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Effect of crocin of *Crocus sativus* L. on serum inflammatory markers (IL-6 and TNF-α) in chronic obstructive pulmonary disease patients: a randomised, double-blind, placebo-controlled trial

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Abstract

Different factors, such as inflammation, oxidative stress, extracellular matrix degradation and apoptosis, affect the pathophysiology of chronic obstructive pulmonary disease (COPD), as a progressive disease characterised by permanent airflow limitation. Herbal supplements with anti-inflammatory and antioxidant properties can help treat certain chronic diseases. The current study aimed at investigating the preventive effects of crocin supplementation on the serum concentrations of IL-6, TNF-α, exercise capacity and pulmonary function tests (PFT) in patients with COPD. The present prospective randomised clinical trial equally divided fifty-seven patients with COPD into a placebo and an intervention group, who respectively received a placebo and crocin (15 mg twice day for 12 weeks) as a supplement. ELISA was used to measure serum levels of IL-6 and TNF-α, also PFT and exercise capacity based on 6-min walking distance test (6MWD), which was performed at the beginning and end of the study. Crocin improved the results of PFT ($P < 0{\cdot}05$) and 6-MWD ($P < 0{\cdot}001$) and exerted preventive effects by increasing the serum levels of IL-6 in patients with COPD compared with those in the placebo group ($P < 0{\cdot}05$). Intervention with crocin significantly lowered serum levels of TNF-α at the end of the study ($P < 0{\cdot}01$). The present findings suggest crocin supplementation improves exercise capacity and PFT in patients with COPD by reducing serum levels of inflammatory factors.

Key words: **Crocin: IL-6: TNF-α: COPD management**

Respiratory capacity is limited by chronic obstructive pulmonary disease (COPD) as the fourth leading cause of death, a major and progressive health problem[1] and a systemic inflammatory disease with different pulmonary and extrapulmonary symptoms, in which systemic inflammatory markers play a key role[2,3]. The potential exacerbation mechanisms of COPD comprise increases in inflammatory factors, including C-reactive protein (CRP), IL-6, IL-1β and TNF-α[4]. Inflammation in patients with COPD causes anorexia, increases energy consumption and decreases muscle proteins[5]. Pulmonary function tests (PFT) are negatively correlated with systemic inflammation in these patients[6].

Researchers have made efforts to introduce treatments for COPD and reduce inflammation using non-pharmacological supplements such as the commonly used saffron and its active ingredients in clinical trials[7]. A clinical trial found the anti-inflammatory properties of saffron to be reflected in decreased inflammatory markers and improved PFT in patients with asthma[8]. Many animal and human studies suggest the anti-inflammatory and antioxidant properties of crocin as an active ingredient of saffron[7,9,10]. The anti-inflammatory activities of crocin were also reported in body organs such as respiratory, nervous, cardiovascular, gastrointestinal, urogenital and musculoskeletal systems[11,12]. Moreover, clinical trials have used the so-called Krocina™ or purified crocin (98 %) as a supplement[13]. Its anti-inflammatory and antioxidant effects have also been reported[13]. The present study investigated the anti-inflammatory effects of Krocina™ on patients with COPD. The 6MWD test and spirometry were performed to evaluate its effects on the exercise capacity and PFT.

Participants and methods

Design

This study was a randomised, double-blind, placebo-controlled clinical trial.

Abbreviations: COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; FEV1, forced expiratory volume in the first second; FVC, forced vital capacity; PFT, pulmonary function tests.

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Participants

The present trial recruited fifty-seven male patients with COPD presenting from December 2019 to October 2020 to Imam Khomeini Hospital affiliated with Ardabil University of Medical Sciences, Ardabil, Iran.

Clinically stable COPD patients in the past 8 weeks were included in the study based on the symptoms and criteria suggested by the American Thoracic Society, that is, cough, sputum and chronic dyspnoea with FEV1/FVC < 70 %. The exclusion criteria were as follows: infectious disease, bronchiectasis, chronic inflammatory diseases, active liver and kidney diseases, cancer, myocardial infarction and unstable angina in the previous 6 months and sensitivity to crocin and hospitalisation over the previous 3 months. To match the patients by physical activity, those with structured physical activity or planned exercise were excluded. According to the Global Initiative for Obstructive Lung Disease treatment guidelines, patients do not receive medications other than those associated with COPD grade.

