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Original Research

The Effect of Barley Aqueous Extract on Clinical Signs of COVID-19: An Open-Labeled Randomized Clinical Trial

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Abstract

Coronavirus has spread around the world with high prevalence rate and severe manifestations since 2019 (COVID-19) and imposed global economic, social, and health burden. Many studies were conducted to manage the complications of COVID-19 and to reduce the hospital stay but results were not satisfactory. Hence, according to the high potential of medicinal plants in management of diseases, we aimed to investigate the effects of barley aqueous extract (BAE) on clinical and laboratory features of COVID-19 in hospitalized patients. This study was an open-labeled, randomized, controlled clinical trial performed on 80 hospitalized COVID-19 patients in COVID-19 medical center, Isfahan, Iran, from July to August 2021. All patients received a same standard treatment according to the protocol of the Iranian Ministry of Health and BAE was added to the treatment of intervention group. Patients received BAE 200 mL every 4 hours for 5 days. Seventy-two patients completed the study and no significant differences were reported in the baseline data between groups. Results showed significant improvement of shortness of breath (p=0.012) and cough (p=0.09) after receiving BAE, compared to the control group. On the other hand, there was no significant difference between groups considering body temperature, respiratory rate, oxygen saturation, muscle pain, and laboratory factors. No serious adverse effect was reported. This study suggests that BAE may serve as a safe complementary treatment for alleviating symptoms of COVID-19; however, more clinical trials with higher sample size are needed.

Keywords: COVID-19; Persian medicine; Phytotherapy; Medicinal Plants; *Hordeum*

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Introduction

In late December 2019, several Wuhan Health Centers in China reported patients with pneumonia of unknown cause [1]. This disease spread rapidly and increased in other parts of the world [2,3], and in February 2020, the World Health Organization assigned the term COVID-19 to the disease, which stands for Coronavirus 2019 [3]. While its mortality rate is lower than that of SARS-CoV in 2002 and MERS-CoV in 2012, its higher transmission rate has resulted in a much greater threat, leading to significant social, public health, and economic challenges [4]. The clinical presentation of COVID-19 varies from mild manifestations typically upper respiratory tract viral infection symptoms including low-grade fever, myalgia, sore throat, dry cough, nasal congestion, and headache to more severe cases that progress to pneumonia or develop critical complications including acute respiratory distress syndrome (ARDS) and sepsis [5].

Despite the high prevalence of COVID-19 and the implementation of various treatment modalities, no definite treatment has been identified [9]. On the other hand, there is a growing trend to use traditional medical systems to manage different disease. Traditional Persian medicine have historically served as foundational medical references in Western and Eastern academic institutions [10]. In Persian medicine textbooks, there is various recommendations and remedies to alleviate flu-like symptoms and lung disease such as COVID-19, one of which is barley [6,7]. Based on Persian medicine, barley has a cold and wet temperament and is useful for most acute fevers [8,9], inflammatory lung diseases, pneumonia, and pleuritis [10]. Rhazes mentioned in his book "Al-Hawi" expectorant and mucolytic effects for barley [11]. Barley with the scientific name of *Hordeum vulgare* L. contains 29.5% fiber, 27.3% protein, 4.57% fat, vitamin C 251.6 mg / 100 g, vitamin A 20.5 mg / 100 g, magnesium 183.2 mg / 100 g, calcium 479.4 mg / 100 g, potassium 3384 mg / 100 g, and iron 23.3 mg / 100 g. Barley has also immune-boosting, liver protection, anti-inflammatory, antioxidant, and heart protective effects [12]. Previous studies showed the anti-inflammatory effects of barley through inhibiting the expression of interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)-α [13,14] and suppressing the lipopolysaccharide-induced inflammatory response (LPS) [15]. Barley prevents chronic inflammatory condition in cardiovascular system [16] by modulating the production of proinflammatory cytokines such as interferon (IFN)-γ and IL-17 [17].

Considering the lack of certain treatment for COVID-19 and evidence on potential benefits of barley, we aimed to investigate the effect of barley aqueous extract in management of clinical symptoms of hospitalized COVID-19 patients.

Materials and Methods

Study design

This study was an open-labeled, randomized, controlled clinical trial. Ethical approval was obtained from Isfahan University of Medical Sciences ethics committee (code: IR.MUI.MED.REC.1399.078) and the protocol of the study was registered at Iranian Registry of Clinical Trials (code: IRCT20200428047229N1). Eligible patients were recruited from hospitalized COVID-19 patients at Amin Hospital, Isfahan University of Medical Sciences affiliated, from July to August 2021, and written informed consent was obtained.

