ARTICLE

Cite This: Int. J. Infe. Diseas. Prevent. medicin. 2022, 1-1, 10-21

Sciforce International Journal of Infectious Diseases and Preventive Medicine

Journal homepage: www.sciforce.org

The Effects of Hydroalcholic Extract Polyherbal Formulation On Improving The Symptoms of Patients With COVID-19 In Hospital

Mohammadreza Gholibeikian[1*], Hasan Fallah Huseini[2], Afsaneh Kaffash[3], Majid Ghorbani Nohooji[2]

[1]*Department of Organic Chemistry, Faculty of Chemistry, University of Kashan, Kashan, I.R.Iran.*

[2]*Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, I.R.Iran.*

[3]*Infectious Diseases Department, Mashhad University of Medical Sciences, Mashhad, Iran.*

ARTICLE INFO

Article history:
Received: 20221011
Received in revised form: 20221020
Accepted: 20221115
Available online: 20221125

Keywords:
hydroalcholic extract;
double-blind placebo;
Herbal medicine;
Imfluna.

ABSTRACT

Objectives:

The pandemic of Coronavirus disease and severity of the infection and high mortality have almost unprecedented challenges in the health systems of most countries around the world. The present study aimed to evaluate the effect of hydroalcholic extract polyherbal formulation as entitled Imfluna on symptoms of COVID-19 infected patients. The polyherbal remedy for Imfluna had a significant effect on pulmonary involvement and reduced pulmonary involvement, the severity of shortness of breath, alanine aminotransferase (ALT), sodium (Na) in the post-test phase. Also, the average CBC count and percentage of blood oxygen saturation increased in both experimental and control groups. In addition to, the mean CBC count and percentage of blood oxygen saturation of the control group increased significantly.

Methods:

In this randomized double-blind placebo-controlled clinical trial a total of 60 voluntary confirmed COVID-19 patients were randomly assigned to placebo and Imfluna groups. Patients in each groups, in addition to receiving standard medications, took two 500 mg Imfluna capsules or placebo every 8 hours for 2 weeks. The patient's vital signs, pulmonary involvement, severity of shortness of breath, average blood CBC count, Percentage of blood oxygen saturation, liver and kidney function tests and study Na were evaluated.

Results:

The results showed that patients in the Imfluna-treated group had significantly greater improvement in pulmonary involvement, severity of shortness of breath, average blood CBC count, Percentage of blood oxygen saturation compared with the placebo group.

Conclusion:

Patients with COVID-19 who were treated with a Imfluna for 2 weeks had better comfort and fewer symptoms associated with the disease with no drug side effects.

∗Corresponding author. e-mail: mgholibeikian@gmail.com

Introduction

COVID-19 infection since 2019 is a global pandemic that has challenged the health care system worldwide. The symptoms of coronavirus are usually fever, cough, sore throat, breathlessness, fatigue and malaise among others. The disease is mild in most people; in some may progress to pneumonia, acute respiratory distress syndrome and multi organ dysfunction. Most infected patients are asymptomatic or have only mild symptoms. (China Novel Coronavirus I, 2019).

The lung is the main affected organ in severe coronavirus disease caused by the novel coronavirus and undergoes an acute pneumonia process accompanied by diffuse alveolar injury, inflammatory infiltrates, and microvascular thrombosis that is the leading cause of death in the vast majority of patients. (Yuki et al., 2020).

Although the recommended treatments with drugs for COVID-19 and its side effects are antiviral, antithrombotic, anti-inflammatory, antibiotic, anti-malarial, immunomodulatory, and anticoagulant, there is currently a number of drugs used for treatments of this virus, but their safety and efficacy are under investigation (Wu et al., 2020).

Many of the immune responses and respiratory symptoms of COVID-19 patients are common with other respiratory viral diseases (Yuki et al., 2020). In Iranian traditional medicine, several medicinal plants with antiviral, imunomodelating, anti-inflammatory, antioxidant and antimicrobial properties are known to improve pulmonary and systemic symptoms of viral infections, which may be effective in preventing and improving the symptoms of COVID infection (Kenari et al., 2021). Herbal product under the brand name Imfluna is one of the herbal mixtures used similarly by some other herbal ingredients (Manavari SHR, 1386). The main components in the formulation of Imfluna herbal product are included *Echinacea angustifolia* aerial part (Echinacea), *Stachys lavandulifolia* aerial part (Stachys), *Artemisia annua* aerial part (Artemisia), *Hyssopus officinalis* aerial part (Hyssopus), *Polypodium vulgare* rhizome (Polybody), *Alpinia officinarum* rhizome (Alpinia), *Zingiber officinale* rhizome (Ginger) and *Panax ginseng* root (Ginseng). These are traditionally prescribed to treat cough, immune system disorders, respiratory and inflammatory diseases (HajiSharifi, 2003). Polybody is used for cough and shortness of breath; Stachys is used in viral infections and strengthening the immune system; Echinacea, is used in colds, flu, smallpox, measles, inflammation and for strengthening the immune system; Artemisia, is used to remove pathogens and toxins from the body and cures shortness of breath; Alpinia is prescribed to strengthen the immune system; Hyssopus, is prescribed for the treatment of lung infection, coughs, and shortness of breath; Ginger, is prescribed for severe colds and pneumonia; Ginseng is prescribed for respiratory disorders, shortness of breath and to strengthen the body weakness (HajiSharifi, 2003).

A number of experimental and clinical studies demonstrated several pharmacological effects for aforementioned plants including: anti-inflammatory, immunomodulatory, antiviral, antimicrobial and antifungal effects for Echinacea (Barnes et al., 2005); expectorant and antitasive effects indicated for symptom of respiratory diseases by Polybody (Silveira et al., 2020); analgesic, anti-inflammatory immunomodulatory and antimicrobial effects for Stachys (Hajhashemi et al., 2007), antioxidant and antiviral activities for Alpinia (Pillai et al., 2018); antiviral and immunosuppressive activities for Artemisia (Hou et al., 2009); antiviral, anti-inflammatory, analgesic and antipyretic effects for Ginger (Silveira et al., 2020); antioxidant and anti-viral activities for Hyssopus (Ang et al., 2020) and strengthening of host immunity effects and antiviral for Ginseng (Im et al., 2016).

