

Oral administration of the purple passion fruit peel extract reduces wheeze and cough and improves shortness of breath in adults with asthma

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Abstract

Asthma, affecting as many as 400 million individuals worldwide, is one of the most prevalent chronic health condition in the United States. With an increasing number of patients with asthma and the frequent inability of conventional lifestyle modification and therapy to effectively control the problem, nutritional and dietary therapies are being sought. This study was undertaken to investigate the efficacy of the purple passion fruit peel (PFP) extract, a novel mixture of bioflavonoids, on asthma symptoms. Patients with asthma were studied in a 4-week randomized, placebo-controlled, double-blind trial with oral administration of PFP extract (150 mg/d) or placebo pills. The effects of PFP extract were evaluated by assessing the clinical symptoms of asthma and spirometry tests. Most clinical symptoms of asthma of the PFP extract-treated group were moderated significantly compared to the baseline. The prevalence of wheeze, cough, as well as shortness of breath was reduced significantly in group treated with PFP extract ($P < .05$), whereas the placebo caused no significant improvement. Purple passion fruit peel extract supplementation resulted in a marked increase in forced vital capacity ($P < .05$) as placebo showed no effect. However, no significant improvement was observed in the forced expiratory volume at 1 second of those supplemented with PFP extract. No adverse effect was reported by any of study participants. The PFP extract may be safely offered to asthmatic subjects as an alternative treatment option to reduce clinical symptoms. © 2008 Elsevier Inc. All rights reserved.

Keywords: Purple passion fruit; Asthma; Bioflavonoids; Nitric oxide; Human

Abbreviations: PFP, purple passion fruit peel; NO, nitric oxide; iNOS, inducible nitric oxide synthase; FEV₁, forced expiratory volume at 1 second; FVC, forced vital capacity.

1. Introduction

Asthma is a very common disease, affecting approximately 17 million people in the United States, with incidence increasing rapidly [1]. Asthma is characterized by increased

airway inflammation, mucus secretion, and bronchial responsiveness to a variety of stimuli, leading to intermittent episodes of wheezing, coughing, and dyspnea [2,3]. One of the underlying contributors to airway hyperresponsiveness is endogenous nitric oxide (NO). Nitric oxide elicits several functions in airways such as the modulation of airway smooth muscle tone, bronchial hyperresponsiveness, and antiinflammatory activity [4,5]. There is large body of evidence that NO, produced by neural NO synthase in response to physiological

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stimuli, is a potent bronchodilator, counteracting the cholinergic bronchoconstriction [6]. Inhibition of the enzyme NO synthases also increased airway responsiveness and histamine in patients with asthma [7]. However, large amounts of NO produced by inducible NO synthase (iNOS) in response to inflammatory cytokines may have detrimental role in asthma pathogenesis [8,9]. Asthmatic patients showed an increased expression of iNOS in airway epithelial cells and an increased level of NO in exhaled air [10], in proportion to bronchial wall inflammation or induced-sputum eosinophilia as well as to airway responsiveness [11].

The purple passion fruit (*Passiflora edulis* Sims f *edulis*), native to South America, is now grown around the world. Extracts from passion fruit have been used widely in folk medicine in South America to treat anxiety, insomnia, bronchitis, and asthma [12]. The purple passion fruit peel (PFP) extract contains 3 major components: cyanidin-3-O-glucoside, quercetin-3-O-glucoside, and edulilic acid [13]. Purple passion fruit peel extract treatment of spontaneously hypertensive rats, modeling human essential hypertension with increased expression and activity of iNOS, significantly attenuated blood pressure through NO modulation [13]. Purple passion fruit peel treatment of moderate to severe hypertensive patients also markedly reduced both systolic and diastolic blood pressure [13]. However, no data are available with respect to the clinical usefulness of PFP extract in treating asthma. The fact that (a) antioxidants have beneficial effects both on hypertension [14] and asthma [15] and PFP extract has significant antioxidants [13], (b) the effector role of iNOS in asthma pathology, and (c) the antihypertensive effect of PFP extract [13] prompted us to assess the clinical usefulness of the PFP extract in treating asthma. We hypothesized that the PFP extract, a novel mixture of antioxidant bioflavonoids with modulatory effects on iNOS, may improve the symptoms of the asthma.

