



An evaluation of the effect of saffron supplementation on the antibody titer to heat-shock protein (HSP) 70, hsCRP and spirometry test in patients with mild and moderate persistent allergic asthma: A triple-blind, randomized placebo-controlled trial

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ABSTRACT

Background: Asthma is a heterogeneous disease, which usually associated with chronic airway inflammation. The anti-heat shock protein (anti-HSP) 70 is a novel risk factor for asthma. The aim of the present study was to survey the effect of saffron supplementation on anti-HSP70, high-sensitivity C-reactive protein (hs-CRP) and spirometry test in patients with allergic asthma.

Basic procedures: In this clinical trial, patients (N = 80, 32 women and 48 men, 18–65 years old) with mild and moderate allergic asthma were randomized into two groups: a group of patients who received two capsules of saffron (100 mg/d) and a control group who received two capsules of placebo for 8 weeks. Anti-HSP70, hs-CRP and spirometry test were determined in patients before (week 0) and after (week 8) intervention. SPSS software (version 16.0; Inc, Chicago, IL) was used for data analysis.

Main findings: Saffron in comparison with placebo significantly reduced the hs-CRP ($p < 0.001$) and anti-HSP70 ($p < 0.001$) concentrations. In spirometry test, forced expiratory volume in first second (FEV1), forced vital capacity (FVC), FEV1/FVC ratio and forced expiratory flow 25–75% (FEF 25–75) increased significantly in saffron in comparison to placebo group ($p < 0.05$).

Principal conclusions: Results of the present study suggested that saffron supplementation in patients with allergic asthma decreased significantly anti-HSPs 70 and hs-CRP and also improved some spirometry test factors.

1. Introduction

Asthma is usually associated with chronic inflammation of airway [1]. In asthmatic patients, bronchial hyper-responsiveness, airway inflammation and also airway remodeling, are the prominent features. This chronic respiratory disease affects over 300 million people worldwide and this is estimated that it will probably become more than 400 million by 202 [2]. The prevalence of asthma in Iran is reported about 5.5% [3]. The WHO has estimated that 15 million disability-adjusted life-years lost annually due to asthma [4].

Allergic asthma is a phenotype of asthma that easily recognized

which often is related to a past and/or family history of eczema, food or drug allergy or allergic rhinitis and some allergic diseases [1]. Inflammation has an important role in the development of asthma and in the severity of clinical symptoms [5]. In the process of inflammation of airway tract in asthmatic patients, a variety of immunologic cells, such as eosinophils, mast cells, lymphocytes, and neutrophils are involved [6]. This is reported that the expression of gene of anti-heat shock protein antibody (anti-HSP) 70 increased in asthmatic patients [5]. HSPs has a role in inflammation and inflammatory disease and inflammatory mediators such as eosinophils has a role in increase the expression in asthma and the amount of the HSP70 in asthmatic

Abbreviations: HSP, heat shock protein; CRP, C-reactive protein; FEV1, forced expiratory volume in first second; FVC, forced vital capacity; FEF25–75, forced expiratory flow 25–75%

