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A single-center non-blinded randomized clinical trial to assess the safety and effectiveness of PhR160 spray in the treatment of COVID-19 pneumonia

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COVID-19 is an emerging pandemic that caused a very widespread infection with more than 1000000 cases in Iran within a year. The main cause of mortality among patients with COVID-19 is pulmonary failure. In Iranian Traditional Medicine, essences have been used for curing pulmonary diseases. Pinen-Hydronoplacton-Ribonucleic acid (PHR) is an inhaler spray made of seven different plants, which all are used by humans and have desirable pharmacological features for treating pulmonary symptoms of COVID-19 patients. This study was conducted to assess the safety and effectiveness of PHR160 spray in improving pulmonary symptoms of COVID-19 patients. This was a single-centre, non-blinded randomized clinical trial with two parallel groups in two different wards of Baqiyatallah hospital, Tehran, Iran. Participants were 63 male patients diagnosed with COVID-19 pneumonia, divided into 2 groups of 32 in the intervention group and 31 in the control group. The intervention group received 5 days of PHR160 spray, 10 puffs each day, 300 micrograms in each puff in addition to the routine treatment. Oxygen saturation was measured by a pulse oximeter, every six hours and recorded daily. This study showed that administration of PhR 160 in patients of COVID-19 was safe, and it significantly increased the arterial oxygen saturation percentage in COVID-19 patients. In addition, it decreased hospitalization duration, dyspnea score, and cough score significantly in the patients. The statistical modelling test, with adjusting the age and respiratory rate for baseline and 4 days of the intervention, shows that the oxygen saturation percentage mean was significantly more in the intervention group by 5.14 units ($p<0.001$).

Keywords: COVID-19, Hospitalized patients, Iranian Traditional Medicine, Pinen-Hydronoplacton-Ribonucleic acid (PHR160)

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COVID-19, a widespread pandemic, started from Wuhan, China, in December of 2019 and showed a variety of symptoms in patients, mainly pulmonary symptoms[1-3]. Acute respiratory distress syndrome (ARDS) is one of the worst COVID-19 outcomes that can lead to death in patients[4]. There has not been absolute treatment or medications for COVID-19 yet, but symptomatic treatment is available. Antiviral and immune therapies are used these days for COVID-19. For ARDS of COVID-19, early recognition and comprehensive management are two important measures[3]. Also, many studies attempted to decrease the respiratory complications[4] to reduce hospitalization duration and the burden of mortality and complications.

For decades, Iranian Traditional Medicine has been used for the treatment of diseases. In Iranian Traditional Medicine, essences are a mix of volatile oil compounds in plants and have a vast use in curing pulmonary diseases[6,7]. Essences have potential biological and pharmacological activities; therefore, and therefore, have a vast range of applications in the

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medicines and food industry[8-13]. Essences have antimicrobial activities[8,9] and can destroy bacteria, fungi and viruses[10-12], without having any adverse effects on the human cells; they also have anti-inflammatory and antioxidant effects[13].

Pinen-Hydronoplacton-Ribonucleic acid (PHR) is an inhaler spray (Fig. 1). PHR is made of special oils and fatty acids with short, long, singular and multiple chains, which are natural products extracted from six types of plants, *artemisia, mentha, eucalyptus, myrtus, thyme* and *Saffron*, through distillation. All these plants have already been used by humans in singular, dual and triple forms. Some of the essences, used in this product, are eucalyptol/cineol, menthol, crocin and safranal and each of them has pharmacological features desirable for treating COVID-19 patients.

Studies show eucalyptol reduces the severity of chronic obstructive pulmonary disease (COPD)[14], long-term treatment of respiratory tract inflammation in bronchial asthma[6], anti-inflammatory activity (cineol)[3,9] in asthma[6], controlling the respiratory tract mucus by regulating cytokine[7], acute nonpurulent rhinosinusitis[15], treating respiratory tract inflammation diseases, like asthma and COPD, anti-inflammatory, and antioxidant activity, treating nasal congestion and mucus, dyspnea anti-inflammatory[16] and reducing pneumonia, dependent on the ventilator, in patients' under mechanical ventilation[17]. Menthol is useful for treating sore throat, cough, related to the common cold or respiratory inflammation, treating nasal congestion caused by nasal mucus inflammation and soothing dyspnea symptoms[18], (treating mild asthma[19]) and treating asthma, bronchitis, common cold (through inhaling), influenza and other respiratory diseases, and for treating nasal congestion in children. Crocin showed a protective effect on allergic asthma[20], reducing respiratory tract inflammation[21] and lipopolysaccharide-induced acute respiratory distress syndrome[20]. The studies on safranal showed a preventive effect on pulmonary inflammation[22] and that it can alleviate asthma[23]. In addition, safranal is effective in treating asthma and improving the respiratory system's function, pulmonary obstruction, pulmonary inflammation and response, also anti-cough effects[24].

