Effects of Crocin on Diabetic Maculopathy: A Placebo-Controlled Randomized Clinical Trial

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• OBJECTIVE: Diabetic macular edema (DME) is one of the most important sight-threatening complications in patients with diabetes. Owing to neuroprotective properties, crocin, as the main constituent in saffron, is thought to be useful in the treatment and prevention of diabetic maculopathy. The aim of this trial was to evaluate the effects of crocin as a supplement on reducing inflammation in patients with diabetic maculopathy.

• DESIGN: Double-masked, placebo controlled, phase 2 randomized clinical trial.

• METHODS: PARTICIPANTS: In this study, 101 eyes of 60 patients with refractory diabetic maculopathy to conventional therapy including macular photocoagulation and intravitreal injection of anti–vascular endothelial growth factor agent (bevacizumab) with or without steroid (triamcinolone) were studied in 3 groups. INTERVENTION: Patients in the crocin groups received 5 mg or 15 mg crocin tablets per day for 3 months, whereas patients in the placebo group received 1 placebo tablet per day during the study. The best-corrected visual acuity (BCVA) and central macular thickness (CMT) were measured before, every month during, and 3 months after intervention. Biochemical blood tests were also evaluated before and after trial. MAIN OUTCOME MEASURES: The BCVA and CMT were evaluated as the primary outcomes, whereas HbA1c and fasting blood sugar (FBS) were studied as the secondary outcomes in this trial.

• RESULTS: One hundred and one eyes were enrolled in this trial and were divided into 3 groups (crocin 5 mg, n = 34; crocin 15 mg, n = 33; and placebo, n = 34). According to our data, administration of crocin 15 mg tablet per day could significantly decrease HbA1c (*P* value = .024; 95% confidence interval [CI] 0.3-0.96), and CMT (*P* value = .005; 95% CI, 32.75-126.99) and improve BCVA (logMAR changes; *P* value = .012; 95% CI, 0.23-0.69) compared to the placebo group. Although administration of crocin 5 mg tablet per day could clinically improve HbA1c, FBS, CMT, and BCVA, the difference was not significant compared to the placebo group.

• CONCLUSION: This study indicated the effect of crocin as a potent antioxidant and neuroprotective for treatment of refractory DME in the short term; however, the clinical significance is yet to be proved in a study with larger sample size and longer duration of follow-up and also in treatment-naïve patients. (Am J Ophthalmol 2018;190:89–98. © 2018 Elsevier Inc. All rights reserved.)

DIABETIC MACULAR EDEMA (DME) IS AN IMPORtant cause of vision loss in diabetic patients, which can result from diabetic retinopathy.[1,2] Diabetes and high plasma glucose level can damage capillaries, leading to leakage of blood and fluids in the retina.[3–5] Changes resulting from diabetes can affect the macula and lead to DME or diabetic maculopathy.[1,4] The diagnosis of macular edema is based on the retinal thickening observed on slit-lamp examination or on optical coherence tomography (OCT).[6,7]

In the treatment of DME, coupled with intensive systemic control of diabetes, hypertension, and hyperlipidemia, ocular treatment modalities should be initiated. In the past few years, given the central role of vascular endothelial growth factor (VEGF) in the pathogenesis of DME, the treatment modalities of DME have been changed from macular laser photocoagulation to local intravitreal injection of anti-VEGF. Other treatment strategies including intravitreal steroids, as well as oral and topical nonsteroidal anti-inflammatory drugs, have been proposed for the treatment of macular edema.[8,9] The use of drugs for the treatment of diabetic maculopathy continues, but owing to the side effects of medications and unwillingness of some patients to undergo intravitreal injection, the use of supplements of plant origin can be useful in this regard.[10]

The conventional therapeutic strategies for DME include macular laser photocoagulation, which severely reduces vision loss, and anti-VEGFs such as ranibizumab, aflibercept, and bevacizumab.[11] Nowadays, traditional medicine

