine treatments of COVID-19 alone (control group). Routine treatments were determined according to the protocols and guidelines of the Ministry of Health and Medical Education of Iran. In the intervention group, patients were instructed to consume a Saliravira Tablet®, every six hours, 4 times daily (2600–3100 mg per day); Saliravira Nasal® as a nasal-sinuses spray, every four hours, one puff in each nostril (200–320 mg per day); Saliravira Oral® as an oral-pharynx spray, every 4 h, two puffs in the throat, five times per day (200–320 mg per day); and Saliravira Drop® as inhaler drops, 10–15 drops in 500 cc hot water, 20 min inhaling, every 8 h, 2 times daily, under a seamless thick cover (1900–2300 mg per day). The effective inhaling should be over 56 Celsius degrees [25–27]. In total, each patient takes between 4900 and 6040 mg of the effective materials daily.

2.2.2. Randomization

The participants were assigned to two groups by the block randomization method. In order to minimize the probability of sequence prediction, blocks with variable sizes (4 and 6) were used. The randomization ratio was 1:1 and was performed by Random Allocation Software [28]. Allocation concealment was done by assigning unique codes. All participants and outcome evaluators were masked to treatment allocation.

2.2.3. Patients

The clinical study comprised 170 patients 24–80 years old, including 87 patients (treated by Saliravira®) and 56 patients as the control group, who completed CT. 27 patients did not complete CT, mainly because of refusing access to their clinical data during treatment. The criteria to include patients in this study were clinical confirmation of COVID-19 infection by positive RT-PCR test, having some or all of the COVID-19 clinical symptoms such as fever, fatigue, muscle aches (body aches), headache, cough, chest tightness, and shortness of breath and lung involvement below 20% (based on a CT-scan).

The exclusion criteria included pregnancy and lactation, malignant tumors and other acute systemic diseases or special indication patients suffering from autoimmune diseases like psoriasis, ALS and multiple sclerosis patients with comorbidity of respiratory life-threatening problems, drugs and alcohol addiction, use of any other herbal substances, metabolic diseases such as diabetes, kidney, liver and severe cardiovascular diseases, and participation in other clinical trials for COVID-19.

2.2.4. Symptom evaluation procedure

The primary outcome measures included changes from baseline viral load (VL) at two time points, 4th and 8th days after first intake and were tested by patients' "cycle threshold" (Ct) values in both intervention and control groups. VL efficacy was determined by the "mean reduction rate" (MRR) of VL for the patients in both groups.

The secondary outcome was the improvement of COVID-19 clinical symptoms on a daily timeline after the first dose of intervention. The COVID-19 WHO CRF (Case Report Form) is for the symptoms of hospitalized patients (in Stages 4 and 5 of disease), but 14 symptoms are common between hospitalized patients and outpatients. Since this clinical study was for COVID-19 outpatients (Stages 2 and 3, and 4 without shortness of breath symptom), it required to use the common sections of the WHO CRF. As a result, 14 symptoms of COVID-19 were used for this clinical trial.

2.2.5. Statistical analysis

Saliravira®, as a multiple-indication package with four-dosage forms, is designed to treat COVID-19 outpatients in 3 stages, as mentioned above. The main goals of this CT are to statistically evaluate the efficacy of the package.

"Mean reduction rate" (MRR) of VL for treatment and control groups are calculated as $MRR_k = (1/n_k)\sum_{j=1}^{n_k}\left(VL_{jkh} - VL_{jk0}\right)$ where k indicates "treatment" or "control" groups, respectively. n_k stands for the number of patients in group k, and h = 0, 4 or 8 indicates 0th, 4th and 8th days, respectively. Thus, VL_{jkh} is the VL value for patient j in group k in day h. Also, "cumulative probability function" (CDF) of treatment time at a given day T of group k is $CDFT_k = \sum_{t_k=1}^{T_k} Pr(t_k)$, where $Pr(t_k)$ indicates the probability of treatment of patients in day $t_k \leq T_k$.

The required data gathering for analysis had two phases. The first phase included VL measuring and analysis. For each patient real-time PCR tests in the 1st, 4th, and 8th days were taken. Simultaneously, the data of 14 symptoms for the patients were collected each day for the second phase. Patients who did not permit the research team to access their clinical data were excluded from the trials. Finally, statistical analyses of data were accomplished with the R software, version 4.0.2 (The R Project for Statistical Computing).

3. Results

3.1. Preclinical studies results

3.1.1. Physicochemical analyses results

The physicochemical analyses of Saliravira® indicated the total flavonoids determined, based on Hyperoside, was 0.048 mg/ml. Also, based on BP 2017 and using a standard oven, the dry residue of 100 gr of the extract was 2.81 w/w. Using a pycnometer, the density of the solution was 0.82 kg/liter. The total alcohol content in the Saliravira® package was under permitted daily exposure (PDE) based on "APPENDIX 6. TOXICOLOGICAL DATA FOR CLASS 3 SOLVENTS" of the US FDA Guidance. Finally, based on USP-40, the microbial contamination tests of the extract indicate, the numbers of all bacteria were zero and the amount of yeast in the extract was less than 10.