2. Methods and materials

2.1. Sample preparations

The PFP extract was prepared as described in details elsewhere [13]. Briefly, the fresh chopped purple passion fruit shells (*Passiflora edulis* Sims f *edulis*), without juicy pulp, were immersed and soaked in water overnight. The passion fruit extract was produced batchwise by multiple runs through a 10 × 60-cm polymeric resin column, HP-20 (Mitsubishi Chemical Industries Ltd, Yokohama, Japan). The distinctive color of anthocyanins was used to monitor the loading as well as the completion of elution with methanol. The crude filtered extract was loaded onto the column until the red color of the anthocyanins had permeated about two third the length of the bed. The loaded column was then washed with distilled water (about 2 column volumes) until the washing was clear before eluting absorbed compounds with methanol. Then, the eluant concentrated under reduced pressure. Thereafter, with no carrier or excipient added, the concentrate was freeze-dried

yielding the PFP extract as a dark red powder. High-performance liquid chromatography analysis [16] confirms that the product is in every way similar in composition to the original material previously reported [13].

2.2. Study subjects and design

The 4-week, randomized, parallel-group, double-blind, placebo-controlled trial was approved by the human subjects committee of the Mashhad Medical University (Mashhad, Iran). Subjects were eligible for enrollment if they were between 18 and 60 years old and were defined as asthmatic by American Thoracic Society criteria. In addition, they were required to have a forced expiratory volume at 1 second (FEV₁) of 30% to 75% of predicted norm with an increase in FEV₁ of more than 15% above pretreatment values after inhaled salbutamol. Exclusion criteria were as follows: having clinical evidence of chronic obstructive lung disease; any renal, hepatic, cardiac, or endocrine disorders; being pregnant or lactating; use of tobacco or alcohol; and taking oral contraceptives or any dietary supplements other than a single daily multivitamin tablet. They were permitted to take their usual medications except glucocorticoids, leukotriene antagonists, aspirin, or any nonsteroidal anti-inflammatory medicines.

At the screening visit, after explaining the objective of the study, each eligible subject signed written informed consent, followed by a complete medical history and physical examination including checking for the presence of asthma symptoms, and skin prick and liver function tests. Spirometry tests, including forced vital capacity (FVC) and FEV₁, were measured. At the second visit, asthma clinical symptoms and spirometry tests were assessed again, and patients were randomly assigned to receive either the PFP extract pill (150 mg/d) or a similar-appearing placebo pill for 4 weeks. Study pills were prepared kindly by Dr H Afrasiabi at the Pharmacological Department, Mashhad Medical University, by direct compression of the fine-grained PFP extract or placebo powder, using an eccentric tableting machine (Korsch EK0, Korsch Maschinenfabrik, Berlin, Germany). The data for the first 2 visits were combined as baseline values. Patients attended the university's asthma clinic for follow-up every week during study to assess side effects. In the last visit, asthma symptoms and spirometry tests were assessed, and the unused pills were collected and counted to verify compliance. Changes in concomitant medications and clinical adverse events were sought by volunteered comments and by questioning, at baseline and follow-up visits, with none reported. Compliance was evaluated by tablet counting. During the 4-week period of treatment, all the subjects took at least 95% of the pills provided in a blinded fashion. All tests were done 2 to 4 hours after the last consumption of pills.

2.3. Statistical methods

The data are expressed as mean ± SEM. Statistical analyses were performed with SPSS version 11.5 (SPSS

Table 1
Baseline characteristic of the study subjects

Variable	Treatment group	
	Placebo	PFP extract
Number	21	22
Age (y)	35.8 ± 11.5	36.4 ± 15.7
Sex (male/female)	7/14	9/13
Duration of asthma (y)	10.1 ± 6.2	7.1 ± 5.4
Pretrial symptoms (%)		
Wheeze	100	100
Cough	100	95.2
Shortness of breath	78.9	90.0
Spirometry tests		
FEV ₁ (% predicted)	52.1 ± 4.3	51.5 ± 4.2
FVC (L)	2.8 ± 0.2	2.2 ± 0.2
Abnormal liver function tests (%)	0	0

Values are presented as means ± SEM. Student *t* test was used for analysis.

Institute, Chicago, Ill) [17]. For comparison between the groups and the pre- and posttreatment values, Student *t* test was used. Comparable nonparametric tests (the rank sum test) were substituted when tests for normality and equal variance failed [18]. A *P* value of less than .05 was used as a criterion for statistical significance. Analysis was performed according to the intention-to-treat principle.

