Effect of Saffron on Anti-Inflammatory and Oxidative Stress in Asthma

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ABSTRACT

Introduction: This trial was designed to evaluate the efficacy of saffron supplementation in patients with mild to moderate asthma. **Methods: Settings and Design:** This randomized, double-blind, and placebo-controlled trial was conducted on 72 patients with persistent allergic asthma. Patients were assigned into take either 100 mg/day saffron supplement (intervention group) or a placebo capsule (control group) for eight weeks. The participants' anthropometric and biochemical evaluations were measured at the beginning and end of the study. Statistical analysis used: To analyse the data, the SPSS software version 24 was used. The continuous and categorical data were reported in means/standard deviations and frequency (%), respectively. Pre- and post-intervention measures were compared between groups using the paired t-test or the Mann-Whitney U-test for normal distributions. **Results:** At the end of the study, patients who received saffron had lower levels of pro-oxidant/antioxidant balance compared to the group who received placebo (P<0.001). Saffron supplementation caused a significant increase in the Interleukin 10 (IL-10) (P=0.005), Interleukin 35 (IL-35), and transforming growth factor-beta (TGF-?) concentrations (P<0.001). We not found any significant changes in the anthropometric variables between the intervention and placebo groups (P>0.05). **Conclusions:** Our results provide evidence that saffron supplementation can improve anti-inflammatory biomarkers and oxidative stress among patients with asthma. In order to address the precise mechanism of these effects, further studies are needed.

Key Words: *Asthma; Inflammation; Saffron; Prooxidant-antioxidant balance (PAB).*

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INTRODUCTION

Asthma is a chronic inflammatory condition throughout the airways and its global prevalence is more than 300 million, which is also increasing [1].

This disorder is characterized by inflammation and constriction of the intrapulmonary airways with symptoms such as coughing, wheezing, and breathlessness [2]. It has been reported that some of the inflammatory agents specially Interleukin 4 (IL-4) and Interleukin 13 (IL-13) also play a significant role in this disease. These inflammatory factors ultimately lead to tracheal spasm, airway and epithelial cells' damage, more leukocyte accumulation, and inflammatory cycle exacerbation [3]. Despite some medical progress in this regard, no definitive cure has been found for asthma [4].

Nowadays, alternative therapies and herbal medicines are considered to control the symptoms of this disorder. A study over 2763 asthmatic patients in the United States showed that most patients were interested in using complementary and alternative medicines (CAM) [5]. In recent years, the effect of various herbal medicines has been evaluated on the symptoms of this disorder [6].

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Crocus sativus linnaeus, commonly known as saffron, is among the most costly medicinal herbs widely grown in certain countries, particularly Iran [7]. Safran includes numerous active ingredients such as crocin and crocetin, ?-carotene, safranal, and crocusatines. Previous studies confirmed the anti-inflammatory, anticancer, antibacterial, and antidepressant effects of saffron [8].

In addition, saffron has antioxidant, anti-tumor, and radical scavenger features [9]. In traditional and modern medicine, saffron and its active compounds are used as antitussive agents. In the respiratory tract, active constituents of saffron can relax the tracheal smooth muscle [10].

Based on the literature, saffron exerts inhibitory effects on the histamine (H1) receptor and? -adrenoceptors [11]. However, few human trials were carried out on the efficacy of saffron supplementation in participants with asthma. To meet this deficiency, the present trial was designed to examine the efficacy of saffron intake on the modulation of inflammatory responses and oxidative stress in subjects with mild to moderate allergic asthma.

Subjects and Methods Participants We recruited 76 individuals (38 participants in each group) aged 18 to 65 years with mild to moderate allergic asthma based on the Global Initiative for Asthma criteria [12]. Figure 1 shows the enrollment process for the patients. The eligible participants had a Forced Expiratory Volume (FEV1) of 60% to 80 % and clinical manifestations of this disorder.

Exclusion criteria included any of the following conditions: smoking, having food allergies, taking antioxidant supplements, using oral corticosteroid within the past six weeks, having pregnancy, lactation, and diseases such as diabetes, autoimmune diseases, malignancies, gastrointestinal, liver or kidney illnesses, pneumonia, and other lung diseases, as well as being reluctant to continue cooperation in the research.

Study Design This randomized, double-blinded, and placebo-controlled trial (phase II) was conducted from June to August 2018 in the respiratory diseases and asthma clinic. At the beginning of the study, all participants were required to sign written informed consent forms to enter the research. Later, all participants were explained about the study objectives and possible risks.