Fig. 1 — PHR 160 (inhaler spray)

Studies have shown that essences are absorbed quickly through skin, mouth and lungs, and move through the blood-brain barrier, penetrate through the central nervous system, reach out to the receptors[25], and induce biological actions, such as sleep, relaxation, and increased digestion[26]. Most parts of the essence are excreted through the liver, in polar form, following phase one limited enzyme metabolism, by sticking to gluconate or sulfate.

For example, menthol, after prescription through the lungs in the form of food, is excreted in the form of menthol glucuronide (35%)[27-29]. This process applies to thymol, limonin, eugenol, and other essences. After the food prescription of these compounds, sulfate and glucuronide were seen in urine and plasma[30,31]. Fast metabolism and the short lifespan of the active parts of the essences have made this belief that essences have a small accumulation in the body tissues[32].

As seen in many studies, one of the most important factors that cause death in COVID-19 patients is severe inflammation known as a cytokine storm and if it is not controlled, it can cause severe injuries in patients and eventually death. In a way, that inflammation, which is a physiological process and is required for survival, goes into a faulty cycle, and causes these irreparable effects[33]. Another problem in COVID-19 patients is that this disease, due to oxidative stress caused by the disease, can cause a lot of damage to the patient; therefore compounds with antioxidant effects are of great importance.

Therefore, this study was conducted to examine the safety and effectiveness of PHR in improving pulmonary problems in COVID-19 patients.

Methodology

Trial design

This was a single-centre, non-blinded randomized clinical trial with two parallel groups in two different wards in Baqiyatallah hospital. Patients were randomly assigned to either of the two treatment regimens; routine treatment including hydroxychloroquine, oseltamivir capsule, kaletra and ribavirin as the control group and the same routing treatment plus PHR160 spray, as the intervention group. All the patients that were hospitalized in ward number 1 were put into the intervention group, and all the patients that were hospitalized in ward two were put into the control group. There was no difference or any criteria for sending patients to ward one or two.

Participants

64 patients diagnosed as having COVID-19 pneumonia, based on positive pulmonary symptoms in tomography scan (CT scan), according to ground-glass appearance and positive Polymerase chain reaction test (PCR) for COVID-19, in ward one and ward two specified for COVID-19 patients in Baqiyatallah hospital that met the inclusion criteria and did not have any exclusion criteria were entered in the study as intervention (32 patients) and control groups (32 patients). One person in the control group exited from the study, and finally, there were 32 people in the intervention group and 31 people in the control group (Fig. 2).

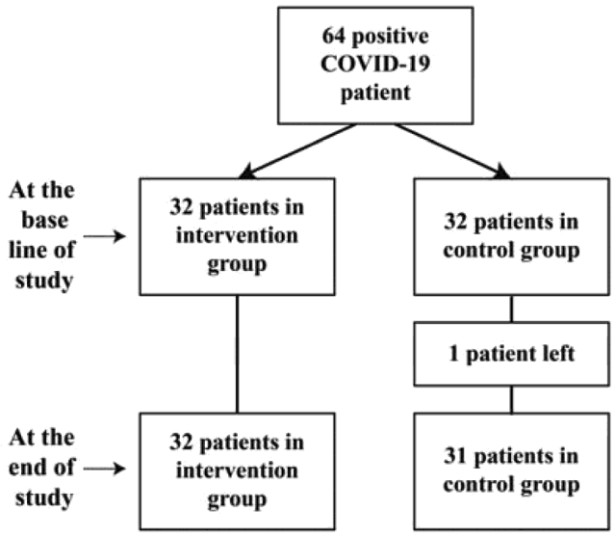

Fig. 2 — Flow chart of participants selection

Inclusion criteria were being hospitalized due to oxygen saturation less than 93%, the age between 18 and 75 years old, complaining of dyspnea and having given consent to participate in the study.

Exclusion criteria included uncontrolled diabetes, asthma, and chemotherapy in the last month, using immunosuppressive drugs, systemic steroids daily, having chronic liver failure, chronic kidney failure, positive HIV, gastrointestinal bleeding, pregnancy, and breastfeeding and being admitted to ICU.

Intervention

The intervention includes receiving Pinen-Hydronoplacton-Ribonucleic acid (PHR160 spray), 300 micrograms in each puff, one every hour for ten hours a day, from day 1 to day 5 (using spacer is compulsory). The interventional treatment was for the intervention group to receive PHR160 spray ten puffs a day, one puff in each hour during day time, in addition to routine treatment, which includes supportive measures like serum therapy and oxygen therapy, according to national protocols and using immunity amplifier compounds and antioxidants, like 75 mg BID Oseltamivir capsule and 200 mg BID Hydroxychloroquine (regimen A) or 75 mg BID Oseltamivir capsule and Kaletra two 50/200 pills every twelve hours (regimen B) or 75 mg BID Oseltamivir capsule, Kaletra two 50/200 pills, 200 mg Ribavirin pills, 1200 mg daily (regimen C), for five days. The study duration was for five days. Hospitalization duration was measured for ten days (five days after ending the intervention). Also, all the patients of this group received 40 mg of pantoprazole pills or capsules daily during the treatment to prevent gastrointestinal diseases.