Subjects

Moderate-to-severe COVID-19 patients with definite diagnosis (positive nasopharynx COVID-19 PCR sample test) with the indication of hospitalization according to the Iran's Ministry of Health protocol for management of COVID-19 were enrolled for primary assessments.

Inclusion and exclusion criteria

Inclusion criteria

Newly admitted COVID-19 patients aged between 18 to 70 years old with moderate-to-severe COVID-19 criteria including fever ≥ 38°C or severe cough or shortness of breath or respiration rate ≥ 24 per minute or oxygen saturation <93%.

Exclusion criteria

More than 24 hours passed from hospital admission, history of allergy to medicinal plants, history of asthma, pregnancy, breastfeeding, recent use of corticosteroids (≥2 mg/kg per day or ≥20 mg daily of prednisone for more than 14 days), immune deficiency, heart failure, or chronic kidney disease.

Exit criteria

Acute allergic reaction to the treatment, worsening of the medical condition, failure to take medication regularly (missing more than 250 mL for one day), unwilling to continue study, or discharge before 5 days of intervention.

Intervention

At admission, standard medications according to the management of COVID-19 protocol was prescribed for every patient and the intervention of this study was added to the standard treatment of the patients. Aqueous extract of barley was prepared based on Persian medicine method [9] by boiling 200 g whole barley without flakes in 2800 g water on a gentle flame until the mixture turned pale pink. Then the strained water was provided to patients with the instruction of con-

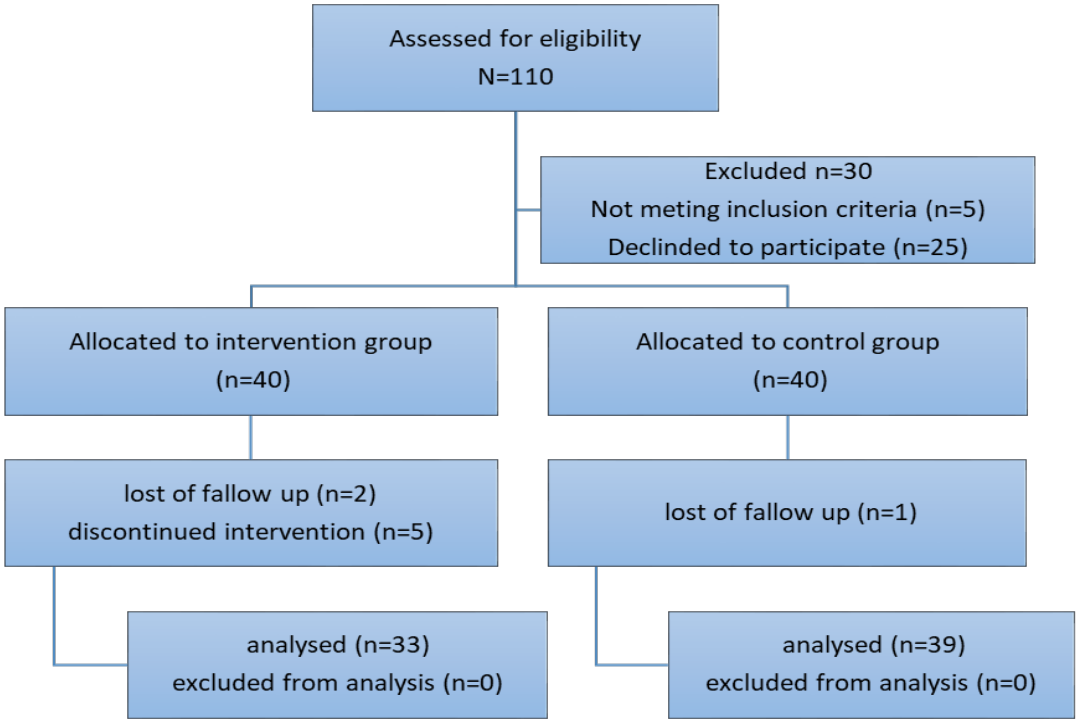

Figure 1. CONSORT flow diagram of the study

sumption: to drink 200 mL every 6 hours for 5 days. The medicine was provided to the patients fresh and warm every morning.

Randomization and blindness

Eligible patients were divided into two groups using 6-block randomization method. Each group of the study assigned a letter A or B and numbers from 1 to 6 assigned for each block (AABB, BBAA, ABBA, BAAB, ABAB, BABA). To achieve the sample size, 20 random numbers from 1 to 6 was generated using online random number generator software to complete the random sequence. Upon admission, patients were sequentially assigned to the first vacant spot. As this study was open-labeled and control group did not receive further intervention, blindness of patients and investigators was not applicable; however, statistical analysis was performed blind based on the label of A or B for the groups.