3. Results and discussion

Of 43 subjects who met the inclusion and exclusion criteria, 42 completed the study with 1 lost to follow-up (in PFP group due to relocation). As shown in Table 1, demographic and clinical characteristics did not differ significantly between the 2 groups. Of the patients having wheeze as an asthma clinical symptom, only 19.1% ± 8.8% still had it after treatment with PFP extract (*P* < .001, compared to the baseline), whereas 78.9% ± 9.6% did in the placebo group (Fig. 1). Similarly, those with cough declined

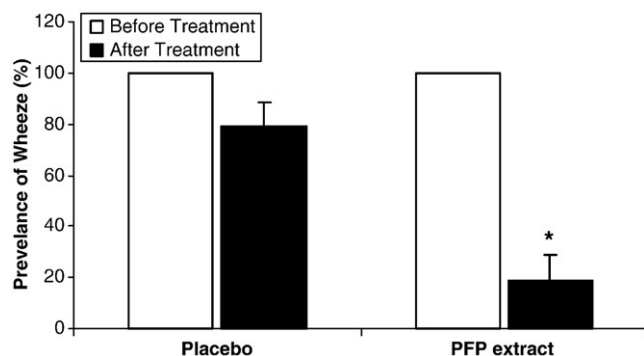


Fig. 1. Effect of the PFP extract on prevalence of wheeze in asthmatic patients. The clinical symptoms were assessed twice at baseline and after 4 weeks of randomized, double-blind, placebo-controlled treatment with PFP extract. These data show that PFP treatment lowered the prevalence of wheeze significantly compared to placebo treatment. Values are means ± SEM (n = 21 subjects in placebo, 22 subjects in PFP extract). Student *t* test was used for analysis (**P* < .001 compared to the control group).

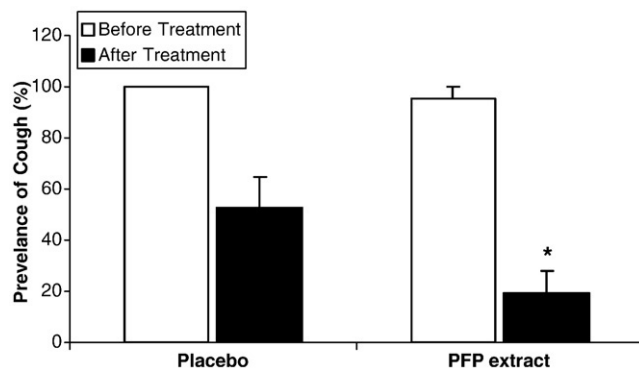


Fig. 2. Effect of the PFP extract on prevalence of cough in asthmatic patients. The clinical symptoms were assessed twice at baseline and after 4 weeks of randomized, double-blind, placebo-controlled treatment with PFP extract. These data show that PFP treatment lowered the prevalence of cough significantly compared to placebo treatment. Values are means ± SEM (n = 21 subjects in placebo, 22 subjects in PFP extract). Student *t* test was used for analysis (**P* < .001 compared to the control group).

76.2% during PFP treatment (*P* < .001, compared to the baseline), whereas cough was reduced only 47.4% in the control group (Fig. 2). Shortness of breath was found in 90.0% ± 6.9% of patients before PFP treatment, whereas it was present in only 10.0% ± 8.9% afterward (*P* < .05, compared to the baseline; Fig. 3). In placebo-treated subject, 78.9% ± 9.6% had shortness of breath before treatment and 36.8% ± 11.4% afterward, a smaller and statistically nonsignificant decline (Fig. 3). The FVC in PFP-treated group increased more than 15.9%, which is considered clinically significant compared to the baseline (*P* < .05, Fig 4). The FEV₁ and FEV₁/FVC ratio did not changed significantly in PFP-treated group (Figs. 5 and 6); however, a significant improvement in FEV₁ was observed in placebo group compared to the baseline (*P* < .05, Fig. 5).

