

## RESEARCH ARTICLE

# Clinical and metabolic responses to crocin in patients under methadone maintenance treatment: A randomized clinical trial

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Patients under methadone maintenance treatment (MMT) programs are susceptible to several complications including metabolic and clinical disorders. This study was designed to determine the effect of crocin supplementation on mental health parameters and metabolic profiles in subjects under MMT. The current randomized, double-blind, placebo-controlled, clinical trial was conducted among 53 patients under MMT to receive either 15 mg/day of crocin ( $n = 26$ ) or placebo ( $n = 27$ ) twice a day for 8 weeks. Crocin administration significantly decreased Beck Depression Inventory score ( $P = 0.01$ ) and Beck Anxiety Inventory score ( $P = 0.008$ ) compared with the placebo. In addition, crocin administration resulted in a significant reduction in fasting glucose ( $P = 0.003$ ), insulin levels ( $P = 0.01$ ), insulin resistance ( $P = 0.008$ ), triglycerides ( $P = 0.001$ ), very low-density lipoprotein ( $P = 0.001$ ), total cholesterol levels ( $P = 0.03$ ), and a significant increase in insulin sensitivity ( $P = 0.003$ ) compared with the placebo. Additionally, crocin intake was associated with a significant reduction in high-sensitivity C-reactive protein ( $p < .001$ ) and malondialdehyde ( $P = 0.001$ ) and a significant rise in total antioxidant capacity levels ( $P = 0.01$ ) compared with the placebo. The findings of this clinical trial indicate that taking crocin for 8 weeks by patients under MMT had beneficial effects on their mental health and improved their metabolic profiles.

## KEYWORDS

crocin, mental health, metabolic profiles, methadone maintenance treatment

**Abbreviations:** BAI, Beck Anxiety Inventory; BDI, Beck Depression Inventory; FPG, fasting plasma glucose; GAPDH, glyceraldehyde-3-Phosphate dehydrogenase; GSH, total glutathione; HDL cholesterol, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model of assessment-insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL cholesterol, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; MDA, malondialdehyde; MMT, methadone maintenance treatment; PPAR- $\gamma$ , peroxisome proliferator-activated receptor gamma; QUICKI, quantitative insulin sensitivity check index; TAC, total antioxidant capacity; TGF- $\beta$ , transforming growth factor beta; TNF- $\alpha$ , tumor necrosis factor alpha; VEGF, vascular endothelial growth factor; VLDL cholesterol, very low-density lipoprotein cholesterol

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## 1 | INTRODUCTION

Opioid abuse disturbance is the most important social economical and clinical problems around the world (Shakibi, 2004). In Iran, the prevalence of drug abuse is rising and was nearly three times higher than the prevalence worldwide. About 1.2 millions of Iranians have opioid dependency (Amin-Esmaili et al., 2016). A methadone maintenance treatment (MMT) program is the most important of medical approach in drug abuse disorder (Gowing & Ali, 2006). In Iran, about 500,000 subjects are under buprenorphine and MMT programs (Danial, Motamedi, Mirhashemi, Kazemi, & Mirhashemi, 2014). MMT induces receptor endocytosis, alters oxidation–reduction activity (Mannelli et al., 2009), and decreases opioid tolerance (Koch et al., 2005). MMT programs may improve public health among cases with opiate dependence (Dolan et al., 2003). Despite doing various practical implementation of MMT programs, many questions and challenges remained unclear. However, there is evidence study that frequent use of opioid and methadone is linked to immune system disturbance, lipid peroxidation, upregulation of inflammatory markers, increased production of reactive oxygen species (ROS), and DNA damage (Chan et al., 2015; Mannelli et al., 2009; Salarian et al., 2018; Vallecillo et al., 2018). MMT programs also might be associated with psychological syndromes including anxiety and depression (Callaly, Trauer, Munro, & Whelan, 2001).

*Crocus sativus* L. (saffron) is known as one of important medicinal plants, which is belonged to Iridaceous family (Ayatollahi, Javan, Khajedaluee, Shahroodian, & Hosseinzadeh, 2014). Saffron could be found, as main crops, in a variety of countries, including New Zealand, France, Switzerland, England, and the United States (Khanali, Shahvaroghi Farahani, Shojaei, & Elhami, 2017). Chemical analyses of saffron there are various compounds (i.e., picrocrocin, safranal, and crocin). The main property of saffron is its antioxidant effect (Rios, Recio, Giner, & Manez, 1996). In this regards, saffron and its compounds particularly crocin could be applied as an antianxiety and anti-depressant agent (Talaie, Hassanpour Moghadam, Sajadi Tabassi, & Mohajeri, 2015). Saffron exerts pharmacological effects via the modulation of insulin metabolism and improves serum lipids, oxidative stress, and inflammatory cytokines that are introduced for the treatment of different disorders (Hosseini, Razavi, & Hosseinzadeh, 2018; Javandoost et al., 2017; Kermani et al., 2017; Razavi & Hosseinzadeh, 2017). We have previously shown that crocin supplementation at a dosage of 15 mg/BID for 2 months to patients undergoing MMT had ameliorating effects on psychological scales (Khalatbari-Mohseni et al., 2019). In an animal study conducted by Yarijani, Pourmotabbed, Pourmotabbed, and Najafi (2017), crocin was introduced as an anti-inflammatory and antioxidant agent in renal ischemia/reperfusion rat models. In addition, saffron administration for 12 weeks improved glycemic status and serum lipids in patients with Type 2 diabetes mellitus (T2DM; Moravej Aleali et al., 2019). Although other researchers failed to find an improvement in insulin and lipid profiles, depression, and metabolic parameters in response to ingesting saffron and crocin supplements (Nosrati et al., 2017; Pourmasoumi, Hadi, Najafgholizadeh, Kafeshani, & Sahebkar, 2019; Sahraian, Jelodar, Javid, Mowla, & Ahmadzadeh, 2016).