Outcomes

Study outcomes, shortness of breath as primary and cough, muscle pain, oxygen saturation, and laboratory findings as secondary outcomes, were assessed three times (before intervention, day 3 and day 5) using a researcher-made questionnaire including demographic characteristics and clinical manifestations (body temperature, dry cough, shortness of breath, muscle pain, and oxygen saturation). Symptoms were scored by the patients as 0= not affected, 1= mild, 2= moderate, and 3= severe. Moreover, in each visit session, 5 mL venous blood was obtained for laboratory assessments including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and complete blood cell count (CBC).

Sample size analysis and statistical analysis

Based on the previous studies, Hasheminasab et al. [18] and Azami et al. [19], and considering α=0.05 and β=0.2 10% dropout, the sample size was calculated 40 subjects in each group .

Qualitative variables were represented by number and percentage and compared between groups using the Chi-square test or Fisher exact test. Quantitative variables were presented by mean and standard deviation (SD) and compared within groups and between groups with appropriate parametric or non-parametric tests. The statistical analysis was performed using SPSS V.26 and *p* value less than 0.05 was considered statistically significant. Given that Bayesian methods provide robust inference especially in studies with small sample sizes, this study utilized a Bayesian approach for data analysis. A Bayesian hierarchical ordered logistic regression model was fitted to analyze the outcome variables The model included day (1, 3, 5), group (Intervention=1, Control=2), and sex (Male=1, Female=2) as predictors. Estimation was performed using Markov Chain Monte Carlo (MCMC)

Table 1. Demographic characteristics and baseline evaluations

Baseline values		Barley group (n=33)	Control group (n=39)	*p* value
Age		50.23±12.89	55.26±11.33	0.057**
Sex	Male	25 (75.8%)	20 (51.3%)	0.033*
	Female	8 (24.2%)	19 (48.7%)	
Body temperature		37.28±0.73	37.42±0.98	0.797**
Respiratory rate		20.06±2.38	20±1.99	0.748**
Oxygen saturation		90.06±4.65	89.92±4.5	0.935**
Shortness of breath		2.39±0.84	1.89±1.01	0.033**
Cough		2.17±0.89	1.77±1	0.112**
Muscle Pain		0.88±0.33	0.67±0.48	0.062**
RBC		4.952 ± 0.594	4.951 ± 1.266	0.294**
Hb		14.288 ± 1.743	13.737 ± 2.256	0.219**
PLT		221.21 ± 95	222.91 ± 12	0.922**
HCT		43.294 ± 4.658	41.989 ± 4.705	0.174**
WBC		1109 ± 396	1392 ± 685	0.063**
Neut%		74.053 ± 7.877	70.450 ± 10.442	0.155**
Lymph%		18.76±7.23	22.52±10.29	0.069**
ESR		45.92±30.09	44.75±26.22	0.988**
CRP		48.66±23.76	45.86±25.99	0.618**

Legend: data were represented by mean±SD or N (%); * Chi-square Test; ** Mann-Whitney U Test; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; Hb: hemoglobin; HCT: hematocrit; Lymph: lymphocyte; Neut: neutrophil; PLT: platelet*10^3; RBC: red blood cell*10^6; WBC: white blood cell.

sampling with Highest Posterior Density (HPD) intervals to quantify parameter uncertainty. This approach accounts for the ordinal nature of the response and allows for robust inference on the effects of the predictors. Since the numerical predictor variables did not follow a normal distribution, we used an ordinal logistic regression model to appropriately handle the ordinal nature of the outcome variables. This method does not assume normality of predictors or the outcome, making it suitable for analyzing ordered categorical data and providing robust estimates of predictor effects.

Results

Demographic characteristics and baseline evaluations

From 80 participants, 72 patients were included for statistical analysis, 39 from control group and 33 from intervention group (Figure 1). None of the patients in barley group reported side effects. Most of the patients were male (62.5%) with mean age of 53.02 years old (Table 1). There was no significant difference between groups considering age (p=0.857); however, despite random allocation, sex distribution differed between groups (p=0.033). At the beginning of the study, there was no significant difference between severity of symptoms between groups (p>0.05), except shortness of breath that was significantly worse in barley group (p=0.033). Furthermore, no significant difference was reported considering laboratory findings (p>0.05).