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and herbal medicines are considered a promising choice for the treatment of various diseases.[12] Animal models have shown that saffron (*Crocus sativus* L.), an important medicinal plant with a broad range of therapeutic properties, has analgesic, anti-inflammatory, and antioxidant properties. Studies on the therapeutic effects of saffron extract in age-related macular degeneration (AMD) presented that daily intake of 20 mg of saffron extract significantly improves focal electroretinogram (fERG) and best-corrected visual acuity (BCVA); it can also induce macular function.[13,14] Further, saffron can increase blood flow to the retina.[10,15,16] Anti-inflammatory and antioxidant effects of saffron are attributed to crocin, the active ingredient of saffron.[10] Considering that crocin has antioxidant, anti-inflammatory, and neuroprotective effects and increases retinal blood flow, we performed this randomized, placebo-controlled clinical trial to ascertain the effects of crocin, as a supplement to the standard treatments, on diabetic retinopathy in patients with refractory DME.

METHODS

• **SUBJECTS AND STUDY DESIGN:** This clinical trial was approved by the Ethics Committee of Mashhad University of Medical Sciences and registered to the Iranian Registry of Clinical Trials (IRCT2015062113058N2 submission code). The study was then registered on the institutional review board organization (IORG) and accepted by the Office for Human Research Protections (code: IORG0009480). Informed consent was obtained from all the patients before entering this study.

DME patients who were poorly responsive to the standard treatment of macular photocoagulation (MPC) and anti-VEGF in the retina service of Khatam-Al-Anbia Eye Hospital, Mashhad University of Medical Sciences, Mashhad, Iran, were studied from August 2015 to May 2016. For randomization, a randomized code number was obtained from Microsoft Excel for each pillbox (treatment and control groups). The inclusion criteria included patients aged ≥18 years with type 1 or 2 diabetes and refractory DME (according to the Early Treatment Diabetic Retinopathy Study criteria), who were unresponsive to at least 3 intravitreal injections of bevacizumab (Avastin; Roche, USA), at least 1 intravitreal triamcinolone injection (IVT), and MPC. All the enrolled patients were receiving intravitreal anti-VEGF injection for several years (with a minimum of 4 years) and their resistance to intravitreal anti-VEGF injection therapy, IVT, and MPC was proven by a retina specialist. The criteria for anti-VEGF resistance were central macular thickness (CMT) more than 296 μm and the presence of intraretinal and subretinal fluids after intravitreal injection with/without MPC. For uniformity of the injection conditions and standardization, the injection in all patients was performed by the same specialist during the 1:00 PM to 3:00 PM time period. The patients received 1 anti-VEGF injection each month during the trial, except those who did not accept intraocular injection. Triamcinolone was administered only in patients who showed no CMT decrease in the first visit 1 week after Avastin injection. Intraocular injection of triamcinolone was applied only in 2 patients (10%) in the crocin 5 mg group, no patients (0%) in the crocin 15 mg group, and 1 patient (5%) in the placebo group. Thus, it had no significant effect on the final results of the study.

The participants included patients in whom CMT in OCT was above 296 μm, had controlled diabetes mellitus (HbA1c ≤ 8.5 over the past 3 months), and had the ability to understand the overview of the study, treatment, and possible side effects.

The exclusion criteria were as follows: (1) diagnosis of other eye abnormalities such as glaucoma and optic neuropathies, which cause no improvement in visual acuity despite improvement in macular edema; (2) other causes of macular edema (retinal vein occlusion, uveitis, and epiretinal membrane); (3) patients with anterior segment abnormalities of the eye (cornea, anterior chamber, iris, or lens); (4) history of vitrectomy or other intraocular surgeries within the past 3 months; (5) deposition of subfoveal hard exudates; (6) pregnancy and breastfeeding; (7) vitreous hemorrhage; and (8) laser therapy, including panretinal photocoagulation (PRP) or MPC, during the past 3 months.

• **SAMPLE SIZE:** SigmaPlot version 12.0 (Systat software, Inc., Germany) was applied for sample size calculation. To have a power of 90%, a significance level of .05, an assumed standard deviation of 45 μm, and a 10% minimum detectable mean difference (40 μm) in CMT changes, a minimum sample size of 32 eyes for each arm was calculated.