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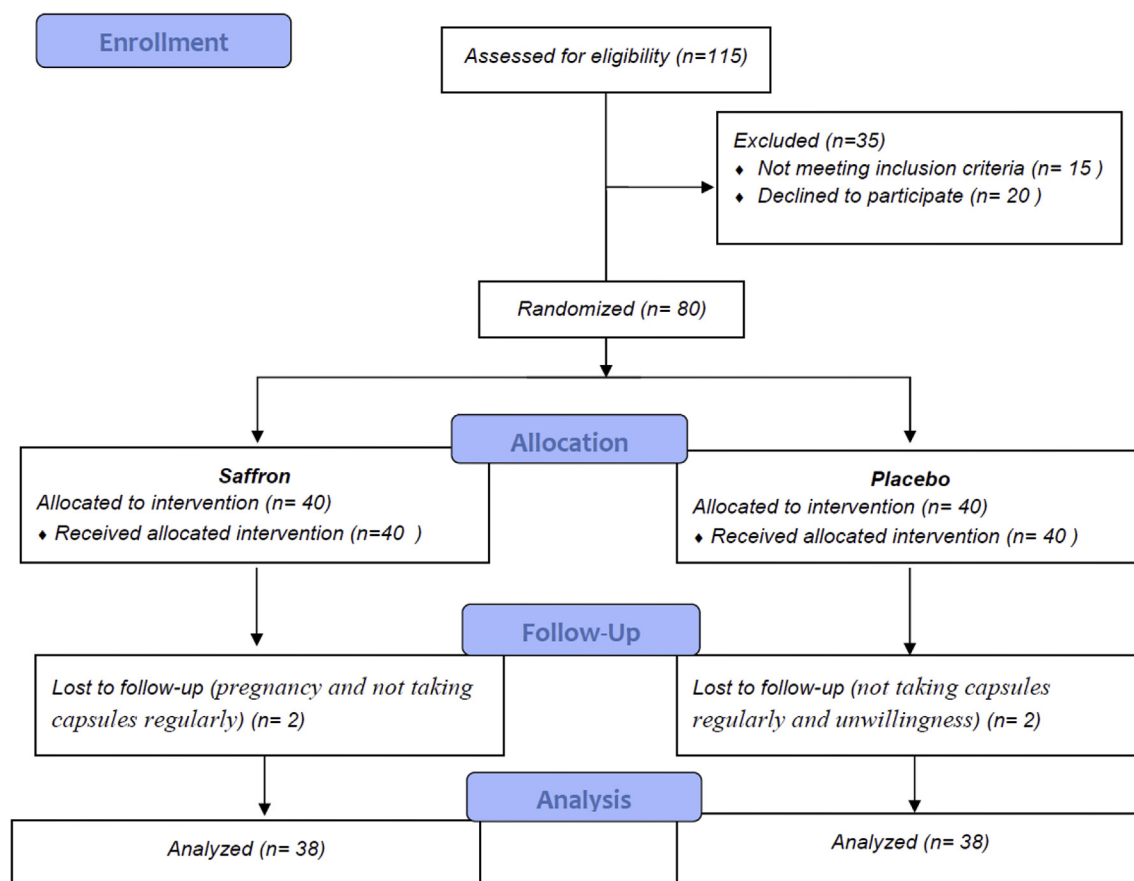


Fig. 1. Flowchart of the patients throughout the study.

patients has correlated with the percent of eosinophils and disease severity [7] and the serum levels of anti-HSP70 has correlated with the asthma severity [8].

This is believed that by efforts by individuals, their healthcare providers, local and national governments and healthcare organizations to improve asthma control, the substantial global burden of asthma can be reduced [1]. Saffron (*Crocus sativus* L.), in traditional medicine has been used for the treatment of depression, stress, heart disease and sleep disorders. Saffron has some beneficial effect about asthma such as antioxidant [9], anti-inflammatory [10] and muscle relaxant effects [11]. Saffron supplementation in animals with allergic asthma reduced eosinophils, basophils and total white blood cells and some of these effects were equal to dexamethasone [12]. In guinea pig with allergic asthma, saffron supplementation reduced the inflammation by reduction in serum level of endothelin1 [13]. Results of the study of Boskabady, et al. showed a muscle relaxant effect of saffron on tracheal chains of guinea-pigs which was comparable to or even higher than that of theophylline [11]. Muscle relaxant effects of saffron are due to anticholinergic and anti-muscarinic effects and also stimulatory effects on β -2 adrenergic receptors [11].

With regard to the significance of allergic asthma, in the present study, we investigated the effect of saffron supplementation on anti-HSP 70, as the novel risk factors of asthma, high-sensitivity C-reactive protein (hs-CRP) and spirometry test in patients with mild and moderate persistent allergic asthma.