Randomisation

The patients were allocated to two groups by simple randomisation. 29 'A' and 28 'B' labeled sealed envelopes were used by nurse to provide medicine and placebo to patients. The patients were randomly assigned to the placebo or intervention group using the RANDBETWEEN function in Microsoft Excel. One of the authors blinded to the grouping randomly inserted the Krocina™ and placebo tablets into numbered bags, which were then distributed by another author among blinded participants who were not involved in the experiment. All authors and participants were blinded using random codes.

Intervention

The intervention and control groups received 15 mg of Krocina™ tablets twice a day (a product of Buali Research Institute of Pharmaceutical Sciences, Pharmaceutical Products Development Center, Sina Pooyesh Drug Company, registration number 48674, and IRC number: 0648126930388841) and the placebo for 12 weeks (Fig. 1). The placebo compounds used were Avicel, polyvinylpyrrolidone and magnesium stearate. The crocin/placebo tablets were taken as two doses in the morning and night with main meals (breakfast and dinner) for 12 weeks.

Outcomes and relevant measures

The primary outcome variable was serum levels of IL-6 and TNF-α after the 12 weeks intervention, and the secondary outcomes were PFT and a 6-min walking distance test (6 MWD).

Demographic and clinical assessments questionnaire

The measured and recorded demographic information of all the subjects included height, weight and BMI. All patients with COPD underwent PFT using spirometry and 6 MWD according to ATS guidelines, at the beginning and end of the study.

Biochemical examinations

Commercial ELISA kits *(Crystal day, China)* were used according to the manufacturer's recommendations, and *v*enous blood samples were taken from all the participants before and after the intervention to measure their serum levels of TNF-α and IL-6.

Sample size estimation

The sample size was calculated based on the formula for mean comparison with $\alpha = 0{\cdot}05$, $\beta = 0{\cdot}05$, $\mu 1 = 5{\cdot}22$, $S1 = 2$, $\mu 2 = 6{\cdot}8$ and $S2 = 2$, based on a previous study on serum levels of IL-6: $n = ([Z1\text{-}\alpha/2 + Z1\text{-}\beta]^2\ [S1^2 + S2^2])/(\mu 1\text{-}\mu 2)$[14]. Based on these calculations, twenty-two participants were included in each group. Considering the probability of sample attrition, twenty-nine participants were recruited from each group.

Ethical considerations

This study was conducted in accordance with the guidelines of the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the ethics committee of the Ardabil University of Medical Sciences (IR.ARUMS.REC.1397.279). Written informed consent was obtained from all patients. This study was also registered in the Iranian Registry of Clinical Trials (IRCT20110109005579N2, https://www.irct.ir/trial/41998).

Statistical analysis

The Kolmogorov–Smirnov test was performed to evaluate the distribution normality of the data. Parametric data were expressed as mean ± SD and non-parametric data as median and 25th–75th percentiles. At the beginning and end of the study, between-group and within-group comparisons were performed using Mann–Whitney and Wilcoxon tests, respectively. The data were statistically analysed using SPSS-21.0 and Graph Pad Prism 7 at a significance level of $P < 0{\cdot}05$. However, intention-to-treat analysis was not used in the analysis of the results because the exclusion of subjects from the study was not due to drug side effects or intolerance of patients that had an effect on the results.

During the study period (12 weeks), all participants were given dietary recommendations, including avoiding fast food, sausages, saffron, smoked and canned foods. The participants were also asked not to change their physical activity or energy expenditure during the experiment. Required information, such as the regular use of tablets and their possible side effects, was followed up every week.

Results

Baseline assessments

Table 1 compares the placebo group with the crocin group, which suggests no significant baseline differences in terms of the mean values of age, height, weight, BMI, PFT, 6 MWD and serum levels of IL-6 and TNF-α.