Evaluation of the effect of Imfluna herbal compound on the improvement of covid-19 pneumonia symptoms in patients were done by Baqiyatallah Hospital in previous study (Fallah Huseini et al., 2022). Effects of herbal drug Imfluna were done on improving the symptoms of patients with COVID-19: A placebo controlled double-blind clinical study. This double-blind, phase 2 clinical trial was performed in 60 patients with covid-19 pneumonia. Patients were randomly assigned to 30 blocks of 2 patients. Each patient in the block then received herbal or placebo capsule with code A or B. So that 30 patients were given herbal compound and 30 people were given placebo. The duration of treatment was two weeks. Patients in intervention group in addition to receiving standard medications, take two 500 mg capsules of the herbal compound three times a day after meals. The herbal capsule is given as a supplement to patients for two weeks along with standard medications. Also, patients in Control group in addition to receiving standard medications, take two 500 mg capsules of the placebo three times a day after meals. The placebo capsule contains a toasted powder. The placebo capsule is given as a supplement to patients for two weeks along with standard medications. The herbal capsule in intervention group contains a mixture of medicinal plant extract powder and in control grop contains a toasted powder are manufactured by the HomaPharmed Pharmaceutical Company.

Patients with COVID-19 who were treated with Imfluna for 2 weeks had better comfort and fewer symptoms associated with the disease with no any drug side effects (Fallah Huseini et al., 2022).
The results showed that patients in the Imfluna-treated group had significantly greater improvement in daily cough, shortness of breath and ESR compared with the placebo group. In addition, lung lesions improved in the Imfluna-treated group, although not significantly (Fallah Huseini et al., 2022).

Therefore, due to the lack of standares effective drug therapy for COVID-19 and the history of Imfluna use in the Iranian traditional medicine for strengthening body immunity and treatment of pulmonary viral infections, this study was conducted in the larger statistical community by Central Organization of Sabzevar University of Medical Sciences. Another placebo controled double-blind clinical study in total of 60 voluntarily approved patients with COVID-19 were randomly assigned to the placebo and Imfluna groups to investigate the efficacy of Imfluna in controlling the symptoms of COVID-19 infected patients.

Study medication

Imfluna and placebo capsules were provided by the Homafarmed Pharmaceutical Company Ltd. The Imfluna active ingredient was a mixture of medicinal plants extracts including: Artemisia, Echinacea, Stachys, Hyssopus aerial part, Polypody, Alpinia, Ginseng root and Zingiber rhizome. The Imfluna was formulated in a 500 mg hard gelatin capsule. The placebo capsules were also prepared similarly using toasted powder.

Standardization of herbal extract

For standardization of the herbal combination extract, Artimisinin concentration was determined by HPLC instrument. Artimisinin concentration was 1.56 ± 0.06 mg/1000 mg of the dry extract of plants mixure.

Trial design and participants

This placebo-controlled double-blind randomized clinical trial, began on November 2020 in Mohammad Vasei Hospital. A total of 60 COVID-19 approved Iranian volunteer patients who were recently admitted to Mohammad Vasei Hospital in in sabzevar city, Razavi Khorasan Province.

This clinical trial was approved by the Ethics Committee of Sabzevar University of Medical Sciences (IR.MEDSAB.REC.1399.115 dated: 01.11.2020) and the trial was registered in the Iranian Registry of Clinical Trials (IRCT20080901001157N17 dated: .29.11.2020).

Inclusion criteria

The inclusion criteria were patients with symptomatic COVID-19 pneumonia including cough and shortness of breath with PCR diagnostic test; ages 20 to 70 years, who are able to use oral medications; declare consent to participate in the study and give written informed consent.

Exclusion criteria

The exclusion criteria were patients with difficulty in swallowing or the possibility of aspiration of food; severe shortness of breath; patients who are unable to take the drug orally; patients with refractory hypoxemia; decreased level of consciousness; hemodynamic instability; hypercapnia; respiratory fatigue who require hospitalization in intensive care units; patients with respiratory failure requiring mechanical ventilation; patients with immunodeficiency, including patients treated with corticosteroids, and chemotherapy; patients with malignancies, organ transplants and HIV; patients with underlying diseases including: cardiovascular disease, uncontrolled hypertension, uncontrolled diabetes and underlying respiratory diseases; patients known to have history of seasonal allergic rhinitis or allegy to asteraceae (compositae) family plants; patients with BMI> 40 and pregnant or breastfeeding women.

Sample Size

A statistical power analysis using GPower 3.1.97 software was performed for sample size estimation, based on comparing two means and the effect size (ES) equal to 0.8, considered to be large using Cohen's (1988) criteria. With an alpha =0.05 and power = 0.80, the projected sample size needed with this effect size is approximately N = 52 (26 in each group) for this simplest between/within group comparison. Thus, our proposed sample size of 52+8=60 will be more than adequate for the main objective of this study and should also allow for expected attrition and our additional objectives of controlling for possible mediating/moderating factors/subgroup analysis, etc.

Intervention

After confirmation of COVID-19 infection, sixty male and female volunteer Iranian patients who met the inclusion criteria signed a writtenin-formed consent form before participating in the study. Patients in both groups received standard medications, including antiviral and anti-inflammatory drugs, and any supportive therapy as required. In addition to standard COVID-19 medications (routine treatment according to the latest national guideline for the treatment of COVID-19), patients in Imfluna group received 2 Imfluna capsules and patients in placebo group received 2 placebo capsules every 8 hours daily for 14 days.

Randomization

A random number table and block randomization method is used. In this method 60 eligible patients are assigned into 30 blocks of 2 patients. Then, each of the 2 patients in the block is randomly assigned to take Imfluna or placebo, so that 30 patients assigned to each group.

Blinding

Package for herbal and placebo capsule were identical in all specifications and labeled with code A or B. No one except technician who has done the capsules packaging is aware of the contents of the packages or what is code A or B. Patients are aware that they are either in the Imfluna or placebo groups, but they are not aware of the type of group they are in.