2. Materials and method

2.1. Trial design

The protocol of our study was approved by the Medical Ethics

Committee at Ahvaz Jundishapur University of Medical Sciences (Registration No. 1395.810.AJUMS.REC.IR). The trial was registered at Iranian Registry of Clinical Trials (IRCT2017012132081N2) and all subjects signed the written informed consent to participate in the study. The present study was a triple blind, randomized and placebo-controlled clinical trial with a parallel design. The sample size was calculated based on the averages comparison formula with $\alpha = 0.05$ and $\beta = 0.2$, $\mu_1 = 0.07$, $S_1 = 0.1$, $\mu_2 = 0.003$, $S_2 = 0.1$ based on the previous study for the serum levels of hs-CRP variable [14].

$$n = ([Z_{1-\alpha/2} + Z_{1-\beta}]^2 [S_1^2 + S_2^2]) / (\mu_1 - \mu_2)^2$$

The sample size of 36 subjects in each group was calculated. Considering the probability of filling of the samples and the length of the treatment, the final sample size of 40 subjects in each group (80 subjects) was calculated. Our patients enrolled the trial according to a computer-generated blocked randomization list with a block size of 6 subjects. For decrease the probable bias, we used an allocation concealment and triple blind technique. A researcher placed the saffron and placebo capsules into numbered bottles based on a random list who did not have any participation in the study. Another researcher assigned the numbered bottles to the patients, who was not aware of the random sequences and was not involved in the trial. By using the random codes, all the researchers, participants and the statisticians (who analyzed the data) were blind to the allocations until the statistical analyses were completed. After allocation, during the baseline visit the participants were asked to fill the questionnaires. In these questionnaires, demographic data, history of using medications and supplements, disease history, history of smoking and physical activity level (assessed by the short form of the International Physical Activity Questionnaire) were assessed. In the next day, the fasting blood samples were collected and the participants were allowed to start consuming the treatment

Table 1

Comparison of the baseline characteristics between the saffron and placebo groups.

Variable	Group		P-value
	Saffron (N = 38)	Placebo (N = 38)	
Gender N			
Male N(%)	24(60)	24(60)	0.99
Female N (%)	16 (40)	16(40)	
BMI (kg/m ²)	26.84 (25.46–27.23)	26.84 (25.05–27.39)	0.79
Age (years)	41.27 ± 9.77	40.77 ± 10.07	0.67
Asthma			0.81
Mild N(%)	14 (35)	15 (37.5)	
Moderate N(%)	26 (65)	25 (62.5)	
Systolic pressure (mm Hg)	11.00 (11.00–12.00)	11.00 (11.00–12.00)	0.15
Diastolic pressure (mm Hg)	8.00 (7.00–8.00)	8.00 (7.00–8.00)	0.85
LDL-C	118.54 ± 36.03	120.74 ± 27.35	0.76
HDL-C	45.32 ± 13.46	45.57 ± 13.01	0.93
TG	159.83 ± 41.20	149.30 ± 48.27	0.31
Total-C	196.82 ± 42.11	192.74 ± 28.93	0.62
Eosinophil (%)	5.60 (3.00–7.45)	4.90 (3.85–6.50)	0.54
Basophil (%)	0.6 (0.3–1.05)	0.6 (0.4–1.20)	0.53
Physical activity (MET-min/week)	676.50 (231–2704)	693 (231–3446.50)	0.71
Symptoms (frequency per day)			
Shortness of breath during the day	1.00 (0.03–1.50)	1.00 (0.04–2.00)	0.73
Shortness of breath during the night	0.71 (0.00–1.87)	0.20 (0.00–1.87)	0.64
Waking up due to asthma symptoms	1.00 (0.00–1.00)	0.00 (0.00–1.00)	0.12
Activity Limitation	0.38 (0.14–1.00)	1.00 (0.10–1.87)	0.22
Salbutamol spray consumption	1.25 (0.02–2.00)	1.00 (0.33–2.00)	0.79
Infant Feeding N (%)			
Breast feeding	30 (75)	32 (80)	0.37
Milk Powder	5 (12.5)	4 [10]	
Breast feeding and milk powder	5 (12.5)	4 [10]	
The age of the onset of asthma (years)	33.50 (23.50–42.50)	30 (25.00–39.00)	0.96

BMI, body mass index; anti-HSP, anti-heat shock protein; CRP, C-reactive protein. LDL-C: low density lipoprotein; HDL-C: high density lipoprotein; TG: Triglyceride; FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; FEF: forced expiratory flow. Values are expressed as mean ± SD, median ± IQR or number and percent. Mann–Whitney and independent sample T-test were used to compare non-parametric and parametric variables between the two groups, respectively.

capsules. Filling the questionnaires and taking blood samples were repeated again after 8 weeks (endpoint).