Ninety-seven participants were included in this clinical trial (Figure 1). Twenty-nine patients were excluded owing to history of vitrectomy, glaucoma, vitreous hemorrhage, and laser therapy, and ultimately 68 patients were enrolled in this clinical trial. The patients were randomly divided into 3 groups: 2 treatment groups (23 patients in crocin 15 mg and 22 patients in crocin 5 mg) and 1 control group (23 patients in placebo). All the patients received the medications for 3 months.

• **CROCIN EXTRACTION AND TABLET PREPARATION:** The powdered saffron stigmas were purchased from Saharkhiz Saffron Company, Mashhad, Iran. The extraction and crystallization of crocin were performed according to our previous study.[17] Crocin is the glycosylated derivative of crocetin (a natural apocarotenoid dicarboxylic acid) found in saffron stigmas. There are 6 forms of crocin with different glycosylations. The main form of crocin is crocetin digentiobioside (about 70% of the total crocin extract).

In our crystallization method, a mixture of total crocins was extracted and used for tablet preparation. The term

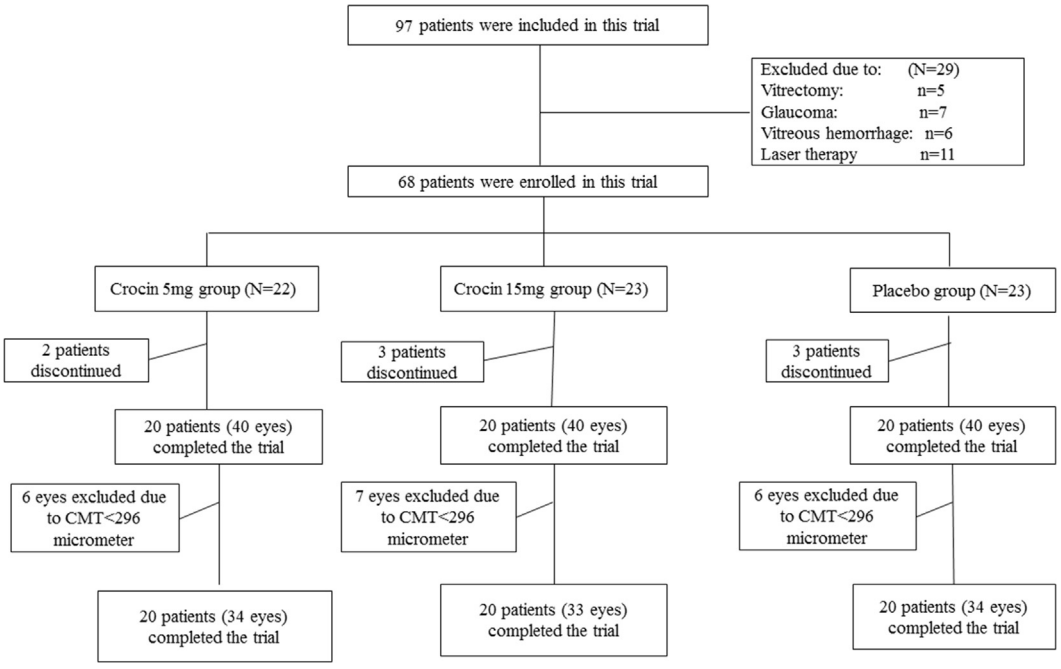

FIGURE 1. Flowchart illustrating patient inclusion/exclusion and group distribution of 101 eyes enrolled in the clinical trial. There were 34 eyes in the crocin 5 mg, 33 eyes in the crocin 15 mg, and 34 eyes in the placebo group.

"crocin" has been used instead of total crocins in this study.[18,19] Briefly, saffron stigmas were powdered, suspended in water/ethanol (20:80 vol/vol), shaken vigorously, and centrifuged (4000 rpm, 10 min). The supernatants were then collected in a glass container and stored at -20°C for crystallization. Then, the crystals were collected after 45 days and dried in the darkness at room temperature. The crocin and placebo tablets were prepared in the pharmaceutical laboratory of the School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran. Two different doses (5 and 15 mg) of crocin tablets were used in this study.