3.1.2. Acute and chronic toxicity study results

The study of hematological factors in the test of control groups in

both acute and chronic treatment did not show a significant difference, which is indicated in Figs. 1A and 2B. Also, among the liver factors, none of the studied enzymes showed a significant difference in activity between the treatment and control groups, which is shown in Figs. 1B, C and 2B, C. Pathological studies did not show a significant difference in inflammation, necrosis and degeneration between the control and treatment groups in both acute and chronic prescriptions. It is noteworthy that the results of studies indicate that the use of SaliraVira® causes no pathological injuries of the brain and liver shown in Figs. 3 and 4.

These preclinical results enhance the idea that Saliravira® is a strong candidate to be an antiviral, anti-inflammatory and/or immune-enhancing agent for COVID-19. Thus, we conducted a randomized, controlled, open-label, single-center, clinical trial of Saliravira® on COVID-19 outpatients.

3.1.3. *In vivo bone marrow assay results*

Bone marrow assay results are summarized and provided in Table 1. As shown in the table, the frequency of micronuclei induced by treatment of an acute dose of the pharmaceutical composition of the invention is nearly similar to those of the animals not given treatment. The results of in vivo bone marrow assay on Saliravira®, imply no genotoxic or clastogenic effects have occurred in the bone marrow of treated mice with the pharmaceutical composition of the invention. Moreover, no change in the ratio of PCE/NCE is observed, indicating no cytotoxic effect of the pharmaceutical composition of the invention in bone marrow (p-value, $P = 0.672$).

3.2. *Clinical Trial study results*

3.2.1. *Demographics and patient characteristics*

Patients included in the CT were outpatients in the age range of 24–80 years, with mean (± standard deviation) age of 50.1 ± 9.2. All patients were white Caucasian. 65% of patients were in mid-lower economic classes, who referred to the governmentally subsidized low-cost hospital. 59% of patients were men. About 23% of the patients had hypertension. The patients were followed up till day 23rd of the treatment.

3.2.2. *Primary outcomes*

MRRs of VL of intervention and control groups are compared in Fig. 5. On the 4th day, the MRR of VL is 27% for patients in the intervention group compared to 10% for patients in the control group. On the 8th day, the MRRs of VL for the patients in the intervention and control groups were 60% and 40%, respectively. Intra- and inter-group comparisons of MRRs of VL in intervention and control groups are used to state the efficacy and significance of VL reduction by the Saliravira® package.

For intra-group comparisons of VL, the p-values for intervention and control groups in the day1-to-day4 period were calculated and are 0.09 and 0.26, respectively. The same p-values for the intervention and control groups in the day1-to-day8 period are 0.0009 and 0.0016, respectively. Also, the inter-group comparison of VL stated by p-value of intervention group versus the control group in day8 case-to-day8 control is 0.005. This indicates that taking Saliravira® has superior efficacy to the routine treatment with a probability of 99.5%.