Saffron might improve mental health symptoms through regulating the neurotransmitters in the brain, including serotonin, dopamine, and norepinephrine (Hossein Hosseinzadeh, Motamedshariaty, & Hadizadeh, 2007; H. Hosseinzadeh, Sadeghnia, Ghaeni, Motamedshariaty, & Mohajeri, 2012). Crocin administration may be favorable in subjects under MMT through its effects on enhancing the immune response, decreased ROS, the activation of peroxisome proliferator-activated receptor alpha, and the regulation of nuclear factor kappa B activation, as there are evidence studies that crocin intake might have anti-apoptotic, anti-inflammatory, and antioxidant effects (Razavi & Hosseinzadeh, 2017; Thushara et al., 2014; Yarijani et al., 2017). These lines of evidence emphasize the importance of crocin on mental health, inflammation parameters, and oxidative stress suggesting crocin intake may have favorable effects in subjects under MMT programs. To our knowledge, data from studies investigating the effects of crocin intake on metabolic biomarkers in MMT patients are limited. Therefore, this study was aimed to evaluate the impacts of crocin supplementation on mental health and metabolic status in MMT patients.

## 2 | METHODS

### 2.1 | Study design and participants

The current randomized, double-blinded, placebo-controlled, clinical trial, registered in the Iranian clinical trials website at (<http://www.irct.ir>: IRCT20170420033551N4), followed the Declaration of Helsinki guideline; informed consent was given by all participants. This investigation was conducted among 53 patients undergoing MMT, who referred to the Golabchi Clinic in Kashan, Iran, between December 2018 and March 2019. The included subjects were aged 18–60 years, currently undergoing MMT and opioid dependence in the past year, evaluated by the drug abuse section of the Structured Clinical Interview for DSM-IV, Beck Depression Scores > 20 and Beck Anxiety > 15, seeking for treatment. Exclusion criteria were as follows: taking crocin and anti-inflammatory and antioxidant supplements during the last 3 months before the intervention and history of metabolic diseases including diabetes, hypertension, thyroid, and cardiovascular disease.

### 2.2 | Study protocol

Participants were randomly allocated to intake either 15 mg of crocin ( $n = 26$ ) or placebo ( $n = 27$ ) twice a day for 8 weeks. We used the mentioned dose of crocin based on a prior study in subjects with major depressive disorder and MMT (Khalatbari-Mohseni et al., 2019; Talaie et al., 2015). Methadone was consumed in the form of syrup by patients. Patients were matched based on the methadone dosage. Randomization was done using computer-generated random numbers by a trained staff at the clinic. Randomization and allocation were concealed to the researchers and participants until the final analyses were completed. The crocin and placebo tablets were prepared in

the same shape, color, size, and texture, and each tablet container had a random code number for this double-blinded trial. Thus, participants, physician, and other investigators were all blind to the treatment group assignment. Saffron stigmas were purchased from Novin Saffron Co. (Mashhad, Iran). Crocin was extracted and crystallized from saffron stigmas, using a previous published protocol (Dorri et al., 2015). Crocin and placebo were similarly formulated into film-coated tablets by the Department of Pharmaceutics, School of Pharmacy in Mashhad University of Medical Science. Each tablet contained 15 mg of crocin or placebo. Also, the purity of crocin in this study was more than 95%, and the main excipient applied in crocin tablet was avicel.

### 2.3 | Outcomes of interest

Mental health indicators including Beck's Depression Inventory (BDI) and Beck Anxiety Inventory (BAI) were considered as the primary outcomes of interest. Metabolic and genetic profiles were considered as the secondary outcomes.

### 2.4 | Anthropometric measures

Participants' weight and height were measured after an overnight fasting status using a standard scale (Seca, Hamburg, Germany) prior to the intervention and after the 8-week treatment. Body mass index was calculated as weight in kilogram divided by height in meters squared.

### 2.5 | Clinical measures

BDI was assessed using a self-compiled questionnaire of 21 items in multiple-choice format (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961). The internal stability of the test among Iranian students (Persian version of BDI-II) was moderate to good (Cronbach's  $\alpha = .58$ ), and its reliability by test-retest was 0.73 (Razavi, Sadeghi, Abnous, Vahdati Hasani, & Hosseinzadeh, 2017). Anxiety measured by BAI-21 questionnaire developed by Beck, Epstein, Brown, and Steer (1988) in order to determine the frequency of anxiety symptoms in adults. Kaviani and Mousavi (2008) approved the validity and reliability of Persian version of BAI among Iranian normal population as well as clinically anxious patients.