Body mass index (BMI) also was determined pre-and post-intervention. During the study period (8 weeks), all the participants were given dietary advices such as avoiding consumption of fast foods, sausage, saffron (from food), smoked and canned foods and also patients were asked not to go out when the air was polluted and use filter masks in emergencies according to the physician recommendation. Patients were also asked not to change their physical activity and energy intake during the trial. To control the energy intake, we used three days 24-hr recalls (2 work days and one weekend day). Our patients had no other disease except asthma and consumed no any drug except asthma drugs. The lung specialist prescribed the same drugs for the patients in our study. There were no significant differences between the drugs which our patients consumed ($p = 0.66$).

Compliance was assessed during a weekly call, by asking the number of capsules consumed and asking them to report possible side effects of the capsules. Those patients who were intolerant to the medication or did not regularly take their capsules were excluded from the study.

2.2. Participants

Our participants included 80 men and women between the ages of 18 and 65 years. Based on the diagnostic criteria of GINA (Global Initiative for Asthma) for asthma, patients with mild and moderate allergic asthma were examined in May and October 2017 by an attendant pulmonologist, and were then recruited from the outpatient clinic at Imam Khomeini Hospital, Ahvaz, Iran. Then, the participants were provided with information about the trial by both written information sheets and verbal explanation. They were asked to consume the capsules regularly and complete the questionnaires and the treatment procedure until the end of the study.

The inclusion criteria were body mass index (BMI) up to 27 kg/m², an age range of 18–65 years with mild and moderate persistent allergic asthma according to the GINA criteria.

2.3. Mild persistent

- Symptoms more than once a week but less than once a day
- Exacerbations may affect activity and sleep Nocturnal symptoms more than twice a month
- FEV1 or PEF ≥ 80% predicted
- PEF or FEV1 variability < 20–30%

2.4. Moderate persistent

- Symptoms daily
- Exacerbations may affect activity and sleep Nocturnal symptoms more than once a week
- Daily use of inhaled short-acting b2-agonist
- FEV1 or PEF 60–80% predicted
- PEF or FEV1 variability > 30%

The exclusion criteria included pregnancy and lactation, smoking, diabetes, autoimmune disease, malignancy and other pulmonary diseases. The whole protocol of our trial met the requirements of Ahvaz Jundishapur University of Medical Sciences Ethics Committee and all the patients provided written informed consent.

2.5. Intervention

All the patients received either two oral capsules containing dries saffron (*Crocus sativus* L.) stigma or a placebo during the intervention. All the capsules were made in the Faculty of Pharmacy at Ahvaz Jundishapur University of Medical Sciences. The saffron capsules contained 50 mg of dried saffron stigma which was taken from Estahban, Fars province, Iran (Herbarium code: JPS018118) and starch (as filler) and the placebo capsules contained edible permitted food colors to trace amount (E104, E110 and E122) and starch similar in color, shape, size, and package to the saffron ones. The amount of crocin in the saffron sample was 304.3 mg/kg (HPLC¹ method) (unpublished data). The patients received the saffron and placebo capsules at the start of the study in the Imam Khomeini hospital pulmonary clinic.

2.6. Outcomes

The primary outcomes of our study were to measure the lung function with spirometry parameters (FEV1, FVC, FEV1/FVC and FEF 25–75) and the serum levels of hs-CRP and anti-HSP 70 at the baseline and post-intervention. Secondary outcomes were to measure blood pressure and lipid profiles pre- and post-intervention (data not shown).

¹ High Performance Liquid Chromatography.