3.2.3. *Secondary outcomes*

Based on the modified WHO CRF for COVID-19 outpatients, 14

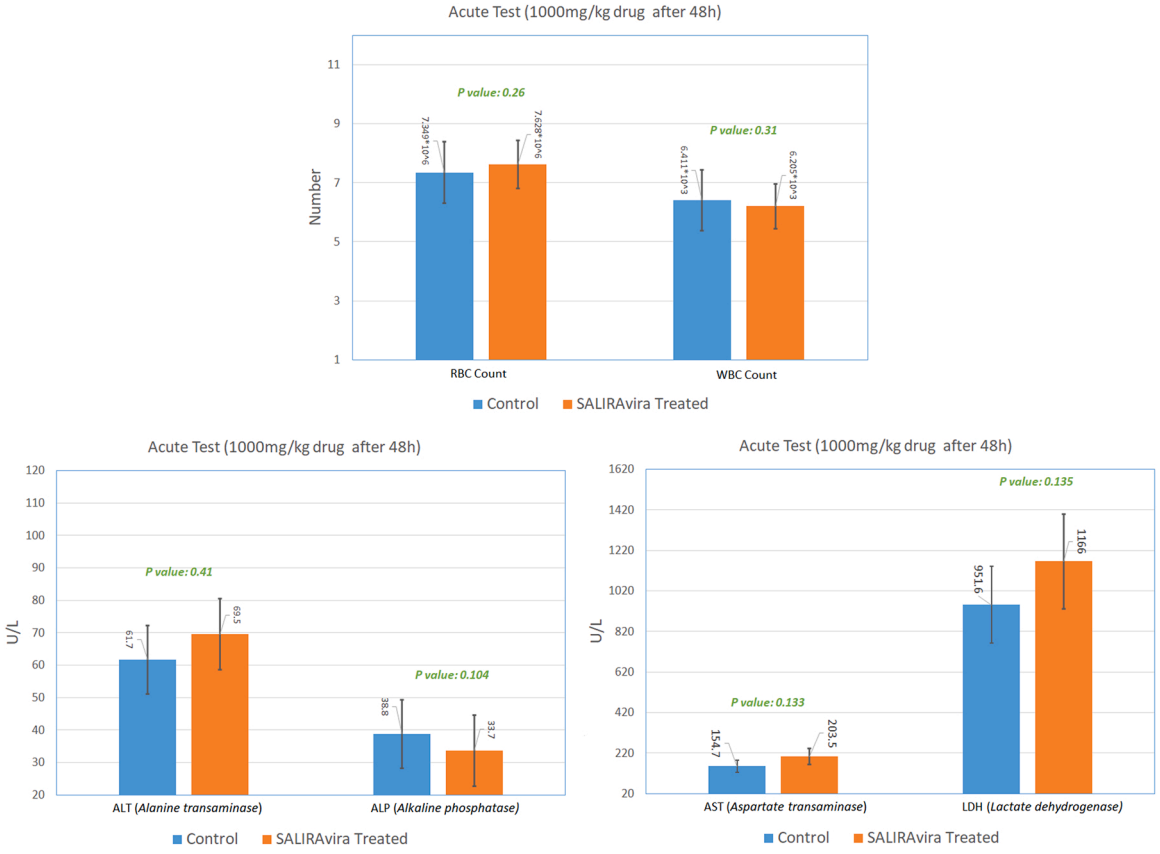

Fig. 1. (A) Comparison of RBC and WBC indices in control and treatment groups, (B) Evaluation of hepatic ALT and ALP enzymes in two groups of control and treatment and (C) Evaluation of AST and LDH enzymes in two groups of control and treatment in acute toxicity tests.

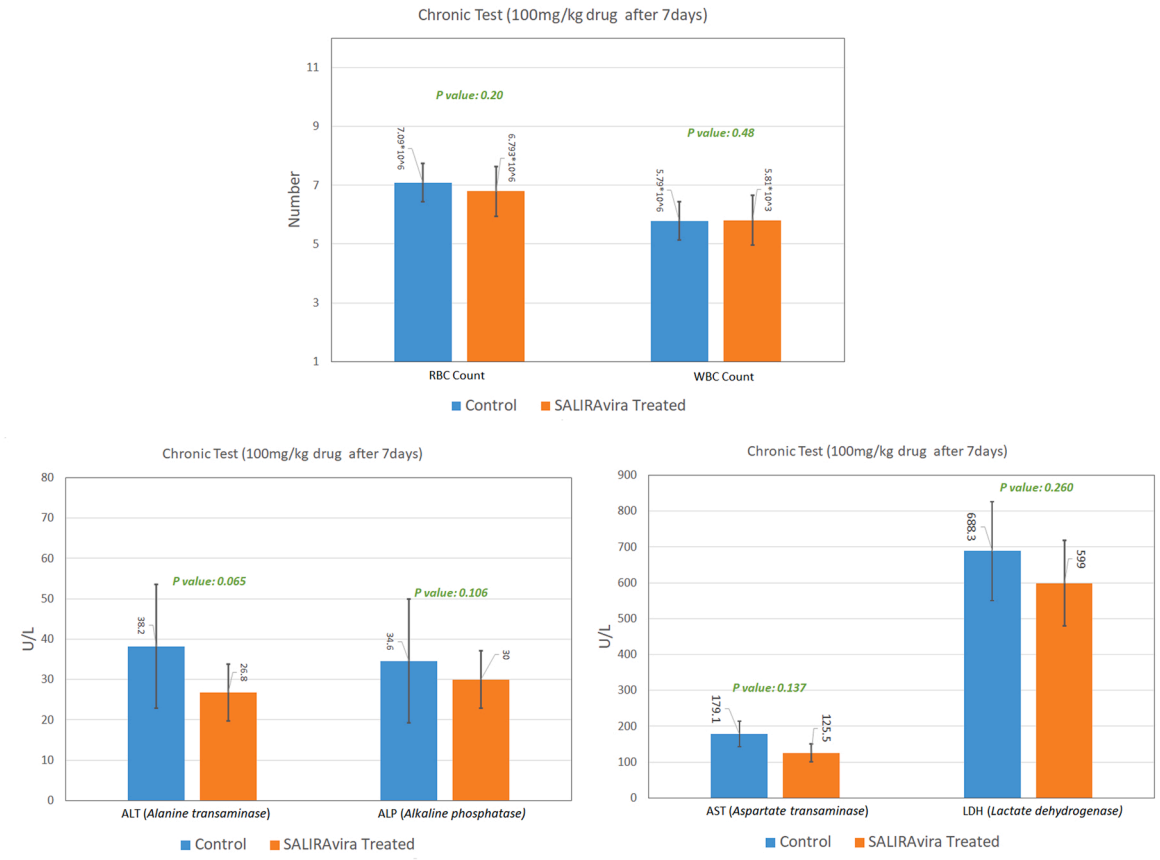

Fig. 2. (A) Comparison of RBC and WBC indices in control and treatment groups, (B) Evaluation of hepatic ALT and ALP enzymes in two groups of control and treatment and (C) Evaluation of AST and LDH enzymes in two groups of control and treatment in chronic toxicity test.