Outcomes

The daily shortness of breath and day and night cough was measured during 14 days of the study as primary outcomes. The Shortness of Breath with Daily Activity Questionnaire and Cough Symptom Scoring Questionnaire were used for data collection of cough and shortness of breath respectively during the study (Hsu et al., 1994, Watkins et al., 2013).

The patient's vital signs, including: blood pressure, heart rate, body temperature, respiratory rate and oxygen saturation were recorded at baseline and then every 8 hours during the study as secondary outcomes. The complete blood count (CBC), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and creatinine (Cr) as secondary outcomes were determined at baseline, day 3, day 8 and endpoint for any hematological, hepatic and renal drug's probable adverse effects. Other secondary outcomes including erythrocyte sedimentation rate (ESR), serum C-reactive protein (CRP) were determined at baseline and endpoint.

Side effects

Artemisia and Echinacea may cause transient allergies in patients who are allergic to the Asteraceae plant family. Although no documented side effects were reported for this combination in our pilot study, in the present study, patients were advised to report any adverse events such as urticaria, hot flashes, wheezing, nasal congestion, and gastrointestinal upset.

Preventive measures

Imfluna or placebo was discontinued if the patient's health or symptoms worsened.

Statistical analysis

The SPSS software (version 17, IBM Corporation) was used for analysis of data. Baseline and post intervention data were analyzed using Kolomograph-Smirnov test, paired sample Kolomograph-Smirnov test and analysis of covariance. Generalized estimating equation (GEE) model with identity link function and exchangeable correlation matrix was used to compare the groups, adjusted for other covariates such as age, BMI and sex. P-value < 0.05 was considered as significant. The data were analyzed by the intention-to-treat approach.

Results:

The main purpose of this study was to investigate the effect of influenza herbal composition on improving the symptoms of patients with COVID-19: a two-sided clinical trial with placebo group. For this purpose, 60 people were randomly selected by stratified sampling method and information was collected using a questionnaire and laboratory research. After collecting data, descriptive statistics and inferential statistics (Kolmogorov-Smirnov test and analysis of covariance) were used using SPSS software to analyze the data

The study was started in november 2021 in Sabzevar University of Medical Sciences Hospital and competed in january 2020. There was no significant difference between age, sex and body mass index of patients (BMI) in the two groups at baseline. The frequency distribution was 48.3% for men and 51.7% for women (31 people). In figures 1 & 2 were observed frequency distribution of respondents' age and frequency distribution of respondents' weight.

Assessment of the patients' vital signs

Pulmonary involvement:

Kolomograph-Smirnov test was used to evaluate the normality of statistical distribution. As can be seen in the pre-test and post-test table 1 & figure 3 of pulmonary involvement in the experimental and control groups, the mean pulmonary involvement of the experimental group decreased significantly from 0.5 to 0.42. But the mean pulmonary involvement of the control group did not change much. This means that the herbal remedy for Imfluna has a significant effect on pulmonary involvement and reduces pulmonary involvement in the post-test phase.

Table 1. Pulmonary involvement

Numbers	Standard deviation	Average	Groups	
30	0.197	0.5	Pulmonary involvement pre-test	Exprimental
30	0.190	0.428	Pulmonary involvement test	
30	0.160	0.506	Pulmonary involvement pre-test	Control
30	0.163	0.485	Pulmonary involvement test	

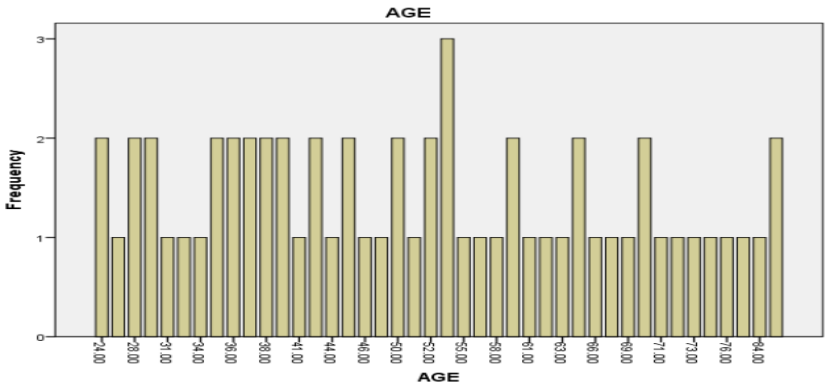

Fig.1. Age

Table 2. Shortness of breath

Numbers	Standard deviation	Average	Groups

30	0.339	0.866	Pre-test for the severity of shortness of breath	Exprimental
30	0.481	0.366	Post-test for the severity of shortness of breath	
30	0.309	0.892	Pre-test for the severity of shortness of breath	Control
30	0.489	0.6	Post-test for the severity of shortness of breath	

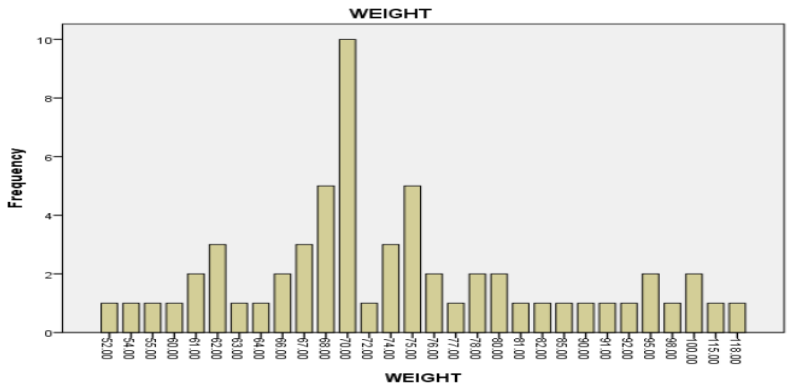

Fig.2. Weight

Table 3. Average blood CBC count and study PL

Numbers	Standard deviation	Average	Groups	
30	56.860	185.448	CBC-PLT counting pre-test	Exprimental
30	43.367	205.827	CBC-PLT post-test	
30	61.898	154.965	CBC-PLT counting pre-test	Control
30	35.846	171.241	CBC-PLT post-test	