### 2.6 | Biochemical measures

At baseline and the end of the treatment, 15 ml of fasting blood was collected from each subject at the Kashan Reference laboratory, Kashan, Iran. Insulin concentrations were measured using ELISA kit (DiaMetra, Milano, Italy) with intra- and inter-assay coefficient variances (CVs) below 5%. The homeostasis model of assessment-insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) were calculated using the established formulas (Pisprasert, Ingram, Lopez-Davila, Munoz, & Garvey, 2013). Enzymatic kits (Pars Azmun,

Tehran, Iran) were used to determine fasting plasma glucose (FPG) and lipid profiles with inter- and intra-assay CVs below 5%. High-sensitivity C-reactive protein (hs-CRP) concentrations were determined using an ELISA kit (LDN, Nordhorn, Germany) with inter- and intra-assay CVs less than 7%. Plasma total nitrite using Griess method (Tatsch et al., 2011), total antioxidant capacity (TAC) using ferric reducing antioxidant power method developed by Benzie and Strain (1996), total glutathione using the method of Beutler and Gelbart (1985), and malondialdehyde (MDA) concentrations by the thiobarbituric acid reactive substances spectrophotometric test (Janero, 1990) were assessed with inter- and intra-assay CVs less than 5%.

### 2.7 | Isolation of lymphocyte cells

Lymphocytes were extracted from blood samples of subjects with MMT at laboratory reference of Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan, Iran using 50% percoll solution (Sigma-Aldrich, Dorset, UK) by centrifugation for 20 min and 3000 rpm at 4°C. The cells of lymphocytes, which were at the interface of percoll and serum, were removed using a pasteur pipette and washed a few times with phosphate buffer saline. Samples were taken for cell count and viability testing by trypan blue, RNA, and DNA extraction.

### 2.8 | RNA extraction and real-time polymerase chain reaction

RNX-plus kit (Cinnacolon, Tehran, Iran) was used to extract RNA from blood samples. The total RNA was precipitated at room temperature for 15 min. The pellet including total RNA was washed using 75% ethanol and centrifuge at 7,500g for 8 min. After drying ethanol, the RNA pellet resuspended in 50  $\mu$ l or less of TE buffer. The concentration of total RNA was calculated based on OD 260/280 ration measurements as a means to means to address purity of RNA (Gmelig-Meyling & Waldmann, 1980). Following the extraction of the total RNAs from each sample, RNA quantification was performed by UV spectrophotometer. Each sample OD 260/280 ratio between 1.7 and 2.1 was intended that shows no contamination with both protein and DNA (Gmelig-Meyling & Waldmann, 1980). The first strand cDNA synthesis can be performed as an individual reaction or as a series of parallel reactions with different RNA templates (Gmelig-Meyling & Waldmann, 1980). The isolated RNA was reverse transcribed to cDNA library using Moloney murine leukemia virus reverse transcriptase.

Gene expressions of tumor necrosis factor alpha (TNF- $\alpha$ ), transforming growth factor beta (TGF- $\beta$ ), vascular endothelial growth factor (VEGF), peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ ), and low-density lipoprotein receptor (LDLR) were assessed by quantitative real-time polymerase chain reaction in peripheral blood mononuclear cells using the LightCycler technology (Roche Diagnostics, Rotkreuz, Switzerland) with SYBR green detection and Amplicon Kit (Table 1). Glyceraldehyde-3-phosphate dehydrogenase primers were used as a housekeeping gene. Primer Express Software

**TABLE 1** Specific primers used for real-time quantitative PCR

Gene	Primer	Product size (bp)	Annealing temperature (C)
GAPDH	F: AAGCTCATTTCTGGTATGACAACG R: TCTTCTCTTGCTCTTGCTGG	126	61.3
PPAR- $\gamma$	F: ATGACAGACCTCAGACAGATTG R: AATGTTGGCAGTGCTCAG	210	54
LDLR	F: ACTTACGGACAGACAGACAG R: GGCCACACATCCCATGATTC	223	57
TNF- $\alpha$	F: GTCAACCTCTCTCTGCCAT R: CCAAAGTAGACCTGCCAG	188	52
TGF- $\beta$	F: TTGAGACTTTCCGTTGCCG R: CGAGGTCTGGGAAAAGTCT	227	56
VEGF	F: CTTCTGAGTTGCCAGGAGA R: CTCACACACACAACCAGG	216	54

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LDLR, low-density lipoprotein receptor; PCR, polymerase chain reaction; PPAR- $\gamma$ , peroxisome proliferator-activated receptor gamma; TNF- $\alpha$ , tumor necrosis factor alpha; TGF- $\beta$ , transforming growth factor beta; VEGF, vascular endothelial growth factor.

(Applied Biosystems, Foster City, USA) and Beacon designer software (Takapost, Tehran, Iran) were used to design primers. Relative transcription levels were calculated using the method of Pfaffl (Gmelig-Meyling & Waldmann, 1980).

## 2.9 | Sample size

Sample size was calculated using the formula suggested for randomized clinical trials. Type 2 ( $\alpha$ ) and Type 2 errors ( $\beta$ ) were defined as 0.05 and 0.20 to have the study power of 80%. The sample size was calculated based on the impacts of crocin administration on BDI score, which was one of the primary outcomes. On the basis of the previously published study (Taleai et al., 2015), we used 2.50 as the effect size of BDI and 3.11 as SD. So 25 participants were required in each group. Considering 20% dropouts, the final sample size was 30 participants in each intervention group.

## 2.10 | Statistical analysis

The Kolmogorov-Smirnov test was done to determine the normality of data. To detect the differences in anthropometric measures and gene expression related to metabolic status between treatment groups, we used the independent samples *t* test. Multiple linear regression model was used to assess treatment effects on study outcomes after adjusting for baseline values of variables. The effect sizes were presented as the mean differences with 95% confidence intervals. *p* values < .05 were considered statistically significant. All statistical analyses were done using the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, Illinois, USA).