If any patient of the interventional group required ICU, the intervention stopped, but as the study was intended to treat, therefore, they were included in the final analysis (there was not even one case that required admission to ICU in the study).

Outcomes

The primary outcome was O_2 saturation without supplemental oxygen.

Oxygen saturation was measured by a pulse oximeter. In case the patient was receiving oxygen, the first oxygen was cut for 5 min and then oxygen saturation was measured again. If oxygen drops lower than 90, oxygen therapy was started again immediately.

The frequency of oxygen saturation measurement:

Oxygen saturation was measured every 6 h and recorded daily. At the end of the treatment period and

at the time of discharge, oxygen saturation was measured again.

Secondary outcomes included

No admission to the intensive care unit.

In the case of the following symptoms, patients were transferred to the ICU ward of the hospital:

- Drop in the level of consciousness (GCS lower than 12)
- Shock (blood pressure lower than 90 or diastolic lower than 60)
- Refractory hypoxia (arterial saturation lower than 90) with a non-rebreather mask

In-hospital mortality: Death cases were counted in the hospital.

Length of hospitalization: Duration of hospitalization (days) since hospitalization until (acceptable) recovery or death.

Fever

Fever was defined as an orally or axillary temperature higher than 37.5 degrees Celsius. This outcome was measured daily.

Cough and shortness of breath

The cough severity and shortness of breath were measured by using the Cough Severity Questionnaire (standardized questionnaire)[34] and Shortness of Breath Questionnaire (standardized questionnaire)[35] among control and intervention groups.

Discharge without intensive care need

Patients were discharged in case their condition met all the criteria including oxygen saturation higher than 93% without receiving oxygen, a normal level of consciousness (Glasgow Coma Scale of 15), normal systolic blood pressure, not having respiratory stress, normal liver and kidney functions and the patient's favourable general conditions for discharge.

The allergic drug reaction and adverse drug reactions in both groups were recorded.

Participant timeline

The study duration was 5 days, and during these 5 days, patients participating in the study were visited twice daily. The study was started on 21st March and ended on 1st April 2021.

Sample size

For comparing two ratios (based on the primary outcome of the study) and assuming a relatively 50% improvement intervention group compared to the control group, the sample size was determined as 64 people, 32 people in each group.

Randomization

Randomizing was done through a geographical cluster method. Considering the large volume of patient visits and the critical conditions and ethical issues, patients who were hospitalized in ward one or were referred to ward one were randomly allocated to the intervention group, and all the patients in unit two were put into the control group. All cases were evaluated based on inclusion criteria. Inclusion to study groups was carried out through a parallel method. There were no criteria for admitting patients in ward one or two; it was based on the vacancy of beds.

Blinding

This study was open-label and was not blinded.

Statistical methods

The main analysis approach in this study was an intention to treat. At first, a descriptive analysis of the variables using mean and standard deviation and frequency and percentage was carried out. In order to ensure the correctness of randomization, all variables (patients' information, including demographic information) were compared with each other in both groups, at the baseline level and at the end of the intervention. Chi-square test was used for comparing the qualitative variables and t-test was used for comparing the scale variables (paired t-test for comparing before and after the intervention within each group and t-test for comparing variables between control and intervention groups at baseline and end of study).

Safety reporting

Cytotoxic effect: Given the size of the outlet nozzle and MDI pumps and the presence of propellant (HFA gas) and alcohol in the product, the concentration of the essence mix and plant compounds in the whole of the MDI is too little. So, the available amount of solved natural materials in each puff is less than 300 nanoliters or 270 mg, which of course, according to scientific principles, a limited part of that reaches the lungs and since the effective amount of PHR160 is in the range of nanograms, basically it does not have any cytotoxic effect. However, the safety reporting was done based on the instructions published by the FDA[36]. Adverse Event (AE), Adverse Drug Reaction (ADR), Serious Adverse Event/Reaction (SAE), and Suspected Unexpected Serious Adverse Reaction (SUSAR) all were recorded and managed based on this guideline.

Ethical approval

The ethical approval was obtained from the ethics committee of Baqiyatallah medical university (2020-03-10). The ethical number is IR.BMSU.REC.1398.387.

Results

Comparing intervention groups and control group characteristics at the baseline level

Table 1 shows the demographic and clinical characteristics of the intervention and control groups at the baseline level. There was not a significant difference in the mean age of patients in the intervention and control groups (p=0.574). All the cases were male in both groups.

Mean oxygen saturation percentage (p=0.008) and respiratory rate (p=0.05) at the baseline level were statistically significant, although the mean oxygen saturation percentage was higher in the control group. The respiratory rate at the baseline level was also significantly higher in the control group. The assignment to different drug regimens A, B and C was compared among the intervention and control groups; there was no significant statistical difference between the groups (Table 1).

As in this study, patients admitted to ward one and ward two were randomly assigned to intervention and control groups, and because some patients were admitted before the initiation of the study in these two wards, therefore, the duration of hospitalization before the intervention was compared among the two groups. There was no significant statistical difference in the hospitalization duration before intervention among control and intervention groups.