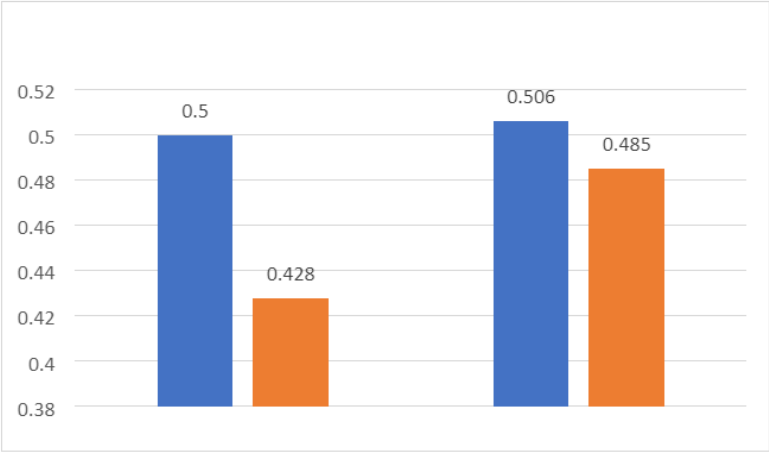

Fig.3. Graph of mean pre- and post-test pulmonary involvement in experimental and control groups

Table 4. Percentage of blood oxygen saturation

Numbers	Standard deviation	Average	Groups	
30	4.606	92.33	Pre-test of blood oxygen saturation percentage	Exprimental
30	2.112	95.266	Post-test of blood oxygen saturation percentage	
30	8.33	90.266	Pre-test of blood oxygen saturation percentage	Control
30	2.676	93.633	Post-test of blood oxygen saturation percentage	

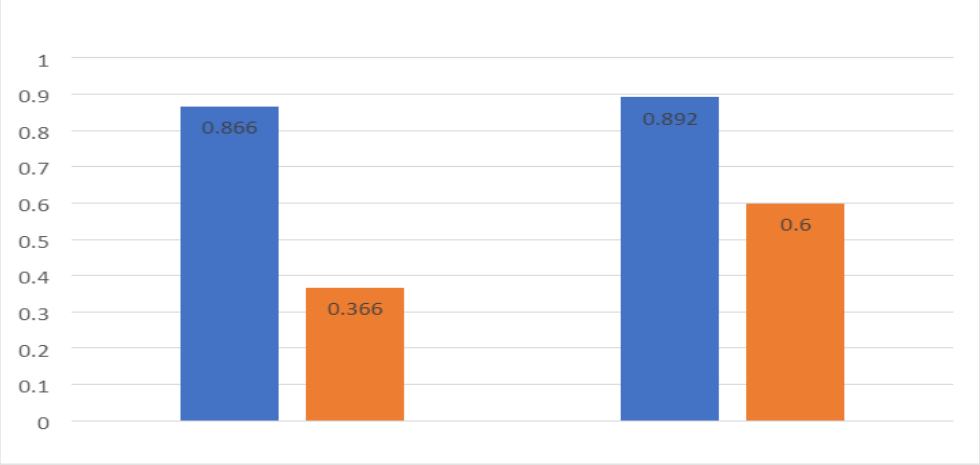

Fig.4. Graph of mean before and after shortness of breath test in experimental and control groups

Table 5. Mean liver enzymes (ALT)

Numbers	Standard deviation	Average	Groups	
30	21.810	47.758	ALT pre-test	Exprimental
30	12.899	37.068	ALT post-test	
30	16.210	42.586	ALT pre-test	Control
30	11.025	38.862	ALT post-test	

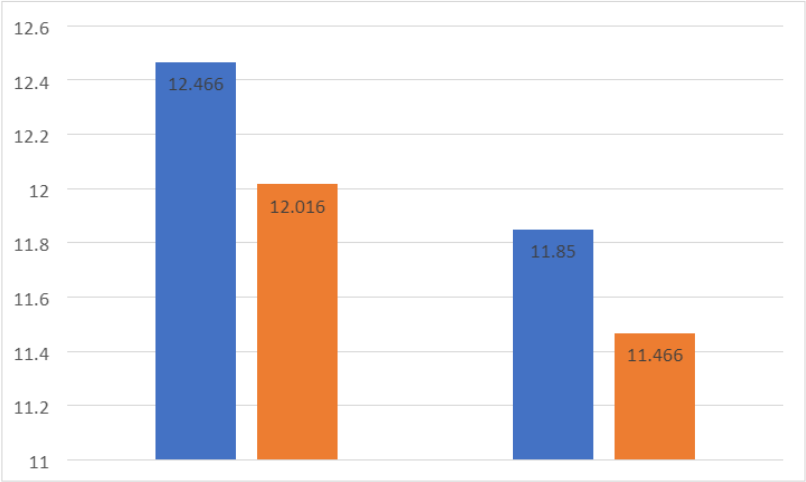

Fig.5. Chart of mean pre- and post-test of CBC count based on PLT in experimental and control groups

Table 6. Study Na

Numbers	Standard deviation	Average	Groups	
30	3.642	142.103	Pre-test Na	Exprimental
30	3.961	139.582	Post-test Na	
30	4.462	137.827	Pre-test Na	Control
30	4.389	134.896	Post-test Na	

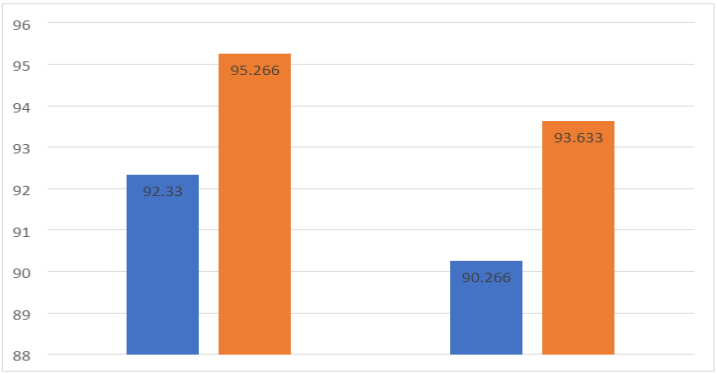

Fig.6. Diagram of mean pre- and post-test percentage of blood oxygen saturation in experimental and control groups

Shortness of breath:

Kolomograph-Smirnov test was used to evaluate the normality of statistical distribution.