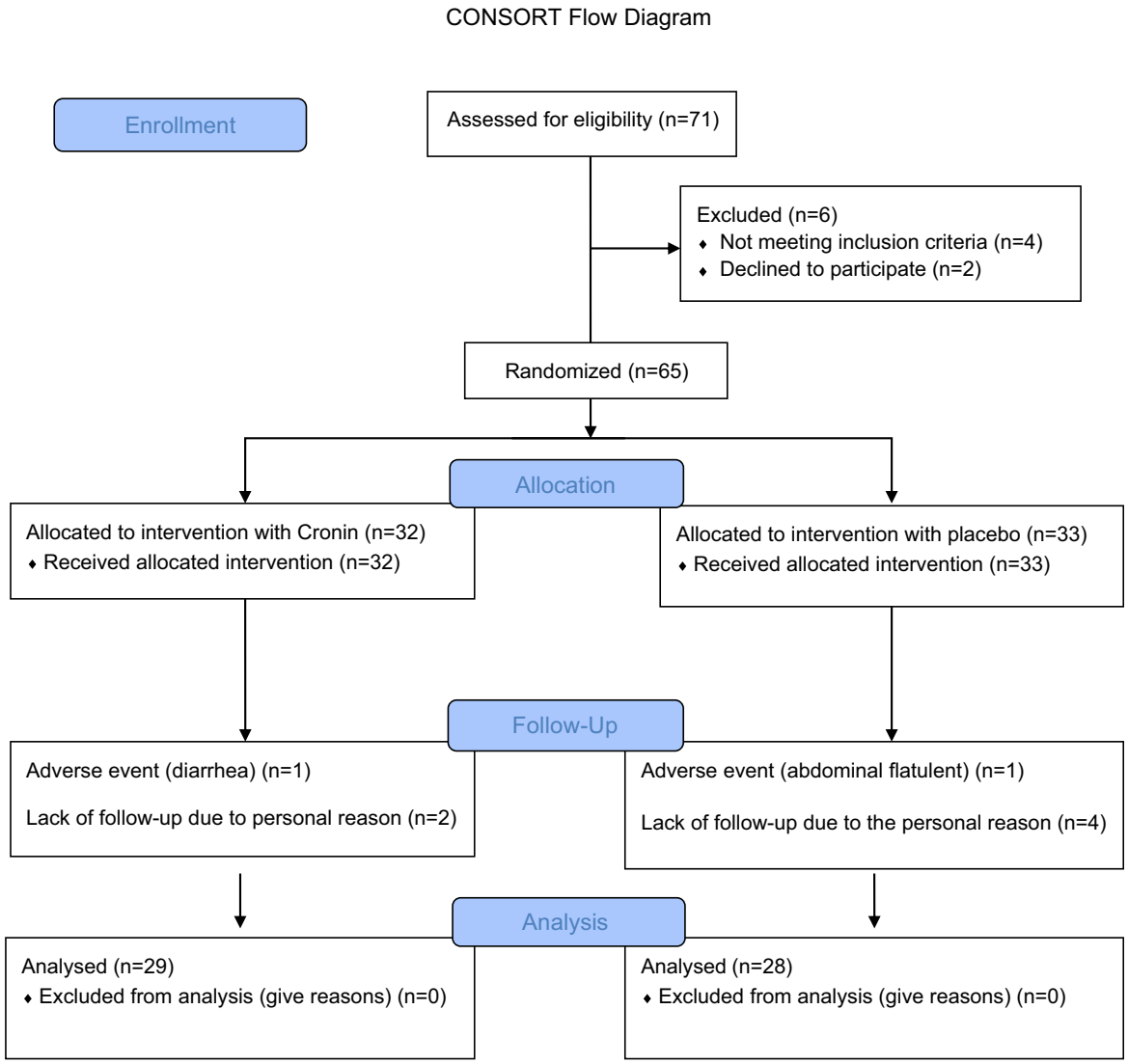

Fig. 1. Flow diagram of the trial.