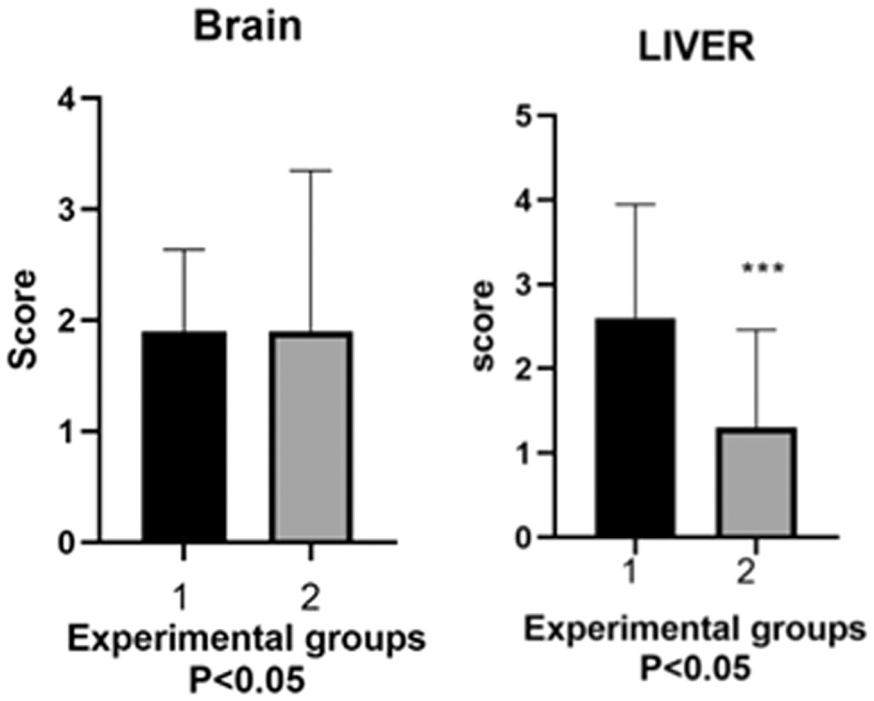

Fig. 3. Acute toxicity group brain and liver tissue pathology study after receiving daily dose of 1000 mg/kg SaliraVira® after 48 h.

symptoms of the patients in both intervention and control groups were tested daily during treatment. By the 8th day of treatment, there was a remarkable improvement in the overall situation of the intervention patients. The comparative results are shown in Table 2. The "efficacy" of treatment is measured by the "probability" of changes in symptoms in both groups, and the "significance" of the efficacy is shown by the

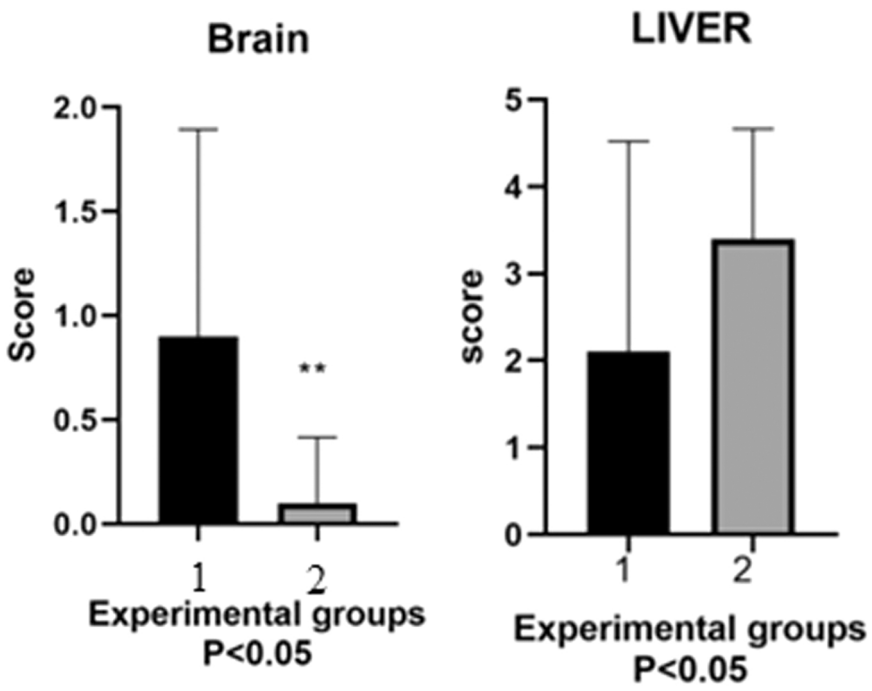

Fig. 4. Chronic toxicity group brain and liver tissue pathology study after receiving daily dose of 1000 mg / kg SaliraVira® after 7 days.

Table 1
Study the cytotoxic effects of the pharmaceutical composition of the invention.

Sample	No. of PCE scored	Total number of MN observed	No. of NCE scored	Ratio PCE/ NCE
Control untreated				
1	500	3	460	1.09
2	500	5	520	0.96
3	500	2	490	0.85
4	500	6	550	0.91
5	500	5	470	1.06
Mean ± SD	500 ± 0.0	4.2 ± 1.47	498 ± 33.1	0.974 ± 0.09
SaliraVira® Treated samples				
1	500	4	480	1.04
2	500	5	530	0.94
3	500	5	510	0.98
4	500	6	540	0.93
5	500	4	480	1.04
6	500	5	470	1.06
7	500	3	480	1.04
8	500	6	520	0.96
9	500	4	510	0.98
10	500	5	530	0.94
Mean ± SD	500 ± 0.0	4.7 ± 0.9	505 ± 24.19	0.991 ± 0.047
Significant Difference		P = 0.462	P = 0.662	P = 0.672

relevant "p-values". The p-value in each group is one-sided and compares the probability distribution of the COVID-19 symptoms on the n^{th} (i.e., 4th, 6th, 7th, and 8th) day to the 1st day of treatment.