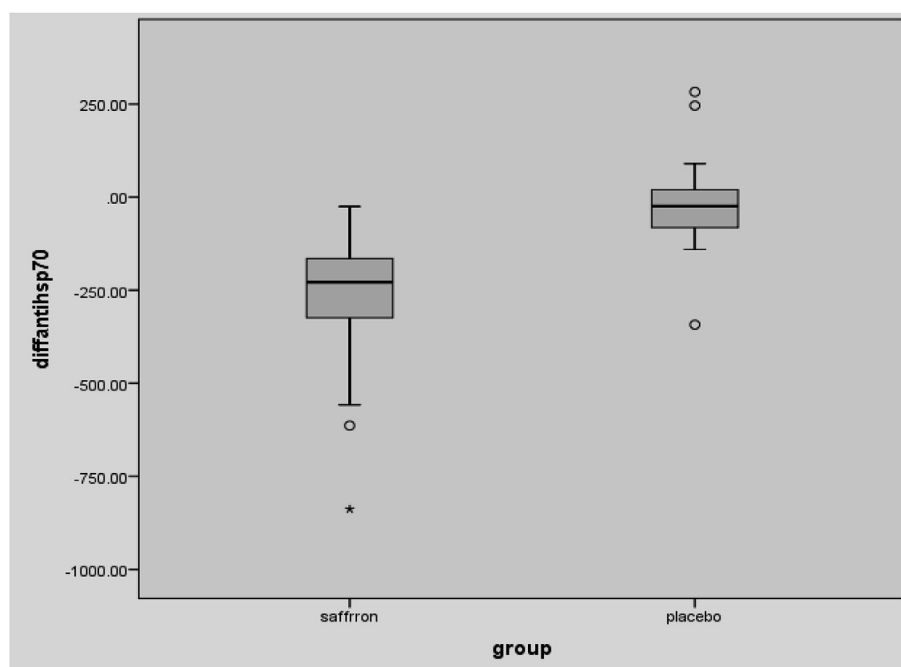


Fig. 2. Comparison the anti-HSP70 changes between saffron and placebo groups during the trial.

Table 2

Within- and between-group comparisons of the changes from baseline to endpoint measures for hs-CRP and anti-HSP70 in saffron and placebo groups of asthmatic patients.

Variables	Saffron (N = 38)		Placebo(N = 38)		P-value*
	Value	P-value [†]	Value	P-value [‡]	
hs-CRP					
Baseline	50.85 (25.76–94.00)	< 0.001	70.16 (37.90–93.25)	0.31	< 0.001
8 weeks (endpoint)	17.90 (9.39–45.77)		72.23 (37.09–45.77)		
Changes	–17.60 (–37.50–(–3.77))		–1.50 (–7.86–(4.79))		
Anti-HSP70					
Baseline	0.46 (0.35–0.82)	0.13	0.54 (0.43–0.78)	< 0.001	< 0.001
8 weeks (endpoint)	0.37 (0.25–0.56)		1.00 (0.73–1.39)		
Changes	–0.06 ± 0.24		0.46 ± 0.27		

*p-value for comparing the changes of variables between the groups. Mann-whitney test was used for hsCRP and independent sample T test for anti-HSP70.†p-value for comparing baseline, with endpoint values within each group. Wilcoxon and paired samples ttest were used. Values are expressed as mean ± SD for parametric data and median (25th, 75th percentiles) for non-parametric data.

2.7. Collection of blood samples

At the baseline and at the end of the trial, blood samples were collected. 5 mL of blood sample each time was drawn. Blood samples of each patient were collected in the morning after a 12-h fasting, and then the hemolyzed samples were excluded from each analysis. The serum samples were frozen at –20 °C immediately, and were then stored at 80 °C until further laboratory analyses were done.

2.8. Laboratory analyses

We used ELISA, as a validated method for measuring hs-CRP (DM E–4600 CRP High Sensitive ELISA kit (LDN[®])) and anti-HSP 70 (ab133063 –anti-HSP70 IgG/A/M Human ELISA Kit (Abcam[®])). During each laboratory analysis, the technicians were blind to the saffron–placebo status. All laboratory tests were done in the Central Laboratory of Ahvaz Jundishapur University of Medical Sciences.