The randomization list was prepared by an independent biostatistician and provided to the Ahvaz Jundishapur University of Medical Science drug service. Randomization codes were only available to the pharmacist and statistician and researchers and patients were blinded from the randomization process. The study procedures were approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Science with the Ethics Code of IR.AJUMS.REC.1396.1095 and registered in the Iranian Registry of Clinical Trials (available at: http://www.irct.ir, identifier: IRCT20180418039354N1).

Intervention Patients who met the eligibility criteria were participated and randomized in the treatment group (1) receiving 100 mg / day saffron (two 50 mg capsules per day) and (2) placebo group that consumed 100 mg/day placebo. The stigma of dried saffron was purchased from Estahban, Fars province, Iran. (Herbarium code: JPS018118).

The Pharmacy School at Ahvaz Jundishapur University of Medical Science produced the saffron capsules and placebos. In the first visit, each participant was provided with the intervention or placebo boxes for four weeks; this process was repeated at the second visit at the end of the fourth week. During the eight weeks of intervention, patients were reminded to consume supplements by phone call.

To evaluate the participants' compliance, they had been asked to return unused supplements. As a result, participants who did not consume more than 10% of their capsules were removed from the research. All participants were asked to avoid eating foods, such as sausage, ham, pepperoni, smoked meat, saffron (from food), and canned foods during the study period.

Patients were also advised not to go outside when the air was polluted and to use filter masks in emergencies according to the physician's recommendation. Anthropometric indices and dietary intake The researchers filled out a three-day dietary history and physical activity questionnaires for all subjects in the baseline and end of the trial.

Furthermore, the general information questionnaire, including age, type of medication, and supplements used by patients and other necessary information was completed by the researchers. We used from the Nutritionist IV software (N Squared Computing, San Bruno, CA, USA) for dietary compounds evaluation. Regarding the physical activity evaluation, a physical activity questionnaire was used designed based on the Metabolic Equivalent of Task (MET) [13].

Anthropometric data were measured at the beginning and termination of the study. Weight and height were recorded to the nearest 0.1 kg and 0.5 cm by a scale, respectively. Participants were required to wear light clothing, without shoes, for anthropometric evaluation. Body mass index (BMI) was calculated after dividing the weight (kg) by height squared (m2).

Blood collection and analyses at the baseline and end of the trial, 10 ml of blood sample was taken from all participants after 12 hours of fasting for biochemical assessments. The samples were centrifuged within 10 minutes at 3000 rpm. The separated serums were divided into small aliquots and frozen at -80°C until biochemical measurements.

Serum concentrations of the anti-inflammatory markers (IL-10, IL-35 and TGF-?) were measured by ELISA kits (Bioassay Technology Laboratory). For Pro-oxidant/Antioxidant Balance (PAB) evaluation, a standard method was used based on a previous study [14]. In this method, there were two different forms of reactions: 1) an enzymatic reaction in which prooxidants oxidize the chromogen Tetramethylbenzidine (TMB) to a color cation and 2) a chemical reaction, in which antioxidants reduce the TMB cation to a colorless compound [15].

For PAB assay, TMB solution was drawn up by dissolving one tablet of TMB in 10 ml of substrate buffer. In the next step, 18 microliters of chloramine T solution were added to one ml of the TMB solution. The exact solution was incubated in a dark place at room temperature for 2 hours; subsequently, 25 U of peroxidase enzyme solution was inserted.

In each well of a 96-well plate, 10 ?L of the sample, standard solution, or control sample (distilled water) was mixed with 200 ?L of the working solution, which was then kept at room temperature for 12 minutes. In the next step, 100 ?L HCl was added to all wells. The plate was kept in the dark for 5 minutes and the measurements were performed using an ELISA reader at 450 nm with a reference wavelength of 620 or 570 nm.

Statistical analyses The Kolmogorov-Smirnov test was run to determine normality of the quantitative variables. The continuous and categorical data were reported in means/standard deviations and frequency (%), respectively. The chi-square test was also applied to compare two groups according to qualitative variables.

Pre- and post-intervention measures were compared between groups using the paired t-test or the Mann-Whitney U-test for normal distributions. Between-group comparisons were done by independent sample t-test or Wilcoxon. Analysis of covariance (ANCOVA) was run in order to control the confounding parameters, including baseline values. Statistical significance was set at $p < 0.05$.

To analyze the data, the SPSS software version 24 (IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY) was used. Results Baseline characteristics Totally, 76 patients with mild to moderate asthma were allocated into the intervention and control groups. During the 56-day follow-up period, two patients dropped out from the intervention group and two patients from the control group.