As indicated, on the 4th day of treatment, with a probability of at least 95%, the improvement of 3 of 14 symptoms of COVID-19 (i.e., joint-pain, muscle-aches, and wheeze) in the intervention group were significantly and efficaciously preferred to the control group. Also, on the 6th day of treatment, with a probability of at least 90%, the improvement of 7 of 14 symptoms (i.e., sore throat, headache, shortness-of-breath, decreased-smell, abdominal-pain, inability-to-walk, and rhinorrhea) in the intervention group were significantly preferred to the control group.

4 out of the 7 symptoms (i.e., headache, abdominal-pain, inability-to-walk and rhinorrhea) in the intervention group were efficaciously preferred to the control group. With a probability of at least 95% on the 6th day of treatment, the improvement of 5 out of 14 symptoms (i.e., sore-throat, headache, shortness-of-breath, abdominal-pain, and inability-to-walk) in the intervention group were significantly preferred to the control group. Also, 4 out the 5 symptoms (i.e., sore-throat, headache, abdominal-pain, and inability-to-walk) in the intervention group were efficaciously preferred to the control group.

On the 7th and 8th treatment days, with a probability of at least 90%, the improvement of 4 out of 14 symptoms (i.e., decreased-taste, fatigue, altered-consciousness-confusion, and chest-pain) in the intervention group were significantly preferred to the control group. Also, 2 out of 4 symptoms (i.e., fatigue and altered-consciousness-confusion) in the intervention group were efficaciously preferred to the control group. In the treatment time span, with a probability of at least 95%, the improvement of 2 out of 14 symptoms (i.e., fatigue and altered-consciousness-confusion) in the intervention group were significantly and efficaciously preferred to the control group.

Results from the evaluation of the COVID-19 symptoms in intervention and control groups in the 8th day treatments with a probability of 90% indicate that Saliravira® is more significant for all 14 symptoms and more significant and efficaciously for 10 of 14 symptoms. The results of 8th-day treatments show that Saliravira®, with a probability of 95%, is more significant for 10 symptoms and more significant and efficaciously for 8 of 10 symptoms.

Fig. 6 indicates that the "CDF of treatment duration" of patients in the intervention group with a mean of 8.8 and standard deviation of 5.5 days. With a probability of 87%, the patients treated by the Saliravira® package were recovered on the 10th day of taking Saliravira®. The difference of the treatment duration of intervention and control with a p-value < 0.05 is tested by both one-sided hypothesis test and mutually inclusiveness of their domains.

The "CDF of treatment duration" of patients in the control group with a mean of 13.7 and standard deviation of 3.3 days was shown in Fig. 3. With the same probability of 87%, patients had recovered on the 17th

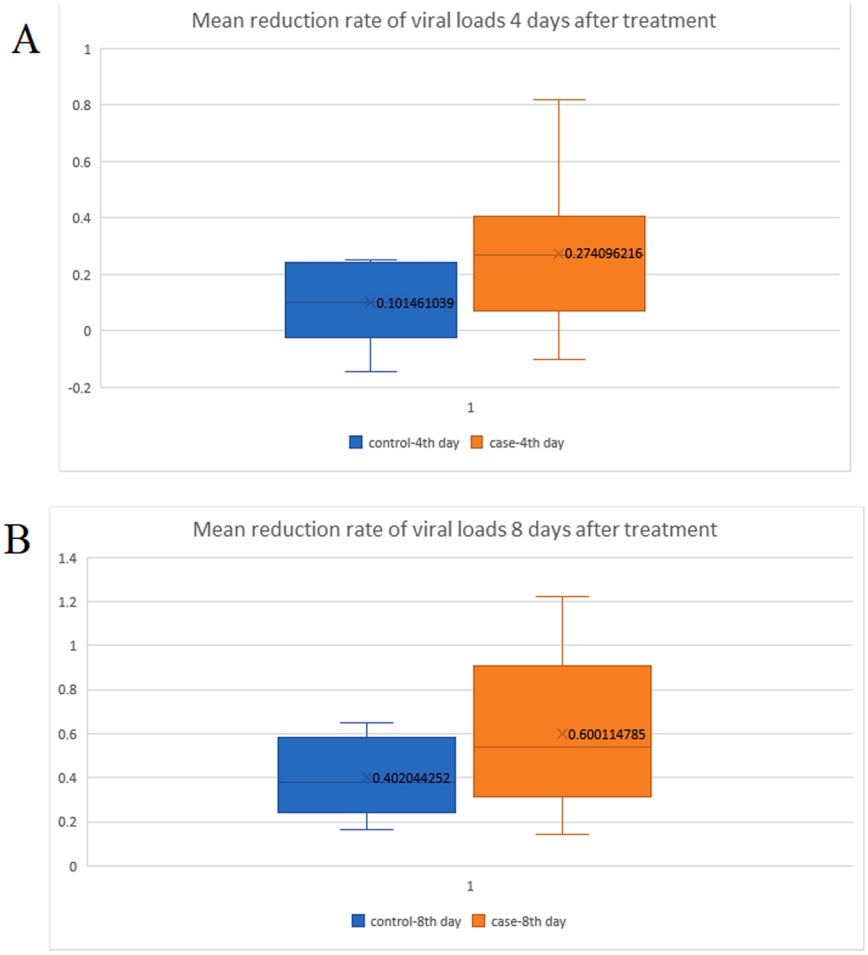

Fig. 5. Comparison of viral load reduction in intervention and control groups A) 4 and B) 8 days after treatment.