Comparing study outcomes

The progression of study outcomes has been presented by Table 2. Results showed shortness of breath, as primary outcome, has significantly improved in barley group, compared with control group (p=0.012), as well as improvement of cough severity in barley group (p=0.029). On the other hand, there was no significant difference between groups considering body temperature (p=0.612), respiratory rate (p=0.187), oxygen saturation (p=0.751), and muscle pain (p=0.159). Likewise, laboratory tests showed no significant difference between groups after 5 days, including ESR (p=0.602), CRP (p=0.860), white blood cell (WBC) (p=0.453), red blood cell (RBC) (p=0.357), hemoglobin (Hb) (p=0.571), hematocrit (p=0.314), platelet (p=0.661), lymphocyte percentage (p=0.639), and neutrophil percentage (p=0.514).

Comparison of study outcomes by gender

According to the significant difference between sex contribution of the patients, we performed a by-gender comparison between groups. In male group, body temperature, shortness of breath, and cough improved in both groups (p<0.05) with no significant difference between groups (p>0.05). Despite no significant change of oxygen saturation in control group, it was increased in barley group (p=0.016). None of the laboratory findings differed between two groups. In female group, no significant difference was reported between

groups considering body temperature, respiratory rate, oxygen saturation, shortness of breath, cough, muscle pain, and laboratory findings (p>0.05).

Bayesian ordered logistic regression parameter estimated that as day increases, the odds of being in a higher temperature category significantly decrease. The entire credible interval is below zero, confirming a negative effect. Moreover, male group is less likely to be in higher categories compared to the other. The effect of day on respiratory rate is slightly negative but the credible interval includes zero, so the evidence is weak or inconclusive for a real effect and there is no strong evidence of significant effect of sex and group on respiratory rate. There is a significant positive effect of day on oxygen levels. As the day increases, the odds of being in a higher oxygen category increase. Sex has a strong positive effect on oxygen saturation and being female is associated with higher oxygen level. There is a strong and significant negative effect of day on shortness of breath. As time passes, the odds of experiencing higher severity of shortness of breath decrease significantly. The effect of sex and group on shortness of breath is uncertain; the credible interval includes zero, indicating no clear evidence of an ef-

Table 2. The progression of study outcomes

	Time	Barley group	Control group	p value*
Body temperature	Before intervention	37.28±0.73	37.42±0.98	0.612
	After 3 days	36.62±0.31	36.74±0.47	
	After 5 days	36.32±1.27	36.58±0.36	
Respiratory rate	Before intervention	20.06±2.38	20.00±1.99	0.187
	After 3 days	19.50±1.08	19.26±1.57	
	After 5 days	19.50±1.47	19.11±1.86	
Oxygen saturation	Before intervention	90.06±4.65	89.92±4.50	0.751
	After 3 days	91.57±3.78	90.31±4.03	
	After 5 days	92.64±2.46	91.40±4.74	
Shortness of breath	Before intervention	2.39±0.84	1.89±1.01	0.012
	After 3 days	1.42±0.96	1.40±1.03	
	After 5 days	0.94±0.64	0.97±0.75	
Cough	Before intervention	2.17±0.89	1.77±1.00	0.029
	After 3 days	1.42±0.72	1.18±0.90	
	After 5 days	0.94±0.77	0.93±0.86	
Muscle pain	Before intervention	0.88±0.33	0.67±0.48	0.159
	After 3 days	0.55±0.51	0.46±0.51	
	After 5 days	0.18±0.40	0.45±0.51	
ESR	Before intervention	45.92±30.09	44.75±26.22	0.602
	After 3 days	32.00±11.79	50.00±2.83	
	After 5 days	-	-	
CRP	Before intervention	48.66±23.76	45.86±25.99	0.860
	After 3 days	47.40±23.09	48.38±25.71	
	After 5 days	31.00±25.16	38.00±21.66	
WBC	Before intervention	6533±2529	6711±2850	0.453
	After 3 days	8400±4327	7421±3855	
	After 5 days	7933±3257	11961±19350	
RBC	Before intervention	4.95±0.59	4.95±1.27	0.357
	After 3 days	4.77±0.51	4.51±0.52	
	After 5 days	4.92±0.57	4.53±0.53	
Hb	Before intervention	14.29±1.74	13.74±2.23	0.571
	After 3 days	13.79±1.69	13.28±1.75	
	After 5 days	13.92±1.61	13.43±2.06	
HCT	Before intervention	43.29±4.66	41.99±4.70	0.314
	After 3 days	41.40±4.75	41.40±9.11	
	After 5 days	42.42±4.41	40.12±5.26	
PLT	Before intervention	221.12±95	222.91±12	0.661
	After 3 days	272.66±13	248.66±12	
	After 5 days	298.67±11	295.27±11	
Lymph%	Before intervention	18.76±7.23	22.52±10.29	0.639
	After 3 days	17.07±12.57	21.93±15.75	
	After 5 days	21.24±15.53	18.17±13.70	
Neut%	Before intervention	74.05±7.88	70.45±10.44	0.514
	After 3 days	75.38±15.48	70.93±20.09	
	After 5 days	69.98±16.38	74.80±15.35	