As can be seen in the pre-test and post-test table 2 & Figure 4, the severity of shortness of breath in both experimental and control groups, the mean severity of shortness of breath in the experimental group has decreased significantly from 0.866 to 0.366. But the mean severity of shortness of breath in the control group did not change much. This means that the administration of the herbal remedy for Imfluna has a significant effect on the severity of shortness of breath and has reduced the severity of shortness of breath in the post-test phase.

Average blood CBC count, Study PLT:

Kolomograph-Smirnov test was used to evaluate the normality of statistical distribution. As can be seen in the pre-test and post-test table 3 & Figure 5 of CBC count in both experimental and control groups, the average CBC count of

the experimental group has increased from 185.448 to 205.827. Also, the mean CBC count of the control group increased significantly.

Percentage of blood oxygen saturation:

Kolomograph-Smirnov test was used to evaluate the normality of statistical distribution.

As can be seen in the pre-test and post-test table 4 & Fig 6, the percentage of blood oxygen saturation in the experimental and control groups, the average percentage of blood oxygen saturation in the experimental group increased from 92.33 to 95.266. Also, the average percentage of blood oxygen saturation in the control group changed from 90.266 to 93.633.

Biochemical analysis of blood:

Mean liver enzymes (ALT):

Kolomograph-Smirnov test was used to evaluate the normality of statistical distribution. As can be seen in the ALT pre-test and post-test table 5 & Fig 7 in the experimental and control groups, the mean ALT of the experimental group changed from 48.758 to 37.068. Also, the mean ALT of the control group changed from 42.568 to 38.862. This means that the administration of the herbal drug Imfluna has a significant effect on ALT and has changed ALT in the post-test phase.

Study Na:

Kolomograph-Smirnov test was used to evaluate the normality of statistical distribution.

As can be seen in the pre-test and post-test Na table 6 & Fig 8 in the experimental and control groups, the mean Na of the experimental group changed from 142.103 to 139.582. Also, the mean Na of the control group changed from 137.827 to 134.896. This means that the administration of the herbal remedy for Imfluna had a significant effect on Na and caused a change in Na in the post-test phase.

Discussion:

The present study reports the safety and efficacy of Imfluna, a natural Iranian traditional formulation containing a mixture of herbs including Polybody, Stachys, Alpinia, Echinacea, Artemisia, Ginger, Hyssopus and Ginseng in controlling the symptoms of patients with COVID-19. Treatment of COVID-19 hospitalized patients with Imfluna improved pulmonary involvement, severity of shortness of breath, average blood CBC count, Percentage of blood oxygen saturation compared with the placebo group without any side effects.

The results of the present study are in accord with result of a clinical trial, reported that infected COVID patients, treated with combination of Echinacea and Ginger had improvement level of dyspnea, coughing, and muscle pain, higher than placebo group (Mesri et al., 2021). The mechanism of action of this polyherbal combination in improving the symptoms of the COVID-19 disease can probably be related to the inhibitory effect its ingradient on the pathophysiological mechansims of creating and exacerbating the symptoms of the disease. Several laboratory and clinical research to date suggest that the body immunomudulatory response to COVID-19 infection is associated with a severe inflammatory response with the release of large amounts of pro-inflammatory cytokines or "cytokine storms" that are directly associated with lung damage (Ye et al., 2020). Therefore, any medication that regulates the aggravation of these two pathways can be effective in reducing the complications of COVID-19 disease. Imfluna is mixture of herbal medicines including Polypody, Stachys, Alpinia, Artemisia, Echinacea, Ginger, Hyssopus and Ginseng. Numerous studies have shown the anti-inflammatory, immunemodlating, anti-viral and antioxidant effects of ingredients in this product that may directly or indirectly affects the immune system and inflammatory infections in the respiratory system.

The available evidence suggests that Echinacea has direct inhibitory effects against a wide range of viruses (Sharma et al., 2009) and the mechanism of its antiviral effects may be related to the immune and anti-inflammatory effects of plant alkaline compounds (Woelkart and Bauer, 2007). As for other components, although no clinical studies have been performed to support the efficacy of Stachys and Polypody in the treatment of complications of COVID infection, the results of previous studies show that the analgesic, antipyretic, antibacterial and antiviral effects of Polypody (Dar et al., 2012, Silveira et al., 2020) and Stachys analgesics, anti-inflammatory and antioxidant activity may be effective in treating COVID complications (Hajhashemi et al., 2007, İşcan et al., 2012). As for Alpinia, another component of Imfluna, in vitro and in vivo studies have reported significant antiviral, anti-inflammatory, and antibacterial properties of this plant (Abubakar et al., 2018, Pillai et al., 2018). However, the anti-inflammatory effects of Alpinia phenolic extract have been attributed to the inhibition of COX-2 (Honmore et al., 2016). COX-2 plays a key role in the inflammatory process and a selective inhibition of COX-2 may help in decreasing the mortality and morbidity rate in COVID-19 patients (Yuki et al., 2020). Artemisia is another component of Imfluna. In accordance to our study safety and efficacy of artemisinin for treatment of patients with mild-to-moderate COVID-19 has been repoted in a clinical trial. In that study, the time to reach undetectable SARS-CoV-2 was significantly shorter in artemisinin treated patients compare to control group (Li et al., 2021). To explain the mechanism of action of Artemisia annua, in the previous studies, artemisinin and its derivatives such as artesunate, have been shown to exert immunomodulatory functions (Hou et al., 2009) and sterols including sitosterol and stigmasterol have been isolated from Artemisia, as virus inhibitory agents (Khan et al., 1991). Ginger, another herbal indredient of Imfluna, showed a wide range of antiviral effects in experimental studies (Silveira et al., 2020). In support to our finding in a clinical trial, intake of

diet supplemented with Ginger in patients with acute respiratory distress syndrome, decreased duration of mechanical ventilation and length of stay in intensive care unit (Shariatpanahi et al., 2013). Ginger plant containes ingredients such as 6-gingerols, 6-shogoals, zhingerol with antioxidant and anti-inflammatory properties, that can reduce inflammatory mediators such as inflammatory cytokines and chemokines (Aryaeian and Tavakkoli, 2015). These inflammatory responses has been exaggerated in COVID infected patients (Yuki et al., 2020). Hyssopus, another herbal ingredient of Imfluna, has demonstrated antiviral effects in laboratory studies. This antiviral effect was against herpes simplex virus and human immunodeficiency virus (Behbahani, 2009). Researchers claim that, this antiviral effect may be due to the inhibition of oxygen free radicals by Hyssopus chemical components (Behbahani, 2009). In the case of Ginseng, another herbal ingredient of Imfluna, in accordance to our study its antiviral effects reported in a placebo-controlled trial in which the Korean red ginseng extract prevents influenza-like illness in healthy adults (Ha et al., 2012). The mechanism for its antiviral effects claim to be due to anti-immunomodulatory and inflammatory in viral infection (Kim et al., 2016).