2.9. Spirometry test

Spirometry is a standard test for evaluation the lung function. In

primary assessment and also before and after the treatment courses, the spirometry could be used [1]. Spirometry could confirm the chronic airway restrictions but the results of this test should be interpreted in combination to the clinical symptoms of asthmatic patients [1]. Because the effects of consumption of the inhaled corticosteroids and long-acting bronchodilators, we asked patients don't using these drugs from one day before the spirometry test. FEV₁,² FVC,³ FEV₁/FVC ratio and FEF_{25–75}⁴ were some factors of the results of spirometry which we used for analysis.

2.10. Statistical analysis

By using the Kolmogorov–Smirnov test, the normal distribution of the data was assessed. Anti-HSP 70, hs-CRP, FEV₁, FVC, FEV₁/FVC ratio and FEF_{25–75} were analyzed by paired t-test or Wilcoxon paired rank test (when data were not normally distributed) for the within-group comparisons (pre-and post-intervention values in each group).

² FEV₁: forced expiratory volume in 1 s.

³ FVC: forced vital capacity.

⁴ FEF: forced expiratory flow.

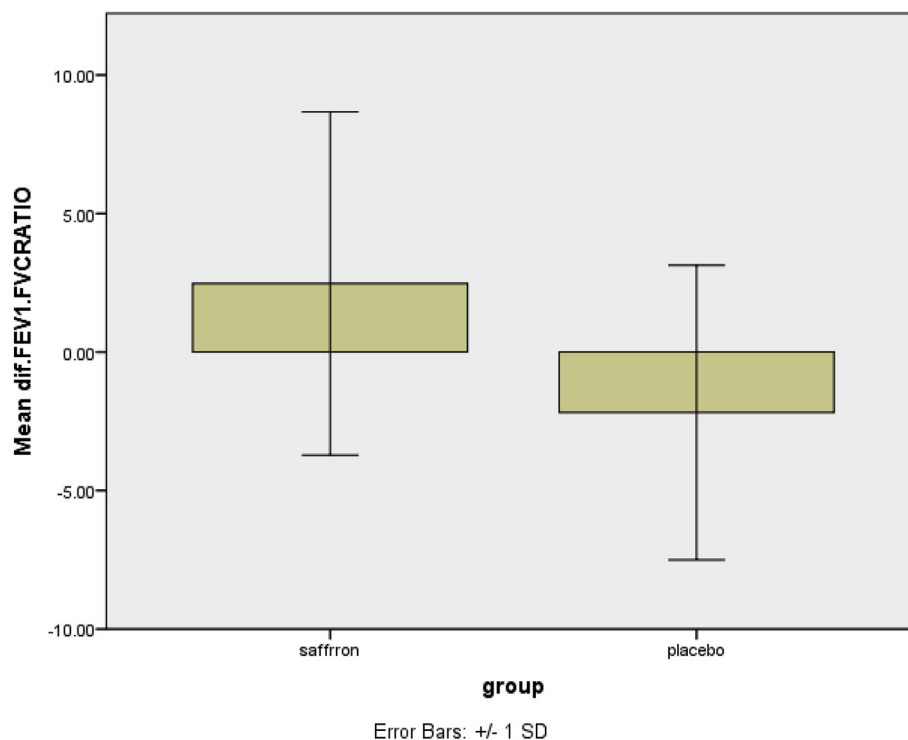
Table 3

Within- and between-group comparisons of the changes from baseline to endpoint measures for spirometry test in saffron and placebo groups of asthmatic patients.