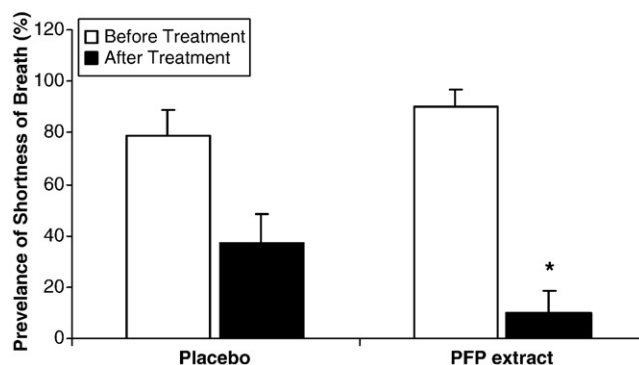


Fig. 3. Effect of the PFP extract on prevalence of shortness of breath in asthmatic patients. The clinical symptoms were assessed twice at baseline and after 4 weeks of randomized, double-blind, placebo-controlled treatment with PFP extract. These data show that PFP treatment lowered the prevalence of shortness of breath significantly compared to placebo treatment. Values are means ± SEM (n = 21 subjects in placebo, 22 subjects in PFP extract). Student *t* test was used for analysis (**P* < .05 compared to the control group).

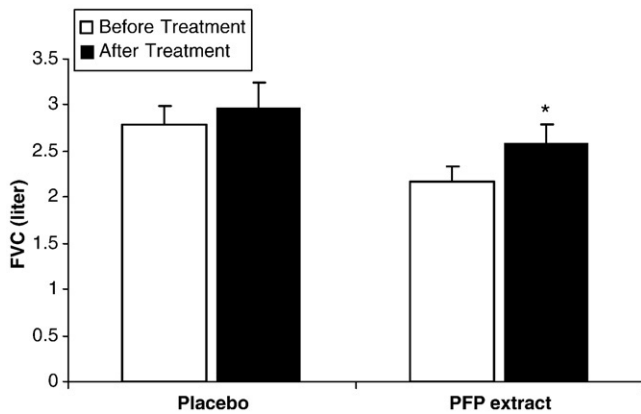


Fig. 4. Effect of the PFP extract on FVC (in liters) before and after treatment in asthmatic patients who received placebo or PFP extract (150 mg/d) for 4 weeks. Values are means $\pm$ SEM ($n = 21$ subjects in placebo, 22 subjects in PFP extract). Student t test was used for analysis (* $P < .05$ compared to the control group).

The present study provides evidence that oral administration of PFP extract produced statistically significant improvement in the symptoms of asthma after only 4 weeks of supplementation. The results of this clinical study indicate that the PFP extract may be a valuable nutraceutical supplement in the management of chronic asthma.

The significant antiasthmatic effect of PFP extract could be based on several mechanisms operating simultaneously. There is substantial evidence that oxidative stress is involved in the pathophysiology of asthma. In asthmatic patients, oxidative stress occurs not only as a result of inflammation but also from environmental exposure to air pollution [19]. There are many studies regarding dietary interventions that confirm the relationship to oxidative stress, bronchial inflammation, the development of asthmatic symptoms, and reduction of cellular functions [20]. Therefore, a role for antioxidants in reducing pathology is

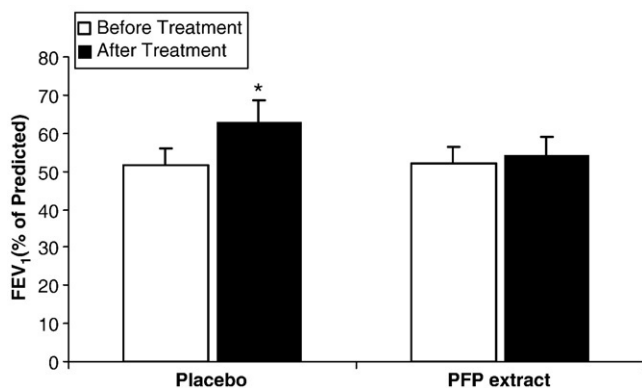


Fig. 5. Effect of the PFP extract on FEV₁ (percentage of predicted) before and after treatment in asthmatic patients who received placebo or PFP extract (150 mg/d) for 4 weeks. Values are means $\pm$ SEM ($n = 21$ subjects in placebo, 22 subjects in PFP extract). Student t test was used for analysis (* $P < .05$ compared to the control group).