Finally, the trial was completed by 72 patients who were included in the final analysis (Figure 1). Table 1 shows the basic characteristics of all patients. No significant difference was observed between the intervention and control groups with regard to the baseline characteristics (P>0.05). in other words, subjects were not significantly different regarding age, height, gender distribution, education levels, smoking history, type of their feeding as infants, the onset age of asthma, systolic blood pressure (SBP), and diastolic blood pressure (DBP) (P>0.05).

Table 2 compares anthropometric measurements, physical activity, total calorie, and macronutrient intake of participants at the baseline and end of the study. We not found any significant differences considering weight (P=0.81), BMI (P=0.39), and physical activity (P=0.3) at the end of the study. Similarly, no significant differences were noted between the saffron and control groups in the calorie (P=0.14) and macronutrients. The PAB levels were 83.06 ± 18.37 (HK unit) and 81.57 ± 20.79 (HK unit) for the intervention and control groups before the study, respectively (Table 3).

No statistically significant difference was found between groups regarding PAB levels before the study (P=0.74). In comparison to the control group, saffron supplementation for 56 days caused a significant reduction in the PAB levels (-22.45 ± 7.74 vs. -1.13 ± 2.96, P<0.001). In the ANCOVA model adjusted for the effect of energy, carbohydrate, protein, and fat, no significant changes were observed in the results (P<0.001).

Table 3 provides the mean ± SD of the serum IL-10, 1L-35, and TGF-? at the baseline and end of the study in the intervention and control groups. No significant difference was found between the two groups regarding the amounts of serum IL-10, 1L-35, and TGF-? at the beginning of the study (P>0.05). However, a significant increase was observed in IL-10 concentration in the intervention group in comparison to the control group (28.70 ± 66.81 vs. -3.94 ± 5.84, P= 0.005).

At the end of the 8-week intervention, the group who underwent saffron treatment had higher concentration of IL-35 in comparison to the control group (4.93 ± 3.08 pg/dL vs. (1.25 ± 0.56 vs. - 0.068 ± 0.19, P<0.001). In comparing the amount of changes between the baseline and end of the study in two groups, we found that saffron supplementation improved the serum levels of IL-35 significantly (1.25 ± 0.56 pg/dL in the intervention group vs. - 0.068 ± 0.19 pg/dL in the control group, P<0.001).

Moreover, the intervention group, had a higher concentration of TGF-? at the end of the study (1098.48 ± 484.43 pg/dL vs. 905.17± 400.79 pg/dL, P= 0.069). Comparison of the TGF-? concentration at the baseline and end of the study showed a significant difference between the two groups (161.50 ± 133.59 pg/dL in the saffron group compared to the -12.17 ± 41.43 pg/dL in the control group, P<0.001).

After adjusting for the effects of energy, protein, carbohydrate, and total fat dietary intake, the results remained unchanged. None of the patients who completed the study had any serious adverse events, indicating tolerance to treatment. Discussion To the best of our knowledge, this was the first randomized, double-blind,

placebo-controlled clinical trial over the effect of saffron supplementation on oxidative stress and anti-inflammatory markers in patients with mild to moderate asthma.

Our findings revealed that 100 mg/day saffron supplementation improved biochemical variables in patients with normal BMI. The efficacy of saffron extract in animal models of asthma was confirmed previously [16]. Asthma is characterized by an inflammatory state that causes over-stimulation of pulmonary tissue cells.

Inflammation causes the over-accumulation of immune cells, especially lymphocytes and eosinophils, as two main features of the inflammatory response induced in allergic asthma [17]. Increased secretion of the inflammatory factors such as interleukins, chemokines, and platelet-activating factor was also observed in these patients, which provides the basis for intensification of the inflammatory process [18].

The association between active constituents of saffron and inflammatory factors was examined in a few studies. In an animal study, Vosooghi S et al. evaluated the effect of different doses of saffron extract on inflammation in the asthmatic rats. Based on the results, saffron extracts could exert anti-inflammatory effects [16].

At the end of the study, a significant increase was found in the concentration of anti-inflammatory factors, including IL-10, IL-35 and TGF-? in the intervention group. However, a non- significant reduction was seen in these factors in the control group. In the allergic cascade, T cells, especially T helper 2 (Th2), exert important roles.

Different cytokine, such as IL-6 is produced by T cells that play a major role in asthma pathogenesis. After entering the foreign antigen to the body of atopic individuals, cytokines release from the Th2 and stimulate the production of immunoglobulin E (IgE) [19]. Our findings revealed that saffron supplementation could increase the concentrations of IL-10, IL-35, and TGF-?, significantly.