Table 1. Baseline parameters in placebo and crocin groups

	Placebo (*n* 28)		Crocin (*n* 29)		
	Baseline		Baseline		
Variables	Mean	SD	Mean	SD	*P*-value
Age (year)	63·17	8·98	61·48	8·49	0·467
Weight (kg)	70·62	12·77	75·89	12·80	0·133
Height (m)	1·72	0·05	1·73	0·04	0·846
BMI (kg/m^2)	23·50	4·38	25·55	4·23	0·078
FEV1 (percent Predict)	56·00	17·02	53·41	15·16	0·548
FVC (percent Predict)	76·39	14·95	70·79	17·08	0·194
FEV1/FVC (Percent)	59·88	11·03	60·64	8·89	0·776
Spo2 (%)	89·03	7·86	89·82	5·39	0·661
6MWD (meter)	376·25	111·69	372·93	106·71	0·909
IL-6 (ng/ml)	3·56	2·97	3·41	1·84	0·829
TNF-α (ng/ml)	13·56	4·01	13·80	2·86	0·795

FEV1, forced expiratory volume in the first second; FVC, forced vital capacity; 6MWD, 6-min walking distance test.

Effects of crocin on BMI and pulmonary function test

There was no significant difference in BMI pre-and post-intervention in both groups. In addition, after 12 weeks of crocin intervention, no significant difference was observed in BMI between the end and beginning of the study.

Before or after the intervention, no significant differences were observed between the two groups in terms of pulmonary function test parameters, including FEV1, FVC and FEV1/FVC. Significantly lower and higher FVC and FEV1/FVC ratios were observed, respectively, after the intervention in the placebo group ($P < 0{\cdot}01$, Fig. 2(c), $P < 0{\cdot}001$, Fig. 2(e), respectively). The post-intervention FEV1 and FEV1/FVC also significantly increased compared with the pre-intervention stage in the crocin group ($P < 0{\cdot}05$, Fig. 2(a), $P < 0{\cdot}001$, Fig. 2(e), respectively). Moreover, the analysis of PFT parameters showed significant post-intervention differences between the two groups in terms

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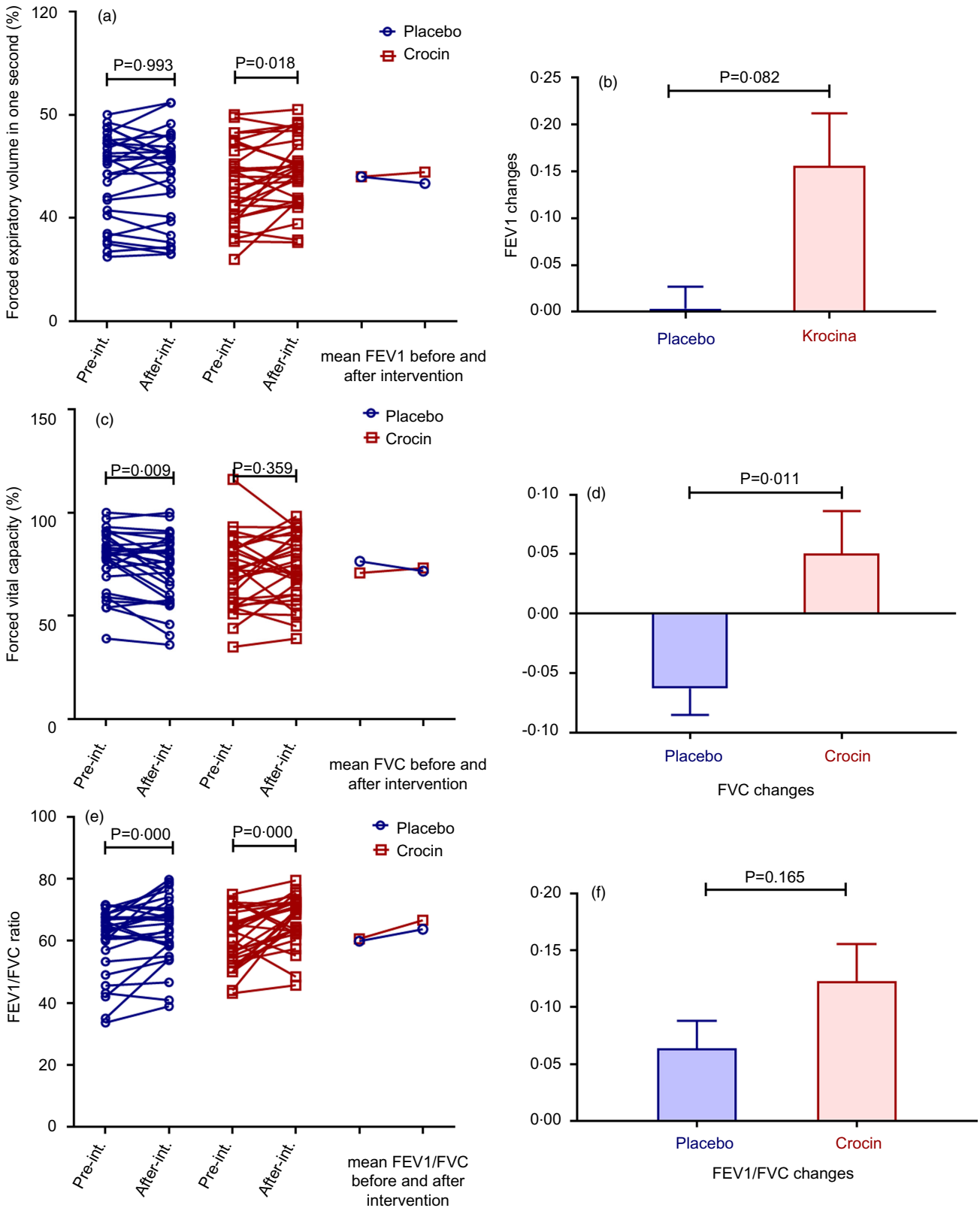