Variables	Saffron (N = 38)		Placebo(N = 38)		P-value*
	Value	P-value [¥]	Value	P-value [¥]	
FEV1					
Baseline	76.02 ± 10.92	0.002	76.40 ± 10.48	0.002	< 0.001
8 weeks (endpoint)	80.15 ± 12.59		73.21 ± 12.15		
Changes	3.00 (0.00–5.25)		–2.00 (–4.25–2.00)		
FVC					
Baseline	81.97 ± 12.93	0.135	81.12 ± 11.68	0.010	0.015
8 weeks (endpoint)	84.31 ± 12.99		79.73 ± 12.65		
Changes	0.00 (–1.00–2.00)		–1.00 (–4.00–0.25)		
FEV1/FVC Ratio					
Baseline	93.10 ± 7.88	0.019	94.12 ± 7.16	0.016	0.001
8 weeks (endpoint)	94.86 ± 9.18		91.57 ± 8.75		
Changes	2.00 (–1.00–6.25)		–1.00 (–4.00–1.00)		
FEF25–75					
Baseline	63.65 ± 19.42	0.067	62.37 ± 16.87	0.101	0.009
8 weeks (endpoint)	68.26 ± 23.95		58.65 ± 19.79		
Changes	3.00 (–2.25–8)		–2.00 (–10.00–2.00)		

FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; FEF: forced expiratory flow.

*p-value for comparing the changes of variables between the groups. Mann-whitney test was used. ¥p-value for comparing baseline, with endpoint values within each group. Paired samples ttest was used. Values are expressed as mean ± SD for parametric data and median (25th, 75th percentiles) for non-parametric data.

**Fig. 3.** Comparison the FEV1/FVC ratio changes between saffron and placebo groups during the trial.

On the other hand, comparisons of changes (endpoint minus baseline) after 8 weeks of intervention between the two groups were done by independent *t*-test or Mann–Whitney *U* test (for non-normal distribution data). Data were reported as mean ± SD or median (25th, 75th percentile) for parametric and non-parametric data, respectively. Chi-squared test, or Fisher's exact test were used for some baseline characteristic data. All statistical analyses were carried out using SPSS version 16 statistical software (SPSS Inc, Chicago, IL, USA) and on the intention-to-treat population corresponding to the subjects having consumed at least one dose of the treatment. The most important outcomes were presented at 95% confidence intervals (CI). For all the tests, two-sided *p*-values < 0.05 were considered statistically significant.

3. Results

During the 8-week supplementation period, 76 from 80 patients who enrolled the trial completed the study. 4 of the enrolled ones, discontinued the study (1 subject in the saffron group because of not taking capsules regularly, and 1 because of pregnancy, and in the placebo group 1 was excluded due to unwillingness, and 1 because of not taking capsules regularly). The flowchart describes the patients' allocation throughout the trial (Fig. 1). There were no serious adverse events during the study. A few minor feeling of warming up were reported in saffron group (but did not lead to discontinuation of the trial).

3.1. Baseline characteristics

The average of the age of our patients was 41.25 years old (range 18–65 years) and 60% of them were male. In the placebo group, 37.5% of the patients and in the saffron group 37.5% had mild asthma. Those patients with moderate asthma were 62.5% and 65% in the placebo and saffron groups, respectively. No significant differences were identified between the two groups in terms of demographic, anthropometric, clinical characteristics, dietary variables (data not shown), asthma severity and physical activity and, as indicated in Table 1 ($p > 0.05$). In addition, there were no differences between the placebo and intervention groups regarding the age of onset of asthma and the history of disease and the use of medication between the trial groups.

3.2. Anti-HSP 70 and hs-CRP

Within group analysis showed that hs-CRP decreased significantly in saffron group (< 0.001) and anti-HSP 70 increased significantly in placebo group (< 0.001) (Fig. 2). The trends of the changes of these factors between saffron and placebo groups were significant. Saffron in comparison to placebo decreased significantly the serum levels of anti-HSP 70 ($p < 0.001$) and hs-CRP ($p < 0.001$) (Table 2).

3.3. Spirometry

About spirometry parameters, we analyzed the changes of the FEV1, FVC, FEV1/FVC ratio and FEF 25–75 during the study between two groups. Results of this trial showed that saffron in comparison the placebo increased the pulmonary volumes (FEV1 ($p < 0.001$), FVC ($p = 0.015$), FEV1/FVC ratio ($p = 0.001$) ratio and FEF 25–75 ($p = 0.009$)) (Table 3 and Fig. 3) and thus improved the clinical symptoms of the allergic asthmatic patients (unpublished data).