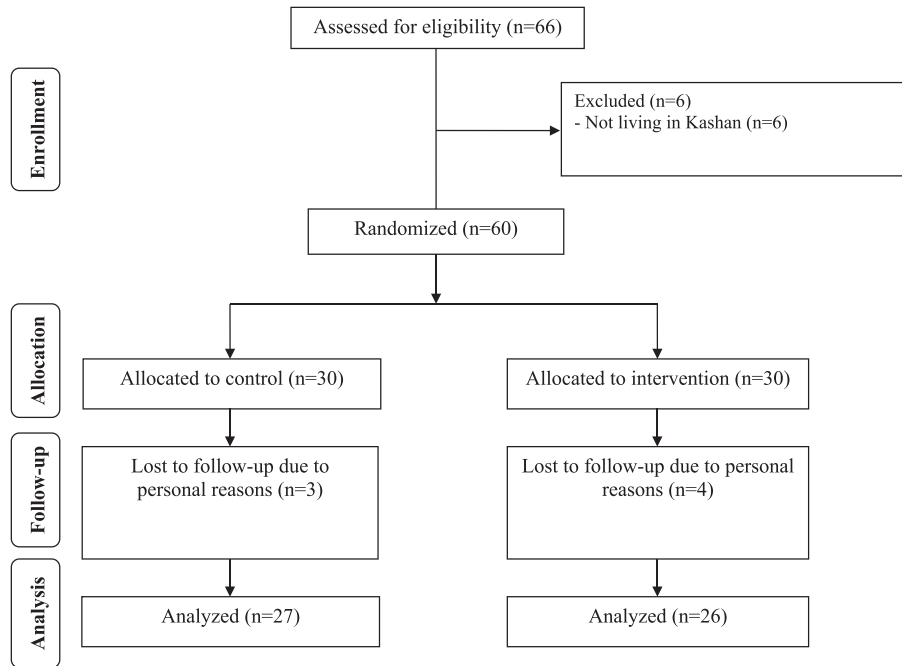
## 3 | RESULTS

Four individuals in the crocin group and three in the placebo group withdraw from the trial, due to personal reasons, and finally, 53 participants (crocin [*n* = 26] and placebo [*n* = 27]) completed the study (Figure 1). The compliance rate in our study was high; more than 90% of capsules were taken during the course of the trial in both groups. No side effects were reported following the consumption of crocin in patients under MMT.

Mean age, anthropometric measures, education, marital status, job, methadone dose, and duration of MMT at baseline and end of trial were not significantly different between crocin and placebo groups (Table 2).

Crocin administration significantly decreased BDI score ( $\beta$  = -2.70; 95% CI [-4.88, -0.53]; .01) and BAI score ( $\beta$  = -2.13; 95% CI [-3.67, -0.59]; .008) compared with the placebo (Table 3). In addition, crocin administration resulted in a significant reduction in FPG ( $\beta$  = -4.78 mg/dL; 95% CI [-7.88, -1.68]; .003), insulin levels ( $\beta$  = -1.09  $\mu$ IU/mL; 95% CI [-1.92, -0.26]; .01), HOMA-IR ( $\beta$  = -0.25; 95% CI [-0.44, -0.07]; .008), triglycerides ( $\beta$  = -21.10 mg/dL; 95% CI [-31.30, -10.91]; .001), very LDL (VLDL;  $\beta$  = -4.24 mg/dL; 95% CI [-6.26, -2.18]; .001), total cholesterol levels ( $\beta$  = -8.39 mg/dL; 95% CI [-16.21, -0.58]; .03), and a significant increase in QUICKI ( $\beta$  = 0.008; 95% CI [0.003, 0.01]; .003) compared with the placebo. Additionally, crocin intake was associated with a significant reduction in hs-CRP ( $\beta$  = -1.07 mg/L; 95% CI [-1.59, -0.54]; *P* < .001) and MDA ( $\beta$  = -0.58  $\mu$ mol/L; 95% CI [-0.90, -0.25]; .001) and a significant rise in TAC levels ( $\beta$  = 50.30 mmol/L; 95% CI [12.38, 88.22]; .01) compared with the placebo. Crocin administration did not affect other metabolic profiles.

Crocin intake upregulated PPAR- $\gamma$  (0.02) and LDLR (0.01) and downregulated gene expression of TNF- $\alpha$  (0.004) in peripheral blood



**FIGURE 1** Summary of patient flow diagram

mononuclear cells of subjects with MMT (Figures 2 and 3). Crocin supplementation did not affect gene expression of TGF- $\beta$  and VEGF.

## 4 | DISCUSSION

In this study, we investigated the effects of crocin on mental health, metabolic, and genetic profiles in subjects under MMT. We found that taking crocin for 8 weeks by subjects under MMT improved mental health, FPG, insulin, HOMA-IR, QUICKI, triglycerides, VLDL, total cholesterol, hs-CRP, TAC, and MDA levels; however, it did not have any effect on other metabolic profiles. In addition, crocin upregulated gene expression of PPAR- $\gamma$ , LDLR, and downregulated gene expression of TNF- $\alpha$  but did not affect TGF- $\beta$  and VEGF expression. Previous evidence has demonstrated that mental and metabolic disturbances were present in opioid use disorder (Gros, Milanak, Brady, & Back, 2013; Vallecillo et al., 2018). To our best knowledge, this evidence for the first time evaluating the effects of crocin on mental scale, metabolic status, and genetic profiles in patients under MMT. It must be kept in mind that in the present study, observed changes in measures of insulin metabolism and serum lipids in the crocin group compared with placebo group were clinically significant. However, observed difference at mental health parameters and few biomarkers of inflammation and oxidative stress in our study was statistically significant; it was not clinically significant. Long-term interventions and higher dosage of crocin might result in greater changes in these variables.