Comparing intervention clinical features after the intervention group and control groups

The mean values of the arterial oxygen saturation before receiving the PHR160 spray and four days of receiving the drug are shown separately for each day and groups in the table below. A significant increase in arterial oxygen saturation percentage was observed in the intervention group (p<0.001) (Table 2).

The statistical modelling test, with adjusting the age for baseline and day-five of the intervention, shows that the oxygen saturation percentage mean was significantly more in the intervention group by 5.14 units (p<0.001). Also, the effect of time was statistically significant (p<0.001), after each day, the oxygen saturation percentage mean increased by 0.59 units (Table 3 and Fig. 3).

The mean respiratory rate before the intervention and for four days of the intervention is shown in Table 4, separately for each day and group.

The statistical modelling test, with adjusting the age, shows that there was no significant difference in mean respiratory rate among the two groups after the intervention (p=0.49), although this rate was significantly higher in the intervention group at the baseline level. Also, the effect of time was not

Table 2 — The comparison of oxygen saturation percentage for baseline and day-five intervention between the control and intervention groups

Group		N	Mean	SD
Control	SPO2.Baseline	31	87.61	3.509
	SPO2.Day-1	31	87.03	2.639
	SPO2.Day-2	28	88.14	3.308
	SPO2.Day-3	26	87.88	4.448
	SPO2.Day-4	26	87.58	4.100
Intervention	SPO2.Baseline	32	85.34	3.023
	SPO2.Day-1	32	90.47	2.782
	SPO2.Day-2	32	92.06	2.723
	SPO2.Day-3	26	92.96	2.163
	SPO2.Day-4	17	93.59	1.770

Abbreviations: SD: standard deviation

Table 1 — Comparison of the demographic and clinical characteristics of the control and intervention groups at the baseline level

	Group	N	Mean	SD	P.V
Hospitalization time before 21st	Control	31	3.13	4.387	0.740
	intervention	32	3.44	2.850	
Age	Control	31	54.97	14.925	0.574
	Intervention	32	52.69	12.132	
Breathing.0	Control	31	22.06	3.021	0.05
	Intervention	32	23.94	4.508	
SPO2.0	Control	31	87.61	3.509	0.008
	Intervention	32	85.34	3.023	
	Group	A	B	C	
		Frequency (percent)	Frequency (percent)	Frequency (percent)	
Drug regimen	Control	9 (28.1)	18 (56.3)	5 (15.6)	0.74
	Intervention	7 (22.6)	17 (54.8)	7 (22.6)	

Abbreviations: SD: standard deviation

significant (p=0.69). Although by studying the two groups separately, as shown in Table 5 and Figure 3, the control group showed a gradual increase in the respiratory rate, the intervention group showed a slight decrease in the respiratory rate.

The mean hospitalization duration after the intervention was significantly more in the control group (8.13 days) compared to the intervention group (4.69 days). Also, the total hospitalization duration was significantly more in the control group (11.19 days) compared to the intervention group (8.13 days) (Table 6).

Table 7 shows the dyspnea score and cough score during the day and night at baseline and 5 days after the PHR160 spray. The ANCOVA test results, with adjusting the effect of dyspnea score, showed that the mean dyspnea score was significantly less in the intervention group compared to the control group at day 5 after the intervention (p<0.001). Also, the mean

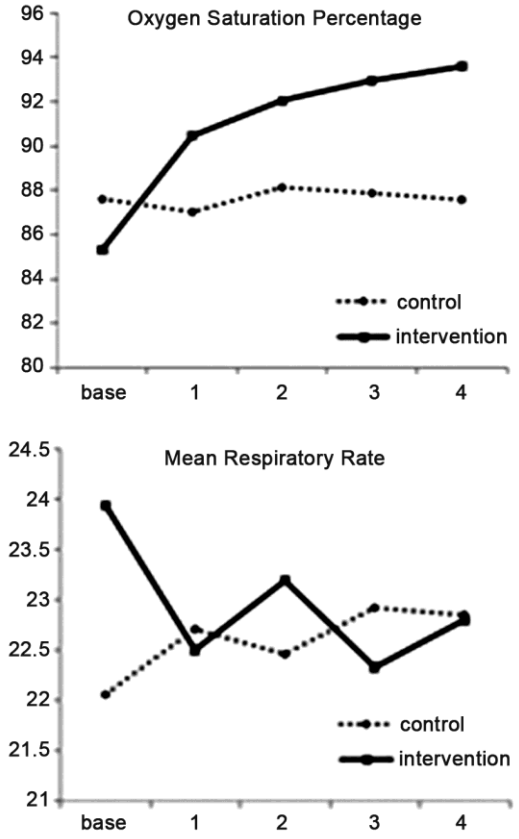

Fig. 3 — The statistical modelling test of oxygen saturation percentage and mean respiratory rate with adjusting the age at baseline and day-five of intervention between the control and intervention groups

Table 3 — The comparison of oxygen saturation percentage, statistical modelling test, with adjusting the age for baseline and day-5 after intervention between the control and intervention groups