4. Discussion

In this study, the effect of saffron on patients with allergic asthma was assessed in a randomized triple blind clinical trial. The findings indicated that saffron supplement therapy has beneficial effects on inflammation and pulmonary volumes (spirometry) including FEV1, FVC, FEV1/FVC ratio and FEF 25–75 as compared to the placebo group in asthmatic patients.

About the safety of the dose of saffron used in present study, a research on healthy humans reported that doses up to 400 mg per day saffron are safe [15] and about the effectiveness, Shemshian, et al. (2014) suggested that in patients with metabolic syndrome, 100 mg saffron reduced anti-HSP 70 (which is a novel risk factor for asthma and is correlated with asthma severity) [8,16]. Thus, based on the evidence, we decided to administer 100 mg/day of dried saffron stigma. To our best knowledge, this is the first clinical trial that has evaluated the effect of saffron supplementation on allergic asthmatic patients.

The importance of saffron in treating asthma is due to anti-asthmatic (antioxidant [9], anti-inflammatory {reduction the eosinophils, basophils and total white blood cells} [12] and muscle relaxant [11]) effects.

Saffron also has immune-modulatory effects which explained the beneficial effects of this plant in allergic asthma. Saffron by activating immune cells that generate free radicals, affects the immune responses. This is reported that treatment with saffron decreased IL-4 and increased IFN- γ levels and IFN- γ /IL-4 ratio (suppressed Th2 and stimulated Th1 cells and increased Th1/Th2 balance) [1].

Vignola, et al. (1995) suggested that in the epithelial cells and alveolar macrophages of asthmatic patients the amount of the gene expression of the HSP 70 was higher than control group and the amount of the expression of the gene of this protein was correlated with the asthma severity and percentage of eosinophils [7]. Tong, et al. (2000) also reported that the amount of the gene expression of the HSP 70 in

asthmatic patients was higher in comparison to the control group [5]. Yang, et al. (2005) in a case-control study in China also showed that the serum levels of anti-HSP 70 was correlated with the risk of asthma and the serum levels of anti-HSP 60 and 70 were correlated with the severity of clinical symptom of asthma [8]. In the present trial (as the first study for assessment the effect of saffron in asthmatic patients in human kinds) saffron (100 mg/day) significantly decreased the serum levels of anti-HSP 70.

Azizmi, et al. (2015) suggested that saffron decreased non-significantly the serum levels of hs-CRP ($p = 0.07$) but has no significant effect in comparison to the control and other plants groups (ginger, cardamom and cinnamon) in patients with type 2 diabetes mellitus [17] but in the present study, saffron in patients with allergic asthma reduced significantly the hs-CRP levels ($p < 0.001$). Mahmoudabady, et al. (2013) in their study showed that saffron supplementation in ovalbumin sensitized rats reduced total white blood cells and the number of eosinophils in pulmonary fluid [18] but in present trial saffron has no significant effect on the eosinophil count in comparison to placebo (unpublished data).

In conclusion, the findings showed the beneficial effects of 100 mg saffron daily on anti-inflammatory factors (HSP 70 and hs-CRP), spirometry parameters (FEV1/FVC ratio and FEF 25–75) and asthma symptoms. In addition, this is believed that saffron supplementation could be effective in a long-term course (2 months). As our trial is the first study investigating the effects of saffron on asthma in human models; thus, more studies with longer supplementation periods are required to provide additional evidence supporting the use of saffron in clinical settings for improving the pulmonary volumes, inflammation and asthma symptoms.

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Author contribution

MZ is the guarantor of the manuscript; MZ, SAH and MHS provided the overall concept and framework for the manuscript, and involved in the drafting and critical revision of the manuscript; SAH supervised the research; MHS served as a medical expert and MGH served as a laboratory specialist. All authors contributed to data interpretation and critically reviewed the manuscript and approved its final version.

Conflicts of interest

The authors have no competing interests. We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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