Fig. 2. Mean ± SD or median (interquartile range) of (a): FEV1, (b): FEV1 changes, (c) FVC, (d): FVC changes, (e): FVE1/FVC and (f): FEV1/FVC changes in placebo (blue colour) and Krocina-treated (red colour) group's pre-intervention and after 12 weeks of intervention. FEV1, forced expiratory volume in the first second; FVC, forced vital capacity.

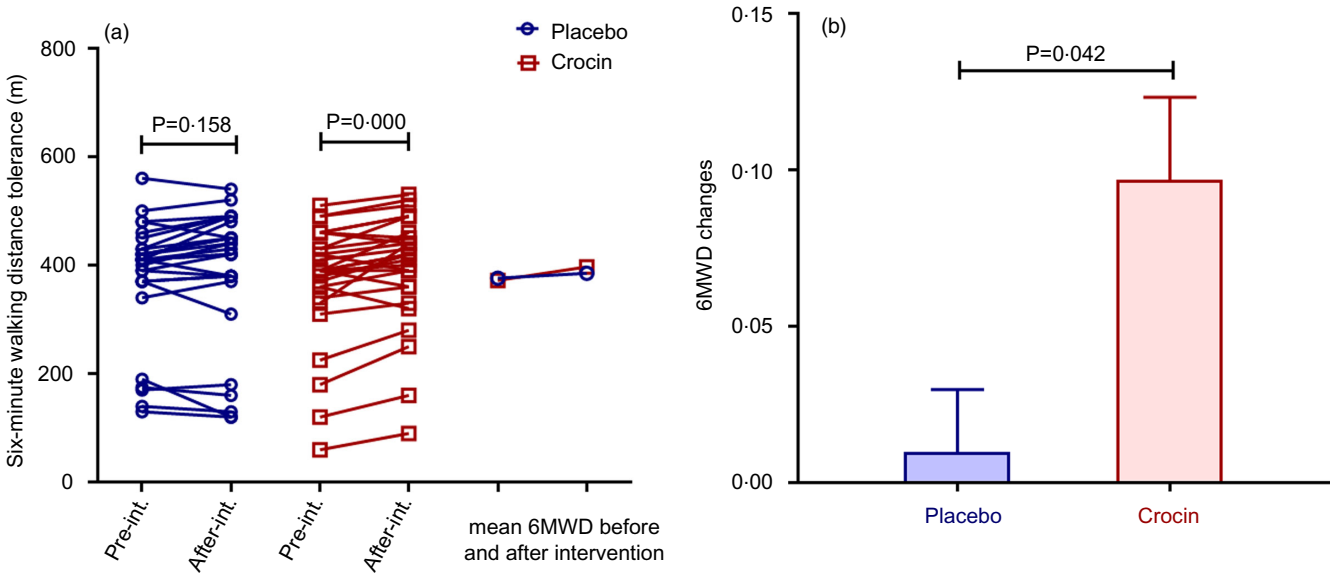

Fig. 3. Mean ± SD or median (interquartile range) of (a): 6MWD and (b): 6MWD changes in placebo (blue colour) and Krocina-treated groups (red colour) pre-intervention and after 12 weeks of intervention. 6MWD: 6-min walking distance test.

of FVC ($P < 0{\cdot}05$, Fig. 2(d)) and no significant difference in terms of FEV1/FVC ($P = 0{\cdot}16$, Fig. 2(f)).

Effects of crocin on 6-min walking distance test

The pre-and post-intervention results of the 6 MWD test were not significantly different between the two groups. Despite the insignificant changes in the 6 MWD between the pre-and post-intervention in the placebo group, the increase in the crocin group was significant ($P < 0{\cdot}001$, Fig. 3(a)). Therefore, the mean post-intervention 6 MWD values were significantly different between the two groups ($P < 0{\cdot}05$, Fig. 3(b)).

Effects of crocin on serum levels of IL-6 and TNF-α

Despite insignificant changes in the crocin group, the serum levels of IL-6 significantly increased in the placebo group after intervention ($P < 0{\cdot}05$, Fig. 4(a)). Significant post-intervention differences were also observed between the two groups in terms of serum levels IL-6 despite the insignificant baseline differences ($P < 0{\cdot}05$, Fig. 4(a)). The mean changes in serum IL-6 levels were significantly different between the placebo and intervention groups ($P < 0{\cdot}01$, Fig. 4(b)).