			95 % Wald Confidence Interval		Hypothesis Test
Parameter	B	Std. Error	Lower	Upper	P. V
(Intercept)	53.361	20.0267	14.110	92.613	0.008
Group	5.141	0.9463	3.286	6.996	0.000
Time	0.594	0.1437	0.313	0.876	0.000
Age	-0.018	0.0173	-0.052	0.016	0.292
SPO2.0	0.328	0.2200	-0.103	0.760	0.136
(Scale)	8.719	-	-	-	-

Dependent variable: SPO2
Model: (Intercept), group, time, age, SPO2.0

Table 4 — The daily mean respiratory rate from baseline to day-five intervention between the control and intervention groups

Group		N	Mean	SD
Control	Respiratory rate. baseline	31	22.06	3.021
	Respiratory rate. Day-1	31	22071	3.671
	Respiratory rate.Day-2	28	22.46	3.328
	Respiratory rate.Day-3	26	22.92	4.471
	Respiratory rate.Day-4	26	22.85	4.115
Intervention	Respiratory rate. baseline	32	23.94	4.508
	Respiratory rate. Day-1	32	22.50	2.540
	Respiratory rate.Day-2	32	23.19	2.583
	Respiratory rate.Day-3	24	22.33	1.834
	Respiratory rate.Day-4	15	22.80	2.366

Abbreviations: SD: standard deviation

Table 5 — The statistical modelling for respiratory rate with adjusting the age for baseline and day-5 after intervention between the control and intervention groups

			95 % Wald Confidence Interval		
Parameter	B	Std. Error	Lower	Upper	P. V
(Intercept)	16.095	2.4284	11.336	20.855	0.000
Group	-0.345	0.5001	-1.325	0.635	0.491
Time	0.072	0.1831	-0.286	0.431	0.693
Age	0.028	0.0163	-0.004	0.060	0.089
Respiratory rate (baseline).0	0.240	0.0796	0.084	0.396	0.003
(Scale)	9.436	-	-	-	-

Dependent variable: Breathing
Model: (Intercept), group, time, age, breathing.0

Table 6 — Comparison of the total hospitalization duration, and hospitalization duration after intervention, between the control and intervention groups

Variable	Intervention (n=32)	Control (n=31)	p Value
	Mean (SD)	Mean (SD)	-
Total hospitalization duration	8.13 (4.1)	11.19 (6.0)	0.020
Hospitalization duration after intervention	4.69 (3.2)	8.06 (3.8)	<0.001

cough score of day and night in the intervention group was significantly less in the control group ($p<0.001$) (Table 7).

The comparison of the body temperature, heartbeat rate, blood pressure, WBC, blood platelet, and CPR was made 5 days after the intervention in the two groups using the ANCOVA test. Although the only significant difference at the baseline level was in the body temperature ($p<0.001$), the results showed that there was no significant difference between the mean of these two groups in all these variables (Table 7).

Safety of the PhR160

The result of this study showed that there was no report of mortality or Adverse Event (AE), Adverse Drug Reaction (ADR), Serious Adverse Events (SAE), and Suspected Unexpected Serious Adverse Reaction (SUSAR) in the intervention or control group during the study period.

Discussion

This study showed that receiving the PHR160 spray for five days, ten puffs a day, significantly increased the arterial oxygen saturation percentage during the five days intervention period in COVID-19 patients. In addition, it decreased hospitalization duration, dyspnea score, and cough score significantly in COVID-19 patients. Many other studies also attempted to use Chinese Traditional Medicine in the treatment of COVID-19, some of which showed

Table 7 — Comparison of the dyspnea and cough score and other clinical symptoms at baseline and day-five after the intervention