Legend: data were represented by mean±SD; * repeated measures ANOVA; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; Hb: hemoglobin; HCT: hematocrit; Lymph: lymphocyte; Neut: neutrophil; PLT: platelet*10^3; RBC: red blood cell*10^6; WBC: white blood cell.

fect. There is a strong and significant negative effect of day on cough severity. As time progresses, the odds of experiencing muscle pain and cough symptoms decrease substantially; however, the effect of sex and group on cough is uncertain because the credible interval includes zero, so no definitive conclusion about group influence can be drawn.

Discussion

The present study showed significant improvements in shortness of breath and cough in patients received BAE compared to control group; however, other outcomes did not change significantly. Furthermore, by gender analysis showed significant changes only in body temperature and oxygen saturation in male group and no remarkable change in female group and logistic regression showed larger effect in magnitude for group parameter compared to sex parameter that means despite significant difference in sex between groups, the results of the study were remarkably related to the group classification, rather than sex. Consistent with the present study, epidemiologic surveys showed that the hospitalization rate of male adults is higher than female adults and males have longer hospital stay, higher rate of intubation, and higher death rate [20] that is maybe due to the fact that males are more likely to have health comorbidities such as hypertension, diabetes, renal failure, congestive heart failure, and liver disease [20]. Hasheminasab et al. showed the therapeutic effects of BAE on decreased oxygen saturation in COVID-19 patients with no change in chough [18]. Although in our study, BAE significantly increased oxygen saturation, it was not comparable with control group; On the other hand, cough recovered significantly. Tavakoli et al. conducted a randomized controlled trial to add-on the BAE extract to the routine treatment regimen. They prescribed 250 mL BAE per day for two weeks in hospitalized COVID-19 patients. BAE decreased the hospitalization duration, body temperature, ESR, and CRP; however, oxygen saturation, cough, respiratory rate, and muscle pain did not change significantly [21]. Despite we prescribed BAE fewer days, patients received 4 times higher daily dose that ameliorated the cough and shortness of breath. On the other hand, in our study laboratory factors were not changed significantly. Derakhshan et al. investigated the effect of BAE on allergic rhinitis, showed that BAE has good effects on the treatment of symptoms such as rhinorrhea, post nasal discharge and itchy nose [22]. As inflammation is the basis of COVID-19, treatments with anti-inflammatory effects can be effective in relieving COVID-19 consequences [23]. Traditional Persian medicine textbooks stated cold and wet temperament for barley and recommended BAE for acute fever and inflammations [9]. Recent studies reported that barley is a rich source of β-glucans, a cluster of polysaccharides with immunomodulatory and anti-inflammatory effects. β-glucans prohibit the formation of lipopolysaccharide inflammatory mediators and suppress the generation of reactive oxygen species (ROS) and gene expression of NF-κB pathway [24]. Study of Iguchi et al. showed that BAE can modulate the production of inflammatory cytokines such as IFN-γ, and IL-17 [13]. Moreover, BAE demonstrated a strong protective effect on oxidative damage and plays an important role in preventing chronic inflammation by inhibiting ROS, monocyte chemotactic protein (MCP)-1, and vascular cell adhesion molecule(VCAM)-1 and upregulating gene expression of superoxide dismutase (SOD), an antioxidant biomarker [16]. Also, Study of Choi et al. showed preventive effects of barley ethanolic extract on expression of TNF-α-induced IL-6 gene [13]; nevertheless, the exact BAE mechanism of action on COVID-19 remained unknown.

Limitations

Consistent with other studies, the present study significantly improved some clinical symptoms, unlike laboratory indices. These discrepancies may be due to different methodologies of studies, especially the duration of the intervention, as our study was a short-course treatment. The ideal design was to just prescribe BAE for the patients, without a basic treatment, but according to the ethical concerns, we could not deprive the patients of the basic treatment. To ensure the potential therapeutic effects of BAE in infectious respiratory disease, more well-designed randomized controlled trials with larger sample size and longer duration is recommended.

Conclusion

This study showed positive effects of barley aqueous extract on clinical respiratory symptoms of COVID-19 patients without any side effects. This study shed a light on Persian Medicine potentials in management of diseases with medicinal plants. While there is restricted treatment methods or the results of the available protocols are not satisfactory, we can use herbal treatments beside the routine treatments, certainly after safety and efficacy approval.

Conflict of Interests

There was no conflict of interest to declare

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