No significant differences were observed in the mean serum levels of TNF-α in the placebo group after intervention. Despite the insignificant baseline between-group differences, the post-intervention TNF-α level was not significantly different between the placebo and crocin groups ($P = 0{\cdot}89$, Fig. 4(c)). Significant differences were also observed between the two groups in terms of the mean changes in serum TNF-α levels ($P < 0{\cdot}01$, Fig. 4(d)).

Discussion

The effects of crocin on patients with COPD include improving PFT, elevating the 6 MWD test and decreasing the serum levels of IL-6 and TNF-α.

Epidemiological, pathological and clinical studies have suggested an association between COPD and systemic inflammation. Even in patients with a stable status, inflammatory proteins such as CRP, TNF-α, IL-6 and IL-8 were found to increase in the systemic circulation[15,16]. Slightly significant increases were also reported in other inflammatory circulatory proteins such as soluble TNF, IL-10 and IL-18 receptors in patients with COPD[17,18]. Epidemiological research suggests a negative association between systemic inflammatory proteins (IL-6 and CRP) and FEV1-based PFT[19,20]. Moreover, systemic inflammatory markers were found to be significantly increased in patients with severe COPD compared with those with stable status[21].

Numerous studies have addressed medicinal plants with anti-inflammatory and antioxidant properties[22]. Nutritional supplements have been found to improve energy and nutrient status in patients with COPD[23]. Supplements with antioxidant and anti-inflammatory properties can be used to treat COPD[24,25]. Research has suggested the anti-inflammatory and free radical scavenging activities of saffron and its compounds[26]. A 12-week crocin intervention significantly reduced the serum levels of IL-6 and TNF-α in patients with COPD compared with those in the placebo group. Crocin also improves systemic inflammatory conditions by reducing inflammatory factors.