• **OPHTHALMIC AND BIOCHEMICAL EXAMINATIONS:** The enrolled patients underwent a complete ophthalmic examination by an ophthalmologist subspecialized in vitreoretinal diseases. The CMT (using OCT Spectralis; Heidelberg Inc., USA) and BCVA (LED visual chart; Medizs Inc., Korea) were measured before and during monthly follow-up. BCVA was reported as logarithm of minimum angle of resolution (logMAR) plot. To reduce the effect of diurnal variation on macular thickness measurement, all the scans were performed between 8 and 12 AM. An ophthalmologist (M.N.) checked all the OCT recordings for possible segmentation errors. The segmentation lines were corrected manually when indicated. The complete biochemical blood test, including high-density lipoprotein (HDL), low-density lipoprotein (LDL), cholesterol (Chol), triglyceride (TG), fasting blood sugar (FBS), glycated hemoglobin A1c (HbA1c), aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatine (Cr), calcium (Ca), phosphorus (Phos), sodium (Na), and potassium (K), was evaluated for all the patients before and 3 months after the intervention.

• **MASKING:** Crocin and placebo tablets were prepared in a similar shape, color, and size; stored in a dark container; and coded by a pharmacist. The treating physician, researcher, and patients were not aware of the code printed on the container.

• **SAFETY:** Patients were followed and questioned for all illnesses, adverse effects, and hospitalizations that occurred during the trial. The complications for interventions other than the main intervention (crocin), including the complications of intravitreal injection in the patients, were reported in both the treatment and placebo groups. The safety assessment, which included physical examination, evaluation of vision, blood pressure, high glucose, routine laboratory tests, and any possible adverse events, was performed at the baseline, during the study, and 3 months after the trial. Also, the patients were asked about the possible side effects of the tablets (crocin and placebo) during the study via phone calls every week. Liver and kidney function tests, as well as metabolic states, were evaluated before and immediately after stopping treatment to detect any possible complications.

• **STATISTICAL ANALYSIS:** Normality of the data was evaluated by Kolmogorov-Smirnov test. Two-way ANOVA

TABLE 1. Demographic Information of 60 Participants (101 Eyes) in This Study

Variable	Group			*P* Value		
	Crocin 5 mg	Crocin 15 mg	Placebo	Crocin 5 mg vs Placebo	Crocin 15 mg vs Placebo	Crocin 5 mg vs Crocin 15 mg
Age, y (mean ± SD)	54.31 ± 6.6	56.09 ± 4.3	57.17 ± 2.9	.74	1.00	1.00
Sex ratio (male/female)	6/14	13/7	10/10	.45	.33	.09
Duration of diabetes, mo (mean ± SD)	216.7 ± 21.9	223.56 ± 18.9	234.07 ± 16.91	.52	.71	.84
Duration of diabetes treatment, mo (mean ± SD)	168.31 ± 11.5	201.12 ± 13.3	213.2 ± 12.1	.46	.63	.55
History of diabetes in family (%)	70%	65%	55%	.48	.73	.98
Type of diabetes (type 1/type 2)	2/18	4/16	4/16	.45	-	.45
Duration of DME, mo (mean ± SD)	72.4 ± 6.7	76.18 ± 9.8	71.9 ± 7.6	.46	.26	.39
Duration of treatment of DME, mo (mean ± SD)	60.2 ± 8.2	66.17 ± 9.8	58.17 ± 7.4	.34	.12	.23
Smoking (%)	45%	55%	50%	.64	.62	.48
Bevacizumab (%)	100%	100%	100%	-	-	-
Average number of bevacizumab injections during trial	2.35 ± 0.26	2.45 ± 0.23	2.55 ± 0.21	1.00	1.00	1.00
Triamcinolone (%)	10%	0	5%	.77	.79	.59
Type of antidiabetic drug						
Metformin/glibenclamide	80%	78%	77%	1.00	1.00	.97
Insulin	17%	19%	20%	.98	1.00	1.00

DME = diabetic macular edema.

followed by Bonferroni post hoc test was performed for the evaluation of the data obtained in CMT and BCVA examinations. One-way ANOVA followed by Tukey post hoc test was applied for inter-group statistical analysis of the data obtained in biochemical experiments (eg, HbA1c and FBS). For better evaluation, changes in each parameter value, before and after the intervention, were considered for statistical analysis. Also, paired *t* test was used for changes in secondary outcomes (eg, HbA1c and FBS) within each group, whereas repeated-measures ANOVA followed by Bonferroni post hoc test was performed for primary outcomes (CMT and BCVA) for statistical evaluation of the data within each group during the intervention. Categorical data (presented in demographic and side effect tables) were compared using Fisher exact test. The variables were reported as mean ± standard error of the mean (SEM). *P* value less than .05 was considered statistically significant.