With the use of Imfluna product compared to the control group was observed improvement in the symptoms of patients with Coronavirus. Impressive results was observed in items such as pulmonary involvement, severity of shortness of breath, average blood CBC count, Percentage of blood oxygen saturation.

Other article reviews a wide variety of research findings with the aim of synthesizing evidence of sodium toxicity as a modifiable dietary factor associated with the nutritional epidemiology and nutritional immunology of COVID-19 and SARS-CoV-2 infection. In addition, sodium toxicity causes pulmonary edema associated with severe acute respiratory syndrome, as well as inflammatory immune responses and other symptoms of COVID-19 such as fever and nasal sinus congestion (Brown, 2021).

Hypertension was identified as a risk factor associated with severe cases of COVID-19 in China and Italy (Cook, et al., 2020). Of relevance, hypertension was found to be a risk factor associated with mortality in both the 2009 (H1N1) swine influenza pandemic (Barakat et al., 2012) and the 2013 avian influenza A (H7N9) virus infection (Ji et al., 2014).

Sodium chloride intake is associated with changes in immune responses that promote organ damage and inflammation, including increased release of inflammatory cytokines, like interleukin (IL)-6, macrophage inflammatory protein-2 (MIP-2), and tumor necrosis factor (TNF)-α (Afsar et al., 2018). Elevated sodium chloride concentrations also increase the proliferation of T-cells, while decreasing the anti-inflammatory responses., for example, anti-inflammatory M2 macrophages are suppressed while pro-inflammatory M1 macrophages are increased by high sodium chloride levels. Furthermore, sodium chloride was found to enhance the production of IL-4 and IL-13, and to suppress the production of interferon in memory T cells (Matthias et al., 2019). Interleukin-17 (IL-17) producing helper T cells (Th17) play a role in clearing the extracellular pathogens, and Th17 cell development is induced by a kinase signaling pathway activated by high sodium chloride concentrations (Wu et al., 2013). Sodium toxicity is also linked to pulmonary edema, a potential transitive link is established between sodium toxicity and ARDS in COVID-19 patients.

A high sodium chloride intake is a risk factor associated with diseases that are also risk factors of underlying conditions associated with COVID-19 morbidity and mortality. The most fatal pathological effect of COVID-19 is acute respiratory distress syndrome with yellow fluid that blocks lung air sacs, and this appears to be the same fluid in pulmonary edema caused by excess sodium chloride. Sodium toxicity is also associated with other symptoms of COVID-19 like fever and nasal sinus congestion (Brown, 2021).

As can be seen in the pre-test and post-test Na table 6 & Fig 8 in the experimental and control groups, the mean Na of the experimental group changed from 142.103 to 139.582. Also, the mean Na of the control group changed from 137.827 to 134.896. This means that the administration of the herbal remedy for Imfluna had a significant effect on Na and caused a change in Na in the post-test phase.

Liver enzymes are frequently deranged in patients admitted with COVID-19. Liver enzymes should be regularly monitored during the course of management of COVID-19, as various medications used in the treatment of COVID-19 may further deteriorate liver enzymes and may cause long-term damage.

In view of the above-mentioned literature, higher levels of liver enzymes are observed in critical COVID-19 patients. Cai et al. stated that with the administration of lopinavir-ritonavir, changes in abnormal liver function tests (LFTs) (Cai et al., 2020).

Similarly, a clinical trial conducted on 199 patients with severe COVID-19 demonstrated that increases in ALT, AST, and total bilirubin levels were more often observed in the lopinavir-ritonavir group compared to those who did not receive this. Remdesivir has also been reported to cause early recovery of COVID-19 patients (Beigel et al., 2020). In a trial assessing Remdesivir treatment for five or 10 days, a considerable increase in ALT/AST levels was observed in 4-6% of patients as well as a life-threatening increase in 2-3% of patients, leading to the discontinuation of the treatment (Goldman et al., 2020).

In the study of the mean ALT of the experimental group changed from 48.758 to 37.068. Also, the mean ALT of the control group changed from 42.568 to 38.862. This means that the administration of the herbal drug Imfluna has a significant effect on ALT and has changed ALT in the post-test phase.

Patients with COVID-19 who had decreased oxygen saturation or increased respiratory rate at hospital admission had a "markedly elevated" risk for mortality, according to researchers. In the study of the average percentage of blood oxygen saturation in the experimental group increased from 92.33 to 95.266. Also, the average percentage of blood oxygen saturation in the control group changed from 90.266 to 93.633. The mean pulmonary involvement of the experimental group decreased significantly from 0.5 to 0.42. But the mean pulmonary involvement of the control group did not change much. This means that the herbal remedy for Imfluna has a significant effect on pulmonary involvement and reduces pulmonary involvement in the post-test phase. In the study of shortness of breath, the mean severity of shortness of breath in the experimental group has decreased significantly from 0.866 to 0.366. But the mean severity of shortness of breath in the control group did not change much. This means that the administration of the herbal remedy for Imfluna has a significant effect on the severity of shortness of breath and has reduced the severity of shortness of breath in the post-test phase. The average CBC count of the experimental group has increased from 185.448 to 205.827. Also, the mean CBC count of the control group increased significantly.