Variable	Intervention group mean±SD (n=32)	Control group mean±SD (n=31)	P.V	P.V ANCOVA
The dyspnea criteria point before intervention	1.50±0.91	1.22±0.65	0.16	-
The dyspnea criteria point after intervention	0.28± 0.31	1.84±0.45	<0.001	-
Difference	(0.81) 1.22	(0.35) -0.62	<0.001	<0.001
Day coughing criteria point (before intervention)	1.25± 0.71	1.55± 0.81	0.11	-
Day coughing criteria point (after intervention)	0.47± 0.50	1.97±0.70	<0.001	-
Difference	(0.55)0.78	(0.67) -0.41	<0.001	<0.001
night coughing criteria point (before intervention)	0.69±0.78	1.39±1.08	0.008	-
night coughing criteria point (after intervention)	0.30±0.17	1.45±0.67	<0.001	-
Difference	(0.74) 0.65	(0.77) -0.06	<0.001	<0.001
Body temperature before intervention	37.46±0.33	37.09± 0.27	<0.001	-
Body temperature after intervention	37.20±0.24	37.31±0.32	0.15	-
Difference	(0.41) 0.25	(0.44) -0.21	<0.001	0.32
Heart beat rate before intervention	89.28±6.70	87.48±4.81	0.22	-
Heart beat rate after intervention	90.28±5.49	90.97±5.81	0.63	-
Difference	(4.72) -1.00	(5.36) -3.48	0.056	0.13
Systolic blood pressure before intervention	116.25±10.23	111.94±6.14	0.04	-
Systolic blood pressure after intervention	116.09±6.18	115.81±7.64	0.87	-
Difference	(10.58) 0.15	(10.30) -3.87	0.13	0.98
Diastolic blood pressure before intervention	72.97±8.11	70.97±4.72	0.23	-
Diastolic blood pressure after intervention	71.09±7.04	70.81±6.59	0.86	-
Difference	(10.9) 1.87	(7.79) 0.16	0.46	0.94
WBC before intervention	5.64±2.31	6.68±3.13	0.13	-
WBC after intervention	7.00±1.93	7.43±2.17	0.49	-
Difference	(2.00) -1.25	(2.69) -1.23	0.97	0.65
Platelets before intervention	174.28±54.77	181.39±72.32	0.66	-
Platelets after intervention	270.00±145.63	249.38±79.81	0.55	-
Difference	(134.41) -102.19	(98.74) -54.83	0.21	0.34
ESR before intervention	41.63±21.55	51.90±27.85	0.10	-
ESR after intervention	34.33±29.14.	36.50±27.38	0.90	-
Difference	(21.65) 7.00	(28.73) 18.43	0.52	0.78
CPR before intervention	42.15±31.79	50.68±34.80	0.31	-
CPR after intervention	4.25±2.02	5.76±2.68	0.30	-
Difference	(11.74) 25.47	(29.77) 42.83	0.27	0.19

promising results[37,38]. These curative effects of PHR Spray might be due to the antibacterial and antiviral effects of compounds in this essence which are shown in previous studies as well[8,9,11-12]. In addition, Menthol, Crocin, Safranal and Safran are among the compound of this essence that has been used for the treatment of pulmonary symptoms in many previous studies.

The study by Eccles showed the effects of menthol on treating sore throat and cough related to the common cold or respiratory inflammation, treating nasal congestion caused by the nasal mucus inflammation and soothing dyspnea symptoms[39]. Also, the study by Tamaoki *et al.*[40] indicated that menthol is effective for treating mild asthma. Some studies showed that Crocin had a protective effect on allergic asthma, reducing lipopolysaccharide-induced acute respiratory distress syndrome and reducing respiratory tract inflammation[20,21].

The studies by Boskabady *et al.*[22] and Bukhari *et al.*[23] showed Safranal could prevent pulmonary inflammation and alleviate asthma in mice in order, respectively. The study by Boskabady *et al.*[24] on Safran showed it is effective in treating asthma and improving the respiratory system's function, pulmonary obstruction, pulmonary inflammation and response, dependent on trachea in animal models, and it has anti-cough effects.

The observed effect of PHR160 spray in improving the condition of COVID-19 patients in the intervention group could be due to its effect on the mechanism caused by Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). CFTR is a membrane protein encoded by the CFTR gene[3] and is a kind of adjustable chloride channel with CAMP that adjusts other ion channels. CFTR preserves the hydration of the secretions in respiratory tracts, by excreting the chloride and regulating the sodium. CFTR dysfunction strongly affects members that secrete liquid mucus, like upper and lower respiratory tracts. Dry and adhesive mucus in the lungs prevents mucociliary clearance. This dysfunction is chromosomal. CFTR protein is a kind of protein tract that controls the flow of water (H_2O) and chlorine ion (^{-}Cl) in the lungs. When CFTR protein is working correctly, ions easily enter or exit these tracts; however, when this tract dysfunctions, due to obstructed tracts, ions can no longer move out of the cell and this causes thick secretion in the lungs. Concerning this, for Coronavirus disease, according to autopsy reports from China[2], the type of mucus and adhesive secretion was seen that were not similar to fibrosis. The similarity of the thickness of secretion in COVID-19 patients can bring up the theory that the increase in the thickness of the secretion in the patients leads to better pulmonary function. PHR160 can lead to recovery, by increasing the thickness of the secretion and increasing clearance and coughing up mucus.

The essence of PHR160 spray is made up of a compound that has shown pulmonary anti-inflammatory activity effects[3,6,21-22], controlling the respiratory tract mucus by regulating cytokine[7], antioxidant activity[16], protective effect on allergic reactions[20] and reducing lipopolysaccharide-induced acute respiratory distress syndrome[20] in different studies. These effects align with other pieces of evidence on the therapeutic effects of the compounds that make the PHR160 spray could explain the effects seen in this study for PHR160 spray.

Conclusion

This study mainly examined the safety of PHR160 spray in the improvement of pulmonary symptoms of COVID-19 patients. The study showed that PHR160 is safe and effective in improving pulmonary symptoms; however, improvement of pulmonary symptoms cannot be generalized, and further study with a larger sample size is required.

Study limitations

The small sample size and small duration of intervention for five days were the main limitations of the study.