### 4.1 | Effects on depression and anxiety

MMT is usually linked into some complications including anxiety and depression (Callaly et al., 2001; Ghaderi et al., 2019; Yin et al.,

2015). Nutritional status and dietary administration of subjects are important factors affecting the development of psychiatric and mental health disturbance (Lim et al., 2016). Opiate abuse significantly impacts metabolic status and the nutritional of users and leads to undernourishment (Forrester, Tucker, & Gorbach, 2004). Several pieces of evidence have reported that drug abuse changes their food intake and nutritional deficiencies (Nazrul Islam, Jahangir Hossain, Ahmed, & Ahsan, 2002; Saeland et al., 2009). It has been documented that saffron was used as a food or spicy plant product for nutritional status and dietary intake (Gohari, Saeidnia, & Mahmoodabadi, 2013). Previously, it was reported that saffron consumption had beneficial effect on mental health parameters in subjects with mental health problems (Shafiee, Arekhi, Omranzadeh, & Sahebkar, 2018). In our previous work, crocin intake at a dosage of 30 mg/day for 8 weeks had ameliorating effects on psychological status in subjects under MMT (Khalatbari-Mohseni et al., 2019). In the study by Jam et al. (2017), consuming crocin of 30 mg/day for 8 weeks by subjects with metabolic syndrome had favorable effects on depression score. Also, crocin administration (30 mg/day) in major depressive for 4 weeks improved depression (Talaie et al., 2015). In another study, after *Crocus sativus* L. administration at a dosage of 30 mg/day for 8 weeks, the median value of depression and anxiety was significantly decreased in woman with postpartum depressive disorder (Tabeshpour et al., 2017). However, saffron supplementation did not affect BDI scale in major depression (Sahraian et al., 2016). All the health effects saffron might have relationship with a synergistic function of several constituents, including crocin, safranal, picrocrocine, and flavonoids (Erfanparast, Tamaddonfard, Taati, & Dabbaghi, 2015). The accurate mechanism of action of crocin in the brain and its effects on psychological parameters is unidentified. Saffron may inhibit reuptake of monoamine neurotransmitters, including norepinephrine, dopamine,

**TABLE 2** General characteristics of the study participants

Characteristics	Placebo group (n = 27)	Crocin group (n = 26)	p <sup>ab</sup>
Age (y)	45.6 ± 9.9	44.5 ± 9.4	.66
Height (cm)	173.7 ± 9.5	172.2 ± 9.0	.56
Weight at study baseline (kg)	75.8 ± 13.0	72.6 ± 12.7	.37
Weight at the end of trial (kg)	76.1 ± 13.1	72.7 ± 13.4	.34
Weight change (kg)	0.3 ± 2.0	0.1 ± 1.9	.63
BMI at study baseline (kg/m <sup>b</sup> )	25.2 ± 4.2	24.5 ± 4.4	.60
BMI at the end of trial (kg/m <sup>b</sup> )	25.3 ± 4.2	24.6 ± 4.6	.56
BMI change (kg/m <sup>b</sup> )	0.1 ± 0.6	0.02 ± 0.6	.68
Education (%)			
Illiterate	6 (22.2)	8 (30.8)	.59 <sup>b</sup>
Elementary	8 (29.6)	9 (34.6)	
Intermediate	13 (48.1)	9 (34.6)	
Marital status (%)			
Single	18 (66.7)	12 (46.2)	.32 <sup>b</sup>
Married	4 (14.8)	6 (23.1)	
Widow/divorced	5 (18.5)	8 (30.8)	
Job (%)			
Unemployed	20 (74.1)	14 (53.8)	.28 <sup>b</sup>
Employed	1 (3.7)	1 (3.8)	
Others	6 (22.2)	11 (42.3)	
Methadone dose (mL/d)	16.5 ± 4.5	15.8 ± 4.7	.57
Duration of MMT (y)	5.2 ± 2.5	4.8 ± 2.2	.46

Note. Data are mean ± SDs.

Abbreviations: BMI, body mass index; MMT, methadone maintenance treatment.

<sup>a</sup>Obtained from independent *t* test.

<sup>b</sup>Obtained from Pearson chi-square test.

and serotonin in synapses (Schmidt, Betti, & Hensel, 2007). Also, the effects saffron on psychological score may provide via its antioxidant effects and inhibiting proinflammatory parameters and free radicals (Hausenblas, Saha, Dubyak, & Anton, 2013; Wang et al., 2010).