In line with the present study, animal research suggests that saffron has anti-inflammatory and antioxidant effects. Oral administration of saffron extract and safranal as active ingredients decreased bronchial epithelial cell apoptosis and serum levels of inducible nitric oxide synthase, IL-5 and IL-13 in animals with asthma[9]. The anti-inflammatory effects of the ethanolic and aqueous extracts of *Crocus sativus* are exerted by reducing the levels of IL-6, IL-1β and TNF-α[27,28]. Research suggests that crocin has anti-inflammatory effects on lung disease. Administration of crocin reduced inflammatory cells and levels of lung inflammatory markers (IL-6, IL-1β and TNF-α) in a

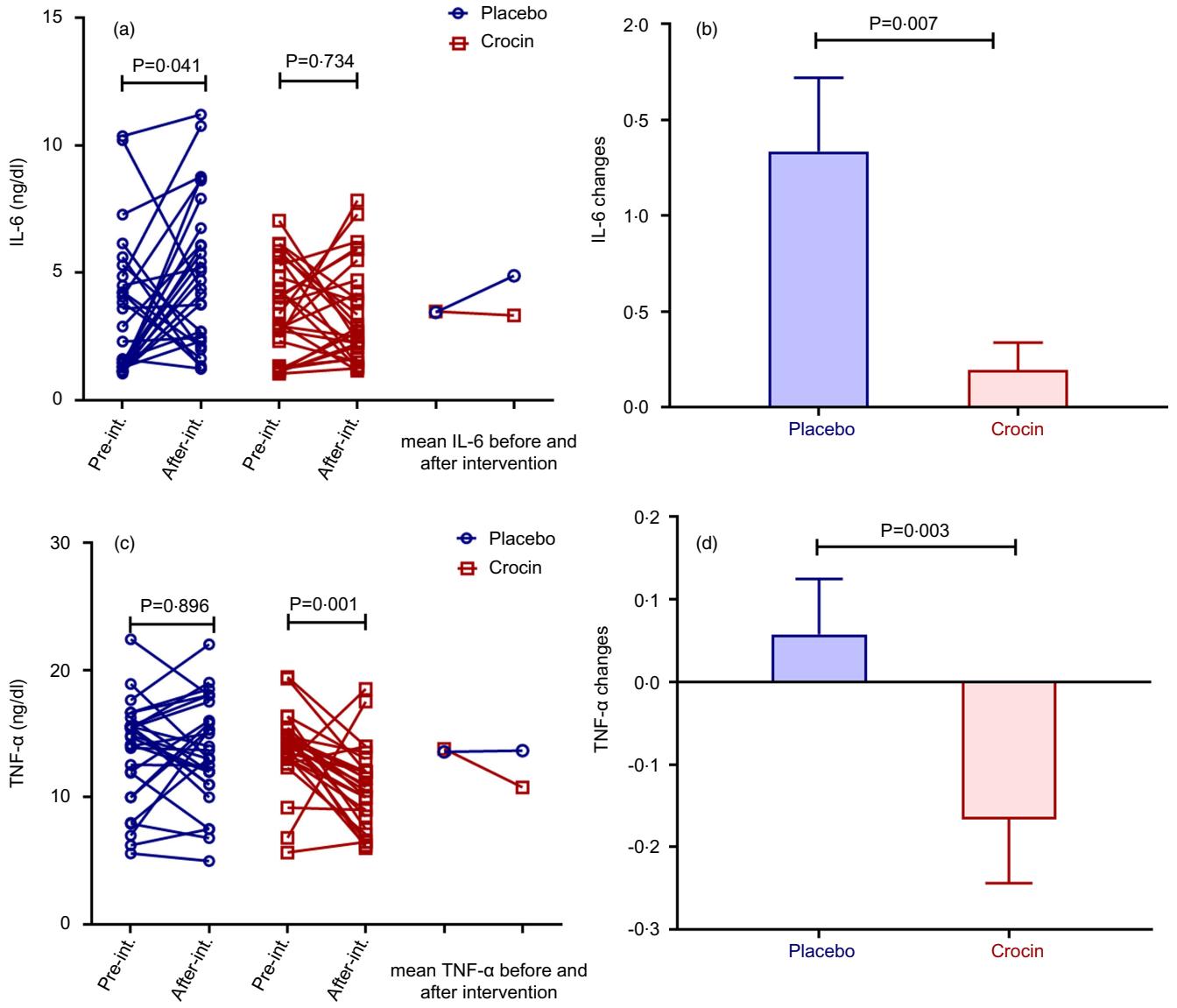

Fig. 4. Mean ± SD or median (interquartile range) of serum levels of (a): IL-6, (b): IL-6 changes, (c): TNF-α and (d): TNF-α changes in placebo (blue colour) and Krocina-treated groups (red colour) pre-intervention and after 12 weeks of intervention..