Conclusion:

Treatment of COVID-19 patients with Imfluna, an Iranian traditional polyherbal medicine, improved symptoms associated with the disease including pulmonary involvement, severity of shortness of breath, average blood CBC count, Percentage of blood oxygen saturation within 14 days of the study without any side effects. It is suggested to conduct a further trials with larger number of patients assessing the efficacy and safety of Imfluna in the treatment of COVID-19 infection, as well as more studies addressing the mechanisms and bioactives involved in the anti-COVID effects of Imfluna seem necessary. It is also suggested to investigate the efficacy of this herbal medicine in the prevention of COVID-19 disease, especially for people who are in contact with newly diagnosed COVID-19 patients.

Funding

This research was financial supported by Homapharmed Pharmacutical Company, Tehran Iran.

Acknowledgments

We thank Dr. Morteza Khairabadi, head of Homapharmad Pharmaceutical Company, for financially supporting this study, Hadi Khairabadi for coordinating the collection of plants and seeking the extraction and preparation of medicine, and Mehdi Khairabadi to bring together the research team and group liaison, Reza Mirzaei for consulting in the selection and dosage of medicinal plants and botanist Dr. Gorbani to identify medicinal plants. We also thank Sabzevar University of Medical Sciences for conducting this clinical trial and the ACECR Institute of Medicinal Plants for providing herbal medicine and placebo.

Author Disclosure Statement

No competing financial interests exist.

References:

1. Abubakar IB.; Malami I.; Yahaya Y.; Sule SM. A review on the ethnomedicinal uses, phytochemistry and pharmacology of Alpinia officinarum Hance. *Journal of ethnopharmacology*. **2018**, 224, 45-62.
2. Afsar, B.; Kuwabara, M.; Ortiz, A.; Yerlikaya, A.; Siriopol, D.; Covic, A.; Rodriguez-Iturbe, B.; Johnson, R.J.; Kanbay, M. Salt intake and immunity. *Hypertension*. **2018**, 72, 19–23. [CrossRef].
3. Ang L.; Song E.; Lee HW.; Lee MS. Herbal medicine for the treatment of coronavirus disease 2019 (COVID-19): a systematic review and meta-analysis of randomized controlled trials. *Journal of clinical medicine*. **2020**, 9(5), 1583.
4. Aryaeian N.; Tavakkoli H. Ginger and its effects on inflammatory diseases. *Adv Food Technol Nutr Sci Open J*. **2015**, 1(4), 97-101.
5. Barakat, A.; Ihazmad, H.; El Falaki, F.; Tempia, S.; Cherkaoui, I.; El Aouad, R. 2009 Pandemic influenza A virus subtype H1N1 in Morocco, 2009–2010: Epidemiology, transmissibility, and factors associated with fatal cases. *J. Infect. Dis.* **2012**, 206, S94–S100.[CrossRef] [PubMed].
6. Barnes J.; Anderson LA.; Gibbons S.; Phillipson JD. Echinacea species (Echinacea angustifolia (DC.) Hell., Echinacea pallida (Nutt.) Nutt., Echinacea purpurea (L.) Moench): a review of their chemistry, pharmacology and clinical properties. *Journal of Pharmacy and Pharmacology*. **2005**, 57(8), 929-954.
7. Beigel JH.; Tomashek KM.; Dodd LE. et al.: Remdesivir for the treatment of COVID-19- final report. *N Engl J Med.* **2020**, 383, 1813-1826. 10.1056/NEJMoa2007764.
8. Behbahani M. Anti-viral activity of the methanolic leaf extract of an Iranian medicinal plant "Hyssopus officinalis" against herpes simplex virus. *J Med Plant Res*. **2009**, 3, 1118-1125.
9. https://www.mdpi.com/1648-9144/57/8/739
10. Cai Q.; Huang D.; Yu H. et al. COVID-19: Abnormal liver function tests. J Hepatol. **2020**, 73, 566-574. 10.1016/j.jhep.2020.04.006.
11. China Novel Coronavirus I RT. A Novel Coronavirus from Patients with Pneumonia in China. *The New England journal of medicine*. 2019, **2020**.
12. Cook, D.J.; Marshall, J.C.; Fowler, R.A. Critical Illness in Patients With COVID-19: Mounting an Effective Clinical and Research Response. *JAMA*. **2020**, 323, 1559–1560. [CrossRef].
13. Dar PA.; Sofi G.; Jafri M. Polypodium vulgare linn. A versatile herbal medicine: A review. International *Journal of Pharmaceutical Sciences and Research*. **2012**, 3(6), 1616.