RESULTS

• **SUBJECTS:** Sixty-eight patients were enrolled in this clinical trial; 8 patients were excluded owing to stomachache (1 patient in crocin 5 mg group), swelling in the feet (1 patient in crocin 5 mg and 1 patient in placebo group), incomplete follow-up (2 patients in crocin 15 mg and 2 patients in placebo group), and previous kidney failure and the need for dialysis (1 patient in crcoin 15 mg). Ultimately, 60 refractory DME cases were recruited into this study; 20 patients (40 eyes) were assigned to each group. Then, 19 eyes were excluded from this trial because of a CMT value < 296 μm; thus, 101 eyes with CMT more than 296 μm were enrolled (Figure 1) (the cause of this exclusion was having no clinically significant macular edema in the first clinical visit). Thirty-three eyes (from 20 patients) were in the crocin 15 mg, 34 eyes (from 20 patients) in the crocin 5 mg, and 34 eyes (from 20 patients) in the placebo group. According to the previous 6 months' medical records of patients before the clinical trial, the number of anti-VEGF injections in the placebo group, crocin 5 mg group, and crocin 15 mg group were 7.9 ± 1.2, 5.8 ± 0.9, and 7.8 ± 1.1, respectively. Statistical analysis did not show significant difference between the crocin-treated groups and placebo regarding the number of previous injections ($P > .05$). Also, there was an average interval of 45 days between the last injection of anti-VEGF and enrollment into the study. The average numbers of anti-VEGF therapies in each group is reported in Table 1. No statistically significant difference was observed between groups.

• **DEMOGRAPHIC INFORMATION OF THE PARTICIPANTS:** The age of the patients ranged between 41 and 82 years, and 48.33% (29) of the patients were male and 51.67% (31) were female. According to the demographic data, no significant difference was observed between the groups in

TABLE 2. Frequency of Side Effects Reported in Treatment and Placebo Groups

Reported Side Effect	Crocin Treatment Group: Crocin 5 mg	Crocin 15 mg	Placebo	*P* Value
Swelling of feet	1	0	1	5 mg vs placebo 1.31 15 mg vs placebo 1.00
Stomach ache	1	0	0	5 mg vs placebo 1.00 15 mg vs placebo -
Increased appetite	2	2	0	5 mg vs placebo 1.00 15 mg vs placebo 1.00
Burning of the eyes	2	0	1	5 mg vs placebo 1.00 15 mg vs placebo 1.00
Eye redness	0	1	1	5 mg vs placebo 1.00 15 mg vs placebo 1.00
Swelling of the eyes	1	1	1	5 mg vs placebo 1.00 15 mg vs placebo 1.00
Subconjunctival hemorrhage	1	2	2	5 mg vs placebo 1.00 15 mg vs placebo 1.00

the mean age, sex, duration of DME, duration of DME treatment, family history of diabetes, smoking status, or types of administered drugs. The demographic data of the patients are summarized in Table 1.

• **SAFETY AND SIDE EFFECTS:** Results showed some side effects, such as increased appetite, swelling of feet, stomach ache, subconjunctival hemorrhage, and swelling, redness, and burning of the eyes, associated with pain in some cases (Table 2). Two patients, in the crocin 5 mg group, complained of foot swelling and stomach ache. One patient in the placebo group suffered from swelling of the feet and 4 patients in the crocin groups complained of increased appetite. No statistical significance was observed between groups for the abovementioned side effects. Also, according to our records, 1 patient in the crocin 15 mg group was excluded from the study owing to previous kidney failure and the need for dialysis.

Moreover, biochemical analysis showed that crocin does not affect lipid profile and biochemical parameters. Hematologic indices were also within the normal range.