Ethical consideration

Baqiyatallah University's ethics board approved research ethics. All participants signed informed consent. A member of the research team explained the purpose and procedures of the study to the patient. The confidentiality principles in collecting, preserving, and disseminating the patients' information were observed in this study.

Conflict of Interest

There was no conflict of interest in those involved in data collection and analysis.

Authors' Contribution

HRSR, NZ, MH, MV and FG contributed to developing the PHR and providing its components for

the manuscript writing purpose. RM contributed to developing research methodology, data analysis, manuscript writing. HRA, PA and ARSO contributed to data collection. MM, MS, MJ and MR contributed to manufacturing of the drug. RM supervised the whole project.

References

1 Zhu N, Zhang D, Wang W, Li X, Yang B, *et al*., A novel coronavirus from patients with pneumonia in China 2019, *N Engl J Med*, 382 (2020) 727-733.

2 Chan J F, Yuan S, Kok K H, To K K, Chu H, *et al*., A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster, *Lancet*, 395 (10223) (2020) 514-523.

3 Li Q, Guan X, Wu P, Wang X, Zhou L, *et al*., Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia, *N Eng J Med*, 382 (2020) 1199-1207.

4 Gibson P G, Qin L & Puah S H, COVID-19 acute respiratory distress syndrome (ARDS): clinical features and differences from typical pre-COVID-19 ARDS, *Med J Aust*, 213 (2) (2020) 54-6.

5 Attaway A H, Scheraga R G, Bhimraj A, Biehl M & Hatipoğlu U. Severe covid-19 pneumonia: pathogenesis and clinical management, *BMJ*, 372 (2021).

6 Juergens U R, Stöber M & Vetter H, The anti-inflammatory activity of L-menthol compared to mint oil in human monocytes in vitro: a novel perspective for its therapeutic use in inflammatory diseases, *Eur J Med Res*, 3 (12) (1998) 539-45.

7 Juergens U R, Engelen T, Racké K, Stöber M, Gillissen A, *et al*., Inhibitory activity of 1, 8-cineol (eucalyptol) on cytokine production in cultured human lymphocytes and monocytes, *Pulm Pharmacol Ther*, 17 (5) (2004) 281-287.

8 Tserennadmid R, Takó M, Galgóczy L, Papp T & Vágvölgyi C, *et al*., Antibacterial effect of essential oils and interaction with food components, *Open Life Sci*, 5 (5) (2010) 641-648.

9 Norajit K, Laohakunjit N & Kerdchoechuen O, Antibacterial effect of five Zingiberaceae essential oils, *Molecules*, 12 (8) (2007) 2047-2060.

10 Reichling J, Schnitzler P, Suschke U & Saller, R, Essential oils of aromatic plants with antibacterial, antifungal, antiviral and cytotoxic properties–an overview, *Complement Med Res*, 16 (2) (2009) 79-90.

11 11.Minami M, Kita M, Nakaya T, Yamamoto T, Kuriyama H, *et al*., The inhibitory effect of essential oils on herpes simplex virus type 1 replication *in vitro*, *Microbiol Immunol*, 47 (9) (2003) 681-684.

12 Astani A, Reichling J & Schnitzler P, Comparative study on the antiviral activity of selected monoterpenes derived from essential oils, *Phytother Res*, 24 (5) (2010) 673-679.

13 Hatamzadeh Esfahani N, Rahimi S, Gharagozloo M & Yakhkashi S, Comparison effects of some chemical and herbal commercial medicines on the immune system and reduce respiratory damage in chickens challenged with infectious bronchitis vaccine, *Vet Res Biol Prod*, 28 (3) (2015) 20-27.

14 Worth H, Schacher C & Dethlefsen U, Concomitant therapy with Cineole (Eucalyptole) reduces exacerbations in COPD: a placebo-controlled double-blind trial, *Respir Res*, 10 (1) (2009) 69.

15 Kehrl W, Sonnemann U & Dethlefsen U, Therapy foracute nonpurulent rhinosinusitis with cineole: results of a double blind, randomized, placebo controlled trial, *Laryngoscope*, 114 (4) (2004) 738-742.

16 Shao J, Yin Z, Wang Y, Yang Y, Tang Q, *et al*., Effects of different doses of Eucalyptus oil from Eucalyptus globulus Labill on respiratory tract immunity and immune function in healthy rats, *Front Pharmacol*, 11 (2020) 1287-1289.

17 Liu H & Rui YE, Observation on the effect of eucalyptol limonene and pinene enteric soft capsules on elder community acquired pneumonia patients, *Chinese J Pri Med Pharm*, 1 (3) (2013) 356-357.

18 Eccles R, Menthol: effects on nasal sensation of airflow and the drive to breathe, *Curr Allergy Asthma Rep*, 3 (3) (2003) 210-214.

19 Tamaoki J, Chiyotani A, Sakai A, Takemura H & Konno K, Effect of menthol vapour on airway hyperresponsiveness in patients with mild asthma, *Respir Med*, 89 (7) (1995) 503-504.