Table 2
Integrated results of COVID-19 symptoms in intervention and control groups.

Symptoms	1st day	nth day	P-Value (intervention)	P-Value (control)	Probability (intervention)	Probability (control)
Joint-pain	1	4	0.046	0.175	0.50	0.42
Muscle-aches	1	4	0.032	0.596	0.50	0.27
Wheeze	1	4	0.012	0.289	0.78	0.50
Sore-throat	1	6	0.054	0.136	0.58	1
Headache	1	6	0.014	0.057	0.61	0.54
Shortness-of-breath	1	6	0.046	0.124	0.50	0.67
Decreased-smell	1	6	0.089	0.161	0.38	0.45
Abdominal-pain	1	6	0.043	0.460	0.75	0.40
Inability-to-walk	1	6	0.006	0.021	0.89	0.78
Rhinorrhea	1	6	0.060	0.700	0.78	0.25
Decreased-taste	1	7	0.062	0.076	0.54	0.71
Fatigue	1	7	0.000	0.069	0.56	0.47
Altered-consciousness-confusion	1	7	0.011	0.025	1	0.86
Chest-pain	1	8	0.084	0.108	0.44	0.46

day of treatment.

"CDFs of treatment duration" of patients in the intervention and the control groups are compared in Fig. 6. The results indicate that the treatment duration of the intervention group has "first-order stochastic dominance" (FSD) or absolutely statistical preference to the control group. The results on the 10th day of the treatment suggest that the patients using the Saliravira® package have recovered then with a probability of 87% compared to the probability of 12% for other patients using routine treatment.

The reliability of performing this CT and the validity of its results were certified by ITDP-TUMS and the certification was officially announced (Reg. no.: 99/11/71/21372) to IR-FDA (Iranian Food and Drug Administration). This resulted in issuing four "marketing authorization codes" (IRCs) for four dosage forms of Saliravira® (Tablets, IRC: 6397073663263955; Nasal spray, IRC: 9537340184415703; Oral spray, IRC: 6051091790519742; Inhaling drops, IRC: 4503619441013809).

4. Discussion

From the results of this clinical trial, it can be concluded that Saliravira® has significant efficacy as an antiviral herbal composition to accelerate the reduction of the VL of coronavirus with a p-value > 0.005.

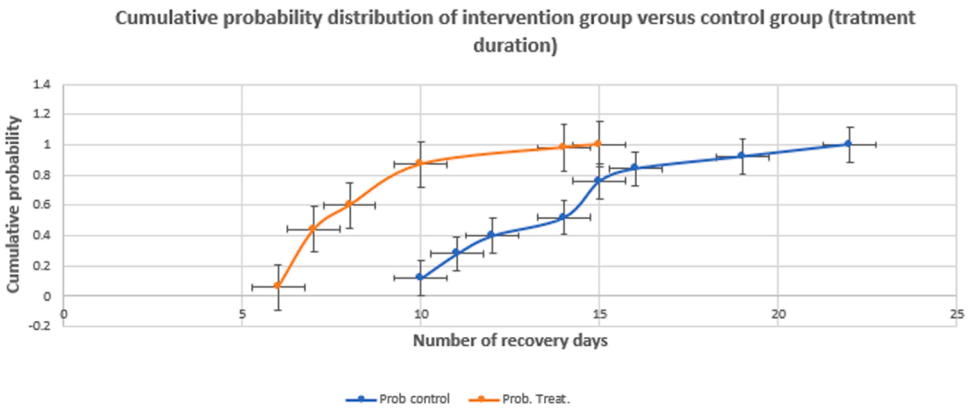

Fig. 6. Cumulative probability distribution of the treatment duration of control and intervention group.

The mechanism of action of Saliravira® is to reduce the viral load in COVID-19 patients. This arises from a hypothesis that Saliravira® contains a set of compounds that inhibit SARS-COV proteins to bind human proteins. These viral bindings result in COVID-19 symptoms. From this point of view, we have studied to determine the COVID-19 viral proteins, inhibitory compounds and the dominant features of Saliravira®.

A broad review of the literature was performed, focusing on the viral proteins of SARS-COV, the inhibitory compounds, and the physical interactions between the compounds and the viral proteins predicted by molecular docking methods [29–34]. This resulted in detecting 54 inhibitory compounds in Saliravira® and 13 inhibited viral proteins in SARS-COV.

In fact, SARS-COV2 has 29 different proteins, including 16 nonstructural proteins, some proteases and polymerases [35]. 13 viral proteins, i.e., NSP1, NSP3, NSP4, NSP5, NSP10, NSP12, NSP13, NSP14, NSP15, M pro, E pro, S pro, N pro are inhibited by Saliravira®. Among these 13 viral proteins, S pro, M pro, and NSP5 are the most affected proteins by inhibitory compounds of Saliravira® That is, these top 3 viral proteins are inhibited by 30, 23 and 21 inhibitory compounds of Saliravira®, respectively.