## 4.2 | Effects on glycemic control and serum lipids

Impaired metabolism insulin, high blood pressure, overweight, and changes in serum lipids in patients under MMT can result in appearance side effects long-term, metabolic abnormalities, infectious complications, chronic obstructive pulmonary disease, risk of drug-related mortality, and the progression to T2DM (Cousins et al., 2011; Maruyama, Macdonald, Borycki, & Zhao, 2013; Vallecillo et al., 2018). In addition, increased serum lipids in patients under MMT would result in enhanced risk of cardiac rhythm disturbance linked into QTc interval prolongation by serving as substrates for excessive production of free radicals and lipid peroxidation, which has neurotoxic effects (Fonseca et al., 2009; Mannelli et al., 2009; Tsai & Huang,

2017). Therefore, crocin administration due to their useful impacts on glycemic status and lipid parameters may be useful to metabolic disorder. Our study demonstrated that taking crocin for 8 weeks in patients under MMT resulted in a significant reduction in FPG, insulin, HOMA-IR, QUICKI, triglycerides, VLDL, and total cholesterol levels; however, it did not influence on other metabolic profiles. In addition, crocin upregulated gene expression of PPAR- $\gamma$  and LDLR. Data documenting the effects of crocin supplementation on glycemic control and lipid profiles in patients under MMT are limited. In an animal study by Shirali, Zahra Bathaie, and Nakhjavani (2013), the injection of crocin (50 or 100 mg/kg) significantly reduced insulin resistance, triglycerides, total and LDL cholesterol, and enhanced high-density lipoprotein (HDL) cholesterol levels in diabetic rats. Recently, it was reported that saffron administration might lead to stable glucose levels, total cholesterol, and triglycerides (Doumouchtsis et al., 2018). Azimi, Ghiasvand, Feizi, Hariri, and Abbasi (2014), shown that herbal medicines (e.g., saffron, cardamom, and ginger) for 8 weeks in patients with T2DM had beneficial effects on lipid profiles but did not affect glycemic control. In another study, after saffron intake for 12 weeks in patients with

**TABLE 3** The effect of crocin administration on mental health parameters and metabolic status in methadone maintenance treatment subjects

Variables	Placebo group (n = 27)		Crocin group (n = 26)		Difference in outcome measures between crocin and placebo treatment groups <sup>a</sup>	
	Baseline	Week 8	Baseline	Week 8	$\beta$ (95% CI)	$p^b$
BDI score	24.1 ± 9.4	22.0 ± 9.6	26.5 ± 9.2	21.4 ± 8.1	-2.70 [-4.88, -0.53]	.01
BAI score	19.6 ± 5.8	18.3 ± 6.1	20.6 ± 6.7	17.0 ± 5.8	-2.13 [-3.67, -0.59]	.008
FPG (mg/dL)	90.2 ± 9.5	90.6 ± 10.1	91.1 ± 11.2	86.5 ± 9.5	-4.78 [-7.88, -1.68]	.003
Insulin ( $\mu$ IU/mL)	11.0 ± 2.1	10.6 ± 2.3	11.8 ± 2.6	10.1 ± 2.4	-1.09 [-1.92, -0.26]	.01
HOMA-IR	2.5 ± 0.6	2.3 ± 0.6	2.7 ± 0.7	2.3 ± 0.5	-0.25 [-0.44, -0.07]	.008
QUICKI	0.33 ± 0.01	0.33 ± 0.01	0.33 ± 0.01	0.34 ± 0.01	0.008 [0.003, 0.01]	.003
Triglycerides (mg/dL)	148.9 ± 57.6	151.5 ± 57.1	169.0 ± 57.1	148.8 ± 44.2	-21.10 [-31.3, -10.91]	.001
VLDL cholesterol (mg/dL)	29.8 ± 11.5	30.3 ± 11.4	33.8 ± 9.4	29.8 ± 8.8	-4.22 [-6.26, -2.18]	.001
Total cholesterol (mg/dL)	186.0 ± 37.8	189.9 ± 37.6	196.8 ± 39.0	191.4 ± 37.8	-8.39 [-16.21, -0.58]	.03
LDL cholesterol (mg/dL)	112.2 ± 36.9	112.7 ± 37.7	117.5 ± 36.0	115.1 ± 33.7	-2.44 [-9.49, 4.60]	.49
HDL cholesterol (mg/dL)	44.0 ± 7.7	46.9 ± 8.9	45.5 ± 6.4	46.6 ± 6.5	-1.65 [-4.47, 1.17]	.24
Total/HDL cholesterol ratio	4.3 ± 1.0	4.2 ± 1.1	4.4 ± 0.9	4.1 ± 0.82	-0.08 [-0.30, 0.13]	.43
hs-CRP (mg/L)	4.3 ± 1.4	4.6 ± 1.5	5.1 ± 1.7	4.3 ± 2.1	-1.07 [-1.59, -0.54]	<.001
Total nitrite ( $\mu$ mol/L)	37.8 ± 4.8	39.2 ± 5.4	38.4 ± 4.9	38.7 ± 5.2	-1.12 [-2.38, 0.13]	.07
TAC (mmol/L)	1009.1 ± 142.1	994.5 ± 141.4	932.8 ± 105.1	985.1 ± 86.5	50.30 [12.38, 88.22]	.01
GSH ( $\mu$ mol/L)	727.1 ± 190.9	748.5 ± 213.5	665.1 ± 119.1	738.3 ± 118.9	45.85 [-8.70, 100.42]	.09
MDA ( $\mu$ mol/L)	2.8 ± 1.3	2.9 ± 1.2	2.9 ± 1.0	2.5 ± 1.0	-0.58 [-0.90, -0.25]	.001

Note. Data are mean  $\pm$  SDs.