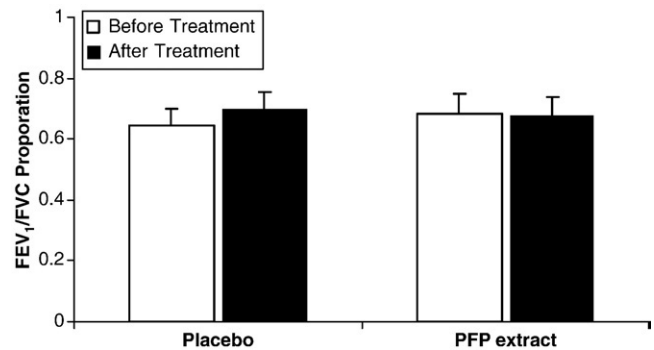


Fig. 6. The effect of the PFP extract on FEV₁/FVC ratio before and after treatment in asthmatic patients who received placebo or PFP extract (150 mg/d) for 4 weeks. Values are means $\pm$ SEM ($n = 21$ subjects in placebo, 22 subjects in PFP extract). Student t test was used for analysis.

likely, particularly during exacerbation of asthma [19]. Although serum vitamin E was not associated with lower prevalence of asthma in children exposed to heightened oxidants from cigarette smoke, levels of other antioxidants, selenium, β -carotene, and vitamin C were [21]. A lower serum concentration of antioxidants in asthmatic patients suggests that dietary interventions may reduce oxidant stress and prevent or minimize asthmatic symptoms [21,22]. Therapeutic interventions such as the PFP extract, a novel mixture of flavonoids with antioxidant and free radical scavenging activity, should inactivate environmental reactive oxygen species or protect endogenous antioxidant defenses, being beneficial as adjunctive therapies in asthmatic patients. As shown by high-performance liquid chromatography, the PFP extract is partially purified, yielding several dozen different constituent peaks [13]. The principal compounds in the PFP extract included edulilic acid, a novel cyclic acid glucoside elucidated by nuclear magnetic resonance spectroscopy, quercetin-3-O-glucoside, and cyanidin-3-O-glucoside [13]. Flavonoids in the PFP extract could plausibly reduce asthmatic inflammation through their known antioxidant, antiallergic, and anti-inflammatory properties. They can inhibit histamine release, arachidonic acid metabolism, and cytokine production [23]. Dietary intakes of several flavonoids, including quercetin, were associated with a lower incidence of asthma [24]. In vitro biological actions of quercetin support this potential respiratory protection including antioxidant effect [25].

In addition, the NO-lowering effect of PFP extract in spontaneously hypertensive rats [13] may explain some of its antiasthmatic activity. The balance between physiological regulation and pathological effect of NO is dependent on its relative concentrations and reactive biological targets. Inducible NO synthase gene expression and activity is up-regulated in asthmatic subjects, sustaining NO production at a greater level [10]. The interaction of excessive iNOS-derived NO with superoxide results in the formation of peroxynitrite, which in turn causes nitration

of protein tyrosine residues and enhances oxidative/nitrosative stress, leading to airway hyperresponsiveness, airway epithelial damage, inflammatory cell recruitment in asthmatic airways, and pulmonary cell death [26,27]. Therefore, the PFP extract may affect asthma by lowering NO production via down-regulation of iNOS expression and the scavenging of NO (as a radical itself) by flavonoids such as quercetin [24,25,28,29]. Such actions would lower peroxynitrite anion generation, thereby protecting the endogenous antioxidant system. However the estimated concentration of the quercetin and related compounds identified in the PFP extract with UV absorption at 325 nm [13] are too low to contribute significantly to observed effects, as occurred with hypertension in rats or humans [13]. Therefore, unidentified compound(s) along with the effects of several polyphenols must be the primary active ingredients with modifying effect.

There are a few potential limitations to this study. The first limitation of our study concerns the accuracy of self-report measurement of asthma symptoms. Another limitation of the study was the relatively small sample size, which may limit the generalizability of the findings. However, the most notable limitation of our study is related to short follow-up period.

Because of rapid industrialization and urbanization throughout the world, the prevalence of asthma will continue to increase. The results of the present study demonstrated the clinical benefit of daily oral administration of PFP in adults with persistent asthma, with safe use. This initial study of asthma therapy expands the understanding of how PFP reduced hypertension [13]. As the PFP extract has shown no toxicity in hypertensive rats and humans [13] and now asthmatic patients, it holds promise to supplement or partially replace standard antiasthmatic drugs. In addition, the PFP extract could serve as an alternative therapy when readily available as a nutritional supplement. As many patients are not able to tolerate the currently available antiasthmatic medications, a natural dietary supplement with low or no toxicity would be an attractive candidate for further development.

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