20 Yosri H, Elkashef W F, Said E & Gameil N M, Crocin modulates IL-4/IL-13 signaling and ameliorates experimentally induced allergic airway asthma in a murine model, *Int Immunopharmacol*, 50 (2017) 305-312.

21 Bastaki S M, Adeghate E, Amir N, Ojha S & Oz M, Menthol inhibits oxidative stress and inflammation in acetic acid-induced colitis in rat colonic mucosa, *Am J Transl Res*, 10 (12) (2018) 4210.

22 Boskabady M H, Tabatabaee A & Byrami G, The effect of the extract of Crocus sativus and its constituent safranal, on lung pathology and lung inflammation of ovalbumin sensitized guinea-pigs, *Phytomedicine*, 19 (10) (2012) 904-911. (https://doi.org/10.1016/j.phymed.2012.05.006)

23 Bukhari S I, Pattnaik B, Rayees S, Kaul S & Dhar M K, Safranal of *Crocus sativus* L. inhibits inducible nitric oxide synthase and attenuates asthma in a mouse model of asthma, *Phytother Res*, 29 (4) (2015) 617-627.

24 Boskabady M H, Gholamnezhad Z, Ghorani V & Saadat S, The effect of *Crocus sativus* (saffron) on the respiratory system: Traditional and experimental evidence, *Sci Spices Culinary Herbs-Latest Lab Pre-Clin Clin Stud*, 1 (2019) 30-54.

25 Ghods A A, Mirmohamadkhani M & Sotodehasl N, Comparison of the effects of lavender essential oil and sesame oil on sleep quality of nurses, *J Babol Univ Medical Sci*, (2016) 13-19.

26 Khazaie F, Modarres M, Rahimikian F, Rahnama P, Bekhradi R, *et al*., The effect of lavender essential oil on anxiety of intra uterine device insertion, *J Med Plants*, 2 (46) (2013) 60-65.

27 Kloucek P, Smid J, Frankova A, Kokoska L, Valterova I, *et al*., Fast screening method for assessment of antimicrobial activity of essential oils in vapor phase, *Food Res Int*, 47 (2) (2012) 161-165.

28 Kumar P, Mishra S, Malik A & Satya S, Insecticidal properties of Mentha species: a review, *Ind Crops Prod*, 34 (1) (2011) 802-817.

29 Van Vuuren SF, Suliman S & Viljoen AM, The antimicrobial activity of four commercial essential oils in combination with conventional antimicrobials, *Lett Appl Microbiol*, 48 (4) (2009) 440-446.

30 Aron de Miranda H A, Gonçalves J C, Cruz J S & Araújo DA, Evaluation of the sesquiterpene (−)-α-bisabolol as a novel peripheral nervous blocker, *Neurosci Lett*, 472 (1) (2010) 5-11.
31 Klevenhusen F, Zeitz J O, Duval S, Kreuzer M & Soliva C R, Garlic oil and its principal component diallyl disulfide fail to mitigate methane, but improve digestibility in sheep, *Anim Feed Sci Technol*, 166 (2011) 356-363.
32 Koba K, Nenonene A Y, Raynaud C, Chaumont J P & Sanda K, Antibacterial Activities of the buds essential oil of *Syzygium aromaticum* (L.) Merr. & Perry from Togo, *J Biol Act Prod Nat*, 1 (1) (2011) 42-51.
33 Merad M & Martin J C, Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages, *Nat Rev Immunol*, 20 (6) (2020) 355-362.
34 Hsu J Y, Stone R A , Logan-Sinclair R B, Worsdell, M, Busst, C M, *et al*., Coughing frequency in patients with persistent cough: assessment using a 24 h ambulatory recorder, *Eur Respir J*, 7 (1994) 1246-53.
35 Wilcox T K, Chen W H, Howard K A, Cates C E, Wiklund I, *et al*., validity of the Shortness of Breath with Daily Activity (SOBDA) questionnaire: A new outcome measure for evaluating dyspnea in COPD, *Ann Am Thorac Soc*, 181 (2010) A5429.
36 US Food and Drug Administration, FDA guidance on conduct of clinical trials of medical products during COVID-19 public health emergency, *Guid Doc*, (2020) 1-29.
37 Zhu R F, Gao Y L, Robert S H, Gao J P, Yang S G *et al*., Systematic review of the registered clinical trials for coronavirus disease 2019 (COVID-19), *J Trans Med*, 18 (1) (2020) 1-9.
38 Luo H, Tang Q L, Shang Y X, Liang S B & Yang M, Can Chinese medicine be used for prevention of Corona Virus Disease 2019 (COVID-19)? A review of historical classics, research evidence and current prevention programs, *Chin J Integr Med*, 26 (4) (2020) 243-250.
39 Eccles R, Menthol: effects on nasal sensation of airflow and the drive to breathe, *Curr Allergy Asthma Rep*, 3 (3) (2003) 210-214.
40 Tamaoki J, Chiyotani A, Sakai A, Takemura H & Konno K, Effect of menthol vapour on airway hyperresponsiveness in patients with mild asthma, *Respir Med*, 89 (7) (1995) 503-4.