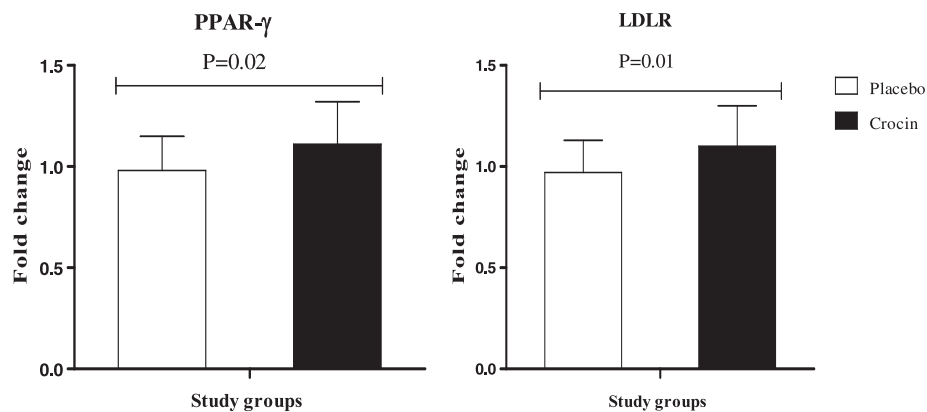
Abbreviations: BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory; FPG, fasting plasma glucose; GSH, total glutathione; HOMA-IR, homeostasis model of assessment-insulin resistance; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; MDA, malondialdehyde; QUICKI, quantitative insulin sensitivity check index; VLDL, very low-density lipoprotein; TAC, total antioxidant capacity.

<sup>a</sup>“Outcome measures” refers to the change in values of measures of interest between baseline and Week 8.  $\beta$  (difference in the mean outcomes measures between treatment groups [crocin group = 1 and placebo group = 0]).

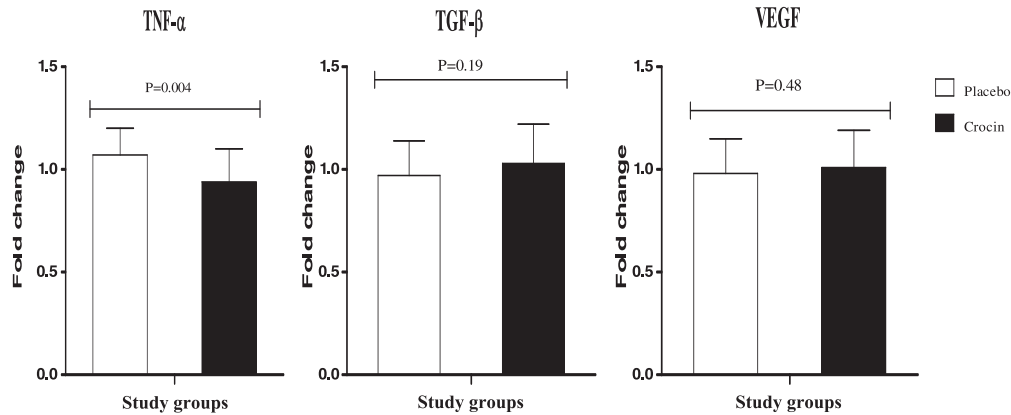
<sup>b</sup>Obtained from multiple regression model (adjusted for baseline values of each variable).

T2DM, it had a significant improvement on glycemic control and serum lipids (Moravej Aleali et al., 2019). Also, Javandoost et al. (2017) demonstrated that 30 mg/day crocin consumption for 8 weeks in subjects with metabolic syndrome was associated with a significant rise in high-density lipoprotein cholesterol levels but did not affect fasting glucose and other lipid profiles. However, saffron

supplementation failed to detect any significant effect on lipid profiles in patients with severe depression (Sahraian et al., 2016). Moreover, a meta-analysis study conducted by Pourmasoumi et al. (2019) did not show any significant change in insulin and serum lipids following saffron supplementation. Several possible mechanisms have been suggested to be involved in the hypoglycemic effect, regulation of



**FIGURE 2** Fold change (means  $\pm$  SDs) in gene expression levels of peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ ) and low-density lipoprotein receptor (LDLR) in patients with methadone maintenance treatment who were received crocin supplements and placebo.  $p$  value was obtained from independent t test



**FIGURE 3** Change (means  $\pm$  SDs) in gene expression levels of tumor necrosis factor alpha (TNF- $\alpha$ ), transforming growth factor beta (TGF- $\beta$ ), and vascular endothelial growth factor (VEGF) in patients with methadone maintenance treatment who were received crocin supplements and placebo. *p* value was obtained from independent *t* test

serum insulin, and lipid metabolism by saffron intake. The hypoglycemic effect of saffron might correlated with glucose-stimulated insulin secretion, antioxidant potential, the inhibition of intestinal glucose absorption, and protein glycation (Farkhondeh & Samarghandian, 2014; Shirali et al., 2013). Also, saffron and active constituents might have key role in improving lipid profiles through several mechanisms such as the activation of peroxisome proliferator-activated receptor alpha, enhancing the status of adiponectin, lipid-lowering, antioxidant, and modulatory effects on the heat shock proteins (Razavi & Hosseinzadeh, 2017).