As previously mentioned, Saliravira® has "immune enhancing", "anti-viral" and "anti-inflammatory" properties. These features are related to the return the normal functionality of "Type 1 interferon pathway", "suppression of viral replication or infection processes", and "NF-kB signaling pathway" in COVID-19 patients, respectively[35]. Each of these three signaling pathways are affected by 10, 12 and 1 viral proteins, respectively. For restoration of "Type 1 interferon pathway", 8 out of 10 viral proteins can be inhibited by inhibitory compounds of Saliravira®. Meanwhile, 7 out of 12 viral proteins related to "suppression of viral replication or infection processes" are inhibited by inhibitory compounds of Saliravira®. The "NF-kB signaling pathway" dysfunction, caused by the spike protein, is also inhibited by several inhibitory compounds in Saliravira®. All things considered, to rank dominant features of Saliravira® based on the number of inhibited viral proteins resulted, the "immune-enhancing" property of Saliravira® is the most dominant feature. The "anti-viral" and "anti-inflammatory" properties are the second and the third dominant features of Saliravira®, respectively.

Furthermore, the evaluation of the efficacy of Saliravira® is based on 14 symptoms of COVID-19 introduced by the WHO CRF. The results of CT show that Saliravira® speeds up the improvement of symptoms. In fact, COVID-patients who take Saliravira® suffer fewer pains from the 4th day of treatment. This is a significant result of this study.

The expected recovery duration of the intervention group is 8.9 $\pm$ 5.5 (standard deviation) days compared to 13.7 $\pm$ 3.3 days of the control group. Therefore, Saliravira® reduces 4.9 days of the treatment duration of the outpatients with a p-value < 0.05. Additionally, the patients in the intervention group did not enter the severe stage of COVID-19 and were protected from the agony of long-run mal-impacts of the pandemic.

In addition, none of the patients in the intervention group needed to be hospitalized, where 28% of the control group were hospitalized. Also, none of the patients in the intervention group died, while because of the lack of access to the clinical data of the hospitalized patients, no data is available about death of patients in the control group.

Moreover, clinical trials of outpatients have several limitations and constraints. The main barrier is the daily availability of outpatients for data gathering of 14 COVID-19 symptoms according to WHO CRF. In addition, short treatment duration, on site VL tests restriction, reliability of PCR tests which may incur retesting, patients' resilience to post-covid follow-up, and a lack of WHO CRF for prophylactic clinical trials are the existing constraints in this research.

5. Conclusion

In conclusion, based on pre-clinical and clinical trial results, Saliravira® is an antiviral and inhibitory agent which with a significant probability saves lives, shortens treatment duration, and reduces the pains of COVID-19 outpatients.

Finally, the viral inhibiting property of Saliravira® projects that it has the potential to be considered as a prophylaxis solution for COVID–19. Particularly, taking Saliravira® nasal-sinuses and oral-pharynx sprays can act as an agent to remove coronavirus from those proliferation focal points. This suggests some new topics for future studies.

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Conflict of interest statement

Dr. Reza Ramazani and Saleh Ramazani are main shareholders of MIM Pharma, Oslo, as a private enterprise. MIM Pharma is the European patent owner and supporter of the project. FMO, FMK, FAZ, FKJ, MAK, ASA, SZA, HMA, MAM, MKH, SSA, APR and MSA were employed for this trial. The expert and faculty members of university and research centers, as indicated in their affiliations, and also AFA, MHB and MKH worked as volunteers in the trial. HAK and the physicians, as the employees of IKHC- an independent governmental entity- worked as defined duties in a national project versus the pandemic situation. The authors declare that there are no conflicts of interest.

Data Availability

Data supporting reported results that is not given here is available on request from the corresponding author. This data is not publicly available due to privacy requirements.

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CRediT authorship contribution statement

RRK was the principal investigator with overall responsibility for conducting the trial and coordinated the writing of the final report. SRK was the operational manager of trial. THO, FMO, FMK were scientific body of trial. FAZ was in charge of coordinating daily data gathering from and interfaces with the patients. HMO was the scientific advisor in viral sampling of the patients. HAK was the head of the TUMS scientific evaluation of the R&D of the project and issue the permission for implementing CT in the infection department (COVID-19 ward) of IKHC. He also reviewed the outcomes and validated the final report of the trials. BTA helped in environment consideration of CT. FKJ helped to shape this publishing. MAK was pharmaceutical consultant of trial. ASA, SZA, HMA, MAM, MKH, SSA, APR and MSA were in the team of interface with and data gathering from the patients. AFA, MGR and MAN helped in organizing and processing data. MHB and MKH helped in operational management of trials. SJA, FGH, ASE, SGH, SAD, MRS, LAB, MHA, MME, MHAJ and ZAH were professional physicians in infection disease, also MPA, ZSA and AMO were residents in IKHC who helped to diagnose the infected patients and randomly introduced to CT.