IL-35, as an anti-inflammatory cytokine, was secreted from several tissues and cells such as monocytes, T cells, B cells, Tregs, and tumor cells under resting conditions. Interleukin-35 has a variety of physiological roles and perhaps one of its most important functions is to inhibit Th2 and Th17 polarization [20]. In an animal study, Niedbala et al. reported that IL-35 could suppress IL-17 production and differentiation in mice [21].

IL-10 is another anti-inflammatory cytokine that inhibits the production and secretion of some proinflammatory cytokines. The concentration of IL-10 in subjects with asthma also decreased [22]. Saffron active ingredients exert anti-inflammatory effects in different ways. Neutrophils, particularly in the lung tissue, play an important role in triggering the inflammatory process.

The active ingredients in saffron prevent the inflammation induced by neutrophil cells. In an animal study, Tamaddonfard et al. evaluated the anti-inflammatory features of crocins and safranol in a different dose. They found that crocin and safranal could exert anti-inflammatory properties by reducing the immune cells [23]. Kang C et al. reported that supplementation with saffron and its aqueous extracts in the animal model of chronic constriction injury could reduce TNF-?, IL-6, and IL-1? levels [24]. Similar findings were also showed in another animal study [25]. Moreover, Zhang et al. found that safranal administration in a dose of 100 mg/kg in animals down-regulated the expression of some inflammatory agents including TNF-?, IL-1?, and mitogen-activated protein kinases (MAPKs) and up regulated the expression of anti-inflammatory cytokines, especially IL-10 [26].

On the contrary, the antioxidant and radical scavenging roles of saffron active ingredients are part of its anti-inflammatory effects [27]. For asthma patients, oxidative stress can stimulate and intensify inflammation of the airway by activating various pro-inflammatory mediators, inducing bronchial hyperresponsiveness, promoting bronchospasm and can mucin release [28].

Our findings showed that 100 mg/day saffron supplementation for eight weeks caused a statistically significant reduction in mean PAB values, showing that saffron supplementation may be associated with a reduction in the levels of oxidative stress. Increased levels of reactive oxygen species alone or in combination with pro-inflammatory cytokines are induced by the inflammatory process.

Recent studies indicated that safranal and other active ingredients had significant antioxidant and radical effects on saffron. The active compounds in saffron exert protective effects against oxidative stress by improving the activity of glutathione reductase NADPH-dependent, superoxide dismutase, and catalase [29, 30]. Kawabata K et al. evaluated the efficacy of crocin, a natural carotenoid in saffron, on inflammatory biomarkers in the mouse model of colitis. They fed 20 male ICR mice with experimental diet containing 200 ppm crocin for four weeks. Kawabata K et al. found that crocin down-regulated the proliferation and expression of nuclear factor- (NF-) ?B, interleukin- (IL-) 1?, IL-6, interferon ?, cyclooxygenase-2, and increased the NF-E2-related factor 2 (Nrf2) expression. The NF-?B signaling pathway had a crucial role in inducing the airways' inflammation [31].

Among different cytokines, TGF-? had a considerable role in the immune system [32]. In another animal study, Faridi S examined the effect of saffron extracts on the pro-inflammatory cytokines in diabetic mice. They reported that administration of 500 mg/kg hydroalcoholic extract of saffron for 21 days caused a considerable reduction in the

IL-17 (a pro-inflammatory cytokine) and increased the concentration of anti-inflammatory cytokines, especially IL-10 and TGF-? [33]. In a human study, Kermani T et al. Evaluated the effect of 100 mg/day crocus-sativus on serum concentrations of 12 serum cytokines in patients with metabolic syndrome. Opposite to our findings, Kermani T et al. revealed that crocus-sativus supplementation could not cause a significant change in the levels of inflammatory factors [34].

Such contradictory results may be due to differences in the doses of administered saffron, types of saffron, studied diseases, and duration of intervention. A major strength of this research was that it was the first clinical trial that evaluated the effect of saffron supplementation on patients with mild to moderate asthma. Furthermore, this study had accurate inclusion and exclusion criteria as well as stratified blocked randomization design.

Our patients also did not receive any concurrent treatment. However, our study had some limitations, such as modest sample size and study duration. In this regard, further research is required on the beneficial effects of saffron among a larger population in a longer duration. Furthermore, we did not evaluate the effects of saffron supplementation on immune response cell counts, which could increase the accuracy of the results.

To put in in a nutshell, the results of the present trial indicate saffron supplementation may attenuate inflammation by increasing the anti-inflammatory cytokines and decreasing oxidative stress in patients with asthma. By confirming these results in future studies, saffron can be used as an alternative therapeutic approach in patients with asthma.

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