### 4.3 | Effects on biomarkers of inflammation and oxidative stress

In subjects undergoing MMT programs, there is an increase in oxidative imbalance (Salarian et al., 2018), and the balance between free radical content and antioxidants capacity is eliminated. ROS enhances matrix metalloproteinase activity and upregulates inflammatory cytokines (Rajagopalan, Meng, Ramasamy, Harrison, & Galis, 1996). Oxidative imbalance can damage neurons through altered cell signaling and lipid peroxidation (Salarian et al., 2018). Opioid and methadone abuse alter redox (oxidation–reduction) activity and induce receptor endocytosis (Koch et al., 2005). Also, recent evidence showed that increasing oxidative damage and upregulating inflammatory parameters are involved in the pathophysiology subjects under MMT program and opioid use (Chan et al., 2015; Mannelli et al., 2009; Salarian et al., 2018). Therefore, crocin supplementation can be the favorable effects on biomarkers of inflammation and oxidative stress. We found that subjects under MMT program who were supplemented with crocin for 8 weeks had a significant reduction in hs-CRP and MDA and a significant elevation in TAC levels but did not improve total nitrite and total glutathione levels. In addition, crocin downregulated TNF- $\alpha$  expression but did not influence TGF- $\beta$  and VEGF expression. Previously, the role of saffron and its active components has been demonstrated on biomarkers of inflammation and oxidative stress in animal and human models. In

animal models with ischemic stroke, crocin (60 mg/kg) significantly decreased MDA values and enhanced activity of superoxide dismutase and glutathione levels (Vakili, Einali, & Bandegi, 2014). In addition, crocin intake downregulated gene expression of TNF- $\alpha$ , interleukin (IL) 8, and IL-10 and significantly suppressed oxidative stress (S. Li et al., 2017). Furthermore, receiving saffron and crocin (30 mg daily) for 12 weeks was correlated with improving metabolic syndrome in patients with schizophrenia (Fadai et al., 2014). Also, reduced production of NO, TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 in rheumatoid arthritis has been demonstrated following the administration of crocin (Li, Jiang, & Zhu, 2017). However, evidence studies have demonstrated that herbal remedies administration had no significant effect on oxidative stress and inflammation patients with T2DM (Azimi et al., 2014). Moreover, Nosrati et al. (2017) observed that crocin intake at a dosage of 15 mg twice a day for 8 weeks did not affect any significant effect on hs-CRP and anti-HSP27 titers in subjects with metabolic syndrome. The evidence demonstrated that saffron might be considered as a preventive or therapeutic agent against oxidative damage and inflammatory markers through several mechanisms, including modulating pro-oxidant–antioxidant balance (Kermani et al., 2015), scavenging ROS, the inhibition of nuclear factor kappa B activation and TNF- $\alpha$  production, decreased lipid peroxidation, enhanced antioxidant capacity, and the activation of PI3K/Akt signaling pathway (Razavi & Hosseinzadeh, 2017; Thushara et al., 2014; Yang et al., 2017; Yarijani et al., 2017). It has also been demonstrated that crocin reduces DNA damage and cell necrosis and prevents the death of PC-12 cells through sphingomyelinase–ceramide signaling by enhancing glutathione synthesis (H. Hosseinzadeh, Abootorabi, & Sadeghnia, 2008; Ochiai et al., 2004). In addition, several reports showed that crocin has different properties on clinical and metabolic disturbance including increases in mRNA and brain-derived neurotrophic factor, protein status of VGF neuropeptide, cAMP response element binding (Dorri et al., 2015; Razavi et al., 2017), downregulation of mitogen-activated protein kinase, mitogen-activated protein kinase-activated protein signaling pathway, miRNA-122 expression (Vahdati Hassani, Mehri, Abnous, Birner-Gruenberger, & Hosseinzadeh, 2017), inhibiting apoptosis



(Razavi, Hosseinzadeh, Abnous, Khoei, & Imenshahidi, 2016), decreased TNF- $\alpha$  and IL-6 levels in striatum (L. Mohammadzadeh, Hosseinzadeh, Abnous, & Razavi, 2018), modulates IL-4/IL-13 signaling (Yosri, Elkashef, Said, & Gameil, 2017), inhibiting TAU protein hyperphosphorylation, and antiapoptotic effects (Leila Mohammadzadeh, Abnous, Razavi, & Hosseinzadeh, 2019), alteration of gene expression profile of T24 (transitional cell carcinoma of bladder) cell (Lv, Luo, Ji, & Zhao, 2008), and regulate the expression of Bcl-2 with simultaneous up regulation of pro apoptotic Bax in MCF-7 cells (Bakshi, Hakkim, & Sam, 2016).

#### 4.4 | Limitations

The present study had some limitations such as long-term intervention is associated with illustrate better effects. In addition, we were not able to investigate the cognitive functions, craving, and withdrawal syndrome in subjects under MMT program. Thus, its performance is suggested in next studies. Also, we did not evaluate the effects of crocin administration on urinary or/and serum crocin.

#### 4.5 | Conclusions

Overall, taking crocin supplements by patients undergoing MMT program had ameliorating effects on mental health scale, metabolic, and genetic parameters. Further studies are needed to demonstrate the relative impact of crocin on patients undergoing MMT program. Crocin could be introduced as an adjunct to MMT program in clinical guidelines for withdrawal protocols because it enhances quality of life and diminishes MMT program side effects.

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#### CONFLICT OF INTEREST

None.

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#### AUTHOR CONTRIBUTIONS

AGh and ZA contributed in design, conception, and statistical analysis. AGh, MR-A, NV, H-RB, AS, FSGH, and AO contributed in data collection and manuscript drafting.

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