14. Fallah Huseini H.; Gholibeikian M.; Shohrati M.; Kaffash A.; Taheri A.; Khajepour H.; Raei M.; Kianbakht S.; Fallah Hosseini A.; Ghorbani Nohooji M.; Mohtashami R. Effects of Imfluna, an Iranian traditional polyherbal medicine, on COVID-19 symptoms: A randomized, double-blind and placebo-controlled clinical trial. *J. Med. Plants*. **2022**, 21(81), 51-66.
15. Goldman JD.; Lye DC.; Hui DS. et al. Remdesivir for 5 or 10 days in patients with severe COVID-19 . *N Engl J Med*. **2020**, 383, 1827-1837. 10.1056/NEJMoa2015301.
16. Ha K-C.; Kim M-G.; Oh M-R.; Choi E-K.; Back H-I.; Kim S-Y. et al. A placebo-controlled trial of Korean red ginseng extract for preventing influenza-like illness in healthy adults. *BMC complementary and alternative medicine*. **2012**, 10, 12.
17. Hajhashemi V Ghannadi A.; Sedighifar S. Analgesic and anti-inflammatory properties of the hydroalcoholic, polyphenolic and boiled extracts of Stachys lavandulifolia. *Research in Pharmaceutical Sciences.* **2007,** 1(2), 92-98.
18. HajiSharifi A. Guide to identifying and using medicinal plants and treating various diseases. In Secrets of Herbal Medicine Noskh-e-Shafa. 1. Tehran, Iran: Hafez Novin Press; **2003**. p. 107, 210, 390, 486, 98, 562, 92, 600.
19. Honmore VS.; Kandhare AD.; Kadam PP.; Khedkar VM.; Sarkar D.; Bodhankar SL. et al. Isolates of Alpinia officinarum Hance as COX-2 inhibitors: Evidence from anti-inflammatory, antioxidant and molecular docking studies. *International Immunopharmacology*. **2016**, 33, 8-17.
20. Hou L-F.; He S-J.; Wang J-X.; Yang Y.; Zhu F-H.; Zhou , et al. SM934, a water-soluble derivative of arteminisin, exerts immunosuppressive functions in vitro and in vivo. *International immunopharmacology*. **2009**, 9(13-14), 1509-1517.
21. Hsu J.; Stone R.; Logan-Sinclair R.; Worsdell M.; Busst C.; Chung K. Coughing frequency in patients with persistent cough: assessment using a 24 hour ambulatory recorder. *European Respiratory Journal*. **1994**, 7(7), 1246-1253.
22. Im K.; Kim J.; Min H. Ginseng, the natural effectual antiviral: Protective effects of Korean Red Ginseng against viral infection. *J Ginseng Res.* **2016,** 40(4), 309-314.
23. İşcan G.; Demirci B.; Demirci F.; Göger F.; Kırımer N.; Köse YB. et al. Antimicrobial and antioxidant activities of Stachys lavandulifolia subsp. lavandulifolia essential oil and its infusion. *Natural product communications.* **2012,** 7(9), 1934578X1200700937.
24. Ji, H.; Gu, Q.; Chen, L.-L.; Xu, K.; Ling, X.; Bao, C.-J.; Tang, F.-Y.; Qi, X.; Wu, Y.-Q.; Ai, J. Epidemiological and clinical characteristics and risk factors for death of patients with avian influenza A H7N9 virus infection from Jiangsu Province, Eastern China. *PLoS ONE*. **2014**, 9, e89581. [CrossRef] [PubMed].
25. Kenari HM.; Yousefsani BS.; Eghbalian F.; Ghobadi A.; Jamshidi AH.; Mahroozade S. Herbal recommendations for treatment of COVID-19 symptoms according to Persian medicine. *Journal of Medicinal Plants*. **2021**, 20(77).
26. Khan MAA.; Jain D.; Bhakuni R.; Zaim M.; Thakur R. Occurrence of some antiviral sterols in Artemisia annua. *Plant Science*. **1991**, 75(2), 161-165.
27. Kim H.; Jang M.; Kim Y.; Choi J.; Jeon J.; Kim J. Red ginseng and vitamin C increase immune cell activity and decrease lung inflammation induced by influenza A virus/H1N1 infection. **2016**, 68(3), 406-420.
28. Li G.; Yuan M.; Li H.; Deng C.; Wang Q.; Tang Y. et al. Safety and efficacy of artemisinin-piperaquine for treatment of COVID-19: an open-label, non-randomised and controlled trial. *International journal of antimicrobial agents.* **2021,** 57(1), 106-216.
29. Manavari SHR HR.; Norouzbabaei Z.; Adibi L.; Norouzi M.; Ziaei SA. Investigation of antiviral effects of twenty-five species of different genera of medicinal plants Iran 1386; *Iranian Journal of Medical Microbiology*. 1(2), 49-59.
30. Matthias, J.; Maul, J.; Noster, R.; Meinl, H.; Chao, Y.-Y.; Gerstenberg, H.; Jeschke, F.; Gasparoni, G.; Welle, A.; Walter, J. Sodium chloride is an ionic checkpoint for human TH2 cells and shapes the atopic skin microenvironment. *Sci. Transl. Med*. **2019**, 11.[CrossRef].
31. Mesri M.; Saber SSE.; Godazi M.; Shirdel AR.; Montazer R.; Koohestani HR. et al. The effects of combination of Zingiber officinale and Echinacea on alleviation of clinical symptoms and hospitalization rate of suspected COVID-19 outpatients: a randomized controlled trial. *Journal of Complementary and Integrative Medicine*. **2021**.
32. Pillai MK.; Young DJ.; Bin Hj Abdul Majid HM. Therapeutic potential of Alpinia officinarum. *Mini reviews in medicinal chemistry*. **2018**, 18(14), 1220-1232.
33. Shariatpanahi ZV.; Mokhtari M.; Taleban FA.; Alavi F.; Surmaghi MHS.; Mehrabi Y.; et al. Effect of enteral feeding with ginger extract in acute respiratory distress syndrome. *Journal of critical care.* **2013**, 28(2), 217. e1-e6.
34. Sharma M.; Anderson SA.; Schoop R.; Hudson JB. Induction of multiple pro-inflammatory cytokines by respiratory viruses and reversal by standardized Echinacea, a potent antiviral herbal extract. *Antiviral research.* **2009**, 83(2), 165-170.
35. Silveira D.; Prieto-Garcia JM.; Boylan F.; Estrada O.; Fonseca-Bazzo YM.; Jamal CM.; et al. COVID-19: is there evidence for the use of herbal medicines as adjuvant symptomatic therapy? *Frontiers in Pharmacology* **2020**, 11, 1479.
36. Watkins ML.; Wilcox TK.; Tabberer M.; Brooks JM.; Donohue JF.; Anzueto A.; et al. Shortness of Breath with Daily Activities questionnaire: validation and responder

thresholds in patients with chronic obstructive pulmonary disease. *BMJ open*. **2013**, 3(10), e003048.
37. Woelkart K.; Bauer R. The role of alkamides as an active principle of Echinacea. *Planta medica*. **2007**, 73(07), 615-623.
38. Wu, C.; Yosef, N.; Thalhamer, T.; Zhu, C.; Xiao, S.; Kishi, Y.; Regev, A.; Kuchroo, V.K. Induction of pathogenic T H 17 cells by inducible salt-sensing kinase SGK1. *Nature*. **2013**, 496, 513–517. [CrossRef].
39. Wu R.; Wang L.; Kuo H-CD.; Shannar A.; Peter R.; Chou PJ.; et al. An update on current therapeutic drugs treating COVID-19. *Current pharmacology reports*. **2020**, 6(3), 56-70.
40. Ye Q.; Wang B.; Mao J. The pathogenesis and treatment of theCytokine Storm'in COVID-19. *Journal of infection*. **2020**, 80(6), 607-613.
41. Yuki K.; Fujiogi M.; Koutsogiannaki S. COVID-19 pathophysiology: A review. *Clinical immunology*. **2020**, 108427.