COPD model of mice exposed to cigarette smoke[29]. Pretreatment with crocin also reduced TNF-α, IL-8, IL-6 and IL-1β levels in human bronchial epithelial cells[30]. An *in vitro* study on murine macrophage RAW 264·7 found that crocin reduced IL-6 and TNF-α and induced IL-4 and IL-10 levels[31]. Moreover, a murine model of asthma showed anti-inflammatory effects of crocin on the lung tissue of ovalbumin-sensitised mice through the prevention of elevated TNF-α, IL-5, IL-1β, IL-13 and IL-4 levels[32].

Different clinical trials on inflammatory factors have reported different effects of saffron and crocin as active ingredients. Shahbazian *et al.* found saffron and crocin to improve serum levels of CRP in healthy individuals and patients with diabetes[14,33,34]. The serum levels of CRP, however, were not significantly changed after the intervention according to Azimi (patients with diabetes)[35], Mousavi (patients with schizophrenia)[36], Kermani (metabolic syndrome subjects)[37] and Ebrahimi (diabetic patients)[38]. Clinical trials have reported different effects on serum levels of TNF-α. According to Kermani *et al.* (metabolic syndrome)[37] and Ebrahimi *et al.* (diabetes)[38], saffron and crocin decrease serum TNF-α levels. In contrast, Shahbazian *et al.* (diabetes)[14] and Ghiasian *et al.* (multiple sclerosis)[39] reported insignificant effects of the intervention. Further studies are required in this context given the contradictory effects of saffron and its active ingredients reported in clinical trials as a result of differences in the disease nature, sample size, concentrations used and intervention duration.

Despite the clinically insignificant changes observed in the present study, 12 weeks of intervention with crocin as a supplement improve PFT results in patients with COPD. Saffron has been found to improve pulmonary symptoms in patients with asthma[8]. Animal studies have suggested that saffron and its active ingredients have anti-inflammatory properties[40,41]. The improved results of PFT in the crocin group can be partly

explained by the anti-inflammatory properties of crocin, which require further research.

The present study reported significant increases in the 6 MWD test in the intervention group compared with the placebo group. Exercise capacity and health-related quality of life are commonly used indicators of respiratory rehabilitation in COPD[42]. Significantly lower 6 MWD was found in patients with COPD[43]. Therefore, prolonged physical activity has been found to help treat patients with COPD. Increased cardiorespiratory fitness has also been observed in COPD patients treated with crocin.

Despite the unknown role of crocin in respiratory diseases, its anti-inflammatory effects are exerted through the modulation of phosphoinositide-3-kinase/Akt, protein kinase C, mitogen-activated protein kinases (MAPK/ERK), nuclear factor erythroid 2-related factor 2 (Nrf2), NF-κB p65, c-Jun N-terminal kinases (JNK), Ca^{2+}/calmodulin-dependent protein kinase 4 (CAMK4), inducible nitric oxide synthase, signal transducer and activator of transcription 6 (STAT6), ER-stress markers and high-mobility group box 1 pathways[29,32,41,44,45,46,47].

This is the first placebo-controlled randomised clinical trial to investigate the effect of crocin on inflammatory markers in COPD patients. However, our study had several limitations. First, the subjects included were all male; further studies are required to assess the effect of crocin in COPD patients of both sexes. Second, there was prolonged recruitment time due to the COVID-19 pandemic and the denial of some patients to participate in the study. Finally, the sample size of our study was moderate, and future studies with larger sample sizes are required. In addition, we did not provide a food reminder for 12 weeks in terms of the content and type of food consumed.

Conclusion

The present clinical trial found that crocin improved systemic inflammation and exercise capacity based on 6 MWD and PFT in patients with COPD. Despite the changes made to improve the patient status, further clinical trials are recommended.

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The authors declare that they have no competing interests.

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