References

[1] World Health Organization Dashboard; Available from: ⟨https://www.covid19.who.int⟩.
[2] L.T. Nolen, S.S. Mukerji, N.I. Mejia, Post-acute neurological consequences of COVID-19: an unequal burden, Nat. Med. (2022) 1–3.
[3] P.K. Zarandi, et al., SARS-CoV-2: from the pathogenesis to potential anti-viral treatments, Biomed. Pharmacother. (2021), 111352.
[4] S.U. Rehman, S.U. Rehman, H.H. Yoo, COVID-19 challenges and its therapeutics, Biomed. Pharmacother. 142 (2021), 112015.
[5] B. Hu, et al., Characteristics of SARS-CoV-2 and COVID-19, Nat. Rev. Microbiol. 19 (3) (2021) 141–154.
[6] C. Wang, J. Han, Will the COVID-19 Pandemic End with the Delta and Omicron Variants? Springer, 2022, pp. 1–11.
[7] C.J. Murray, COVID-19 will continue but the end of the pandemic is near, Lancet (2022).
[8] Y. Jin, et al., Virology, epidemiology, pathogenesis, and control of COVID-19, Viruses 12 (4) (2020) 372.
[9] W.J. Wiersinga, et al., Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review, JAMA 324 (8) (2020) 782–793.
[10] V.S. Salian, et al., COVID-19 transmission, current treatment, and future therapeutic strategies, Mol. Pharm. 18 (3) (2021) 754–771.
[11] A. Piovesan, et al., Human protein-coding genes and gene feature statistics in 2019, BMC Res. Notes 12 (1) (2019) 1–5.
[12] D.E. Gordon, et al., A SARS-CoV-2 protein interaction map reveals targets for drug repurposing, Nature 583 (7816) (2020) 459–468.
[13] Y. Fassin, Research on Covid-19: a disruptive phenomenon for bibliometrics, Scientometrics 126 (6) (2021) 5305–5319.
[14] N. Chams, et al., COVID-19: a multidisciplinary review, Front. Public Health 8 (2020) 383.
[15] Y.-J. Han, et al., Advances and challenges in the prevention and treatment of COVID-19, Int. J. Med. Sci. 17 (12) (2020) 1803.
[16] M.S. Mirtaleb, et al., Potential therapeutic agents to COVID-19: An update review on antiviral therapy, immunotherapy, and cell therapy, Biomed. Pharmacother. (2021), 111518.
[17] S.H. Nile, G. Kai, Recent clinical trials on natural products and traditional Chinese medicine combating the COVID-19, Indian J. Microbiol. 61 (1) (2021) 10–15.
[18] M. Gavriatopoulou, et al., Emerging treatment strategies for COVID-19 infection, Clin. Exp. Med. 21 (2) (2021) 167–179.
[19] J.J. Park, et al., Clinical trials of disease stages in COVID 19: complicated and often misinterpreted, Lancet Glob. Health 8 (10) (2020), e1249.
[20] J.I. Forrest, et al., Early treatment of COVID-19 disease: a missed opportunity, Infect. Dis. Ther. 9 (4) (2020) 715–720.
[21] M.D. Mesarović, Systems Theory and Biology—View of a Theoretician, in Systems Theory and Biology, Springer, 1968, pp. 59–87.
[22] I. Tavassoly, J. Goldfarb, R. Iyengar, Systems biology primer: the basic methods and approaches, Essays Biochem. 62 (4) (2018) 487–500.
[23] R.R.K. Doust, Systems-biology based pharmaceutical composition comprising a mixture of herb extracts as active ingredients, use of said composition as a medicament, particularly in the treatment of human coronavirus infections such as covid-19, M. PHARMA, Editor. 22: Norway.
[24] I.H.T. Guideline, Impurities: guideline for residual solvents Q3C (R5), Curr. Step 4 (2005) 1–25.
[25] C. Batéjat, Q. Grassin, J.-C. Manuguerra, Heat inactivation of the severe acute respiratory syndrome coronavirus 2, J. Biosaf. Biosecurity 3 (1) (2021) 1–3.
[26] M.E. Darnell, D.R. Taylor, Evaluation of inactivation methods for severe acute respiratory syndrome coronavirus in noncellular blood products, Transfusion 46 (10) (2006) 1770–1777.
[27] T.T. Wang, et al., Effective heat inactivation of SARS-CoV-2, MedRxiv (2020).
[28] M. Saghaei, Random allocation software for parallel group randomized trials, BMC Med. Res. Methodol. 4 (1) (2004) 1–6.
[29] S. Ali, et al., Natural products can be used in therapeutic management of COVID-19: Probable mechanistic insights, Biomed. Pharmacother. 147 (2022), 112658.
[30] A. Goc, et al., Inhibitory effects of specific combination of natural compounds against SARS-CoV-2 and its Alpha, Beta, Gamma, Delta, Kappa, and Mu variants, Eur. J. Microbiol. Immunol. (2022).
[31] A. Garg, et al., Virtual screening of natural products inspired in-house library to discover potential lead molecules against the SARS-CoV-2 main protease, J. Biomol. Struct. Dyn. (2022) 1–13.
[32] Y. Kumar, H. Singh, C.N. Patel, In silico prediction of potential inhibitors for the main protease of SARS-CoV-2 using molecular docking and dynamics simulation based drug-repurposing, J. Infect. Public Health 13 (9) (2020) 1210–1223.
[33] T.E. Tallei, et al., Potential of plant bioactive compounds as SARS-CoV-2 main protease (Mpro) and spike (S) glycoprotein inhibitors: a molecular docking study, Scientifica 2020 (2020).
[34] A.K. Mishra, S.P. Tewari, In silico screening of some naturally occurring bioactive compounds predicts potential inhibitors against SARS-CoV-2 (COVID-19) protease, arXiv preprint arXiv:2004.01634 (2020).
[35] R.K. Suryawanshi, et al., Dysregulation of cell signaling by SARS-CoV-2, Trends Microbiol. 29 (3) (2021) 224–237.