Other studies reported no significant side effects for crocin. Safety of crocin has been investigated in patients with schizophrenia,[20] as well as in healthy individuals.[21] No important side effect was observed in these studies. Also, biochemical parameters of blood serum were within the normal range, with no significant changes during the trial.[20,21]

• **BIOCHEMICAL BLOOD TEST:** All the biochemical tests were performed in the laboratory of Ghaem Hospital, Mashhad University of Medical Sciences. According to our results, the levels of LDL, HDL, TG, Chol, BUN, Cr, Ca, Phos, Na, and K showed no significant differences between the treatment and placebo groups and within each group pre- and post-intervention ($P > .05$; Tables 3 and 4). Compared to the placebo group, crocin could significantly diminish both HbA1c and FBS in the crocin 15 mg group. The difference in FBS and HbA1c was significant in the 15 mg and 5 mg crocin groups compared to the placebo group. The mean difference in FBS between crocin 5 mg and placebo was 13 (95% confidence interval [CI]: 6.21-41.2; $P = .035$) and the mean difference in FBS between crocin 15 mg and placebo was 18 (95% CI: 11.33-28.43; $P = .049$). Also, the differences in HbA1c values between crocin 5 mg and placebo groups was 0.11 (95% CI: 0.09-0.87; $P = .051$) and the differences in HbA1c values between crocin 15 mg and placebo groups was 0.76 (95% CI: 0.3-0.96; $P = .024$) (Figures 2 and 3).

• **OPHTHALMIC EXAMINATION:** BCVA and CMT were evaluated for all the patients before and after the intervention and every month during the 3 months of intervention. The difference between the corcin 15 mg and placebo groups in logMAR changes before and after trial was 0.303 (95% CI: 0.23-0.69; $P = .012$), which is significant, whereas the difference between the crocin 5 mg and placebo groups in logMAR changes was 0.122 (95% CI: 0.1-0.88; $P = .09$), which is not significant. According to our data, the difference between the crocin 15 mg and crocin 5 mg groups in logMAR changes after the intervention was statistically significant ($P < .05$; Figure 4).

The difference between the crocin 15 mg and placebo groups in CMT changes was 82 (95% CI: 32.75-126.99; $P = .005$), which is significant, whereas the difference between the crocin 5 mg and placebo groups in CMT changes was 41 (95% CI: 12.9-97.89; $P = .09$), which is not significant. Additionally, the difference between the crocin treatment groups in CMT changes during the intervention was significant ($P < .05$; Figure 5). The CMT and logMAR values were compared within each group before and after the intervention (Table 5). The OCT results of 3 patients (from the placebo, crocin 5 mg, and crocin 15 mg groups) are illustrated in Figure 6.

TABLE 3. Results of Lipid Profile and Liver Enzymes in Patients Before and After Trial

Group	LDL	HDL	TG	Chol	ALT	AST
Placebo (Mean ± SEM)						
Before	113.85 ± 6.02	43.95 ± 0.94	203.75 ± 9.32	189.45 ± 7.24	16.8 ± 0.46	16.85 ± 0.41
After	110.45 ± 5.31	44.35 ± 0.85	200.7 ± 8.09	190.85 ± 7.17	17.05 ± 0.41	16.95 ± 0.37
P value (95% CI)	.12 (102.09-121.1)	.08 (42.35-46.04)	.17 (187.55-227.94)	.17 (175-205.74)	.08 (15.9-17.85)	.07 (16.05-17.7)
Crocin 15 mg (Mean ± SEM)						
Before	118.4 ± 3.46	44.25 ± 1.41	200.95 ± 19.84	199.3 ± 7.7	14.6 ± 0.62	13.9 ± 0.48
After	115.38 ± 5.9	42.7 ± 1.19	201.6 ± 17.43	198.65 ± 7.14	14.9 ± 0.57	13.05 ± 0.32
P value (95% CI)	.19 (106.32-123.09)	.11 (40.55-46.96)	.19 (165.30-238.89)	.21(185.4-213.69)	.15 (13.45-16)	.09 (12.45-14.8)
Crocin 5 mg (Mean ± SEM)						
Before	123.7 ± 8.63	42.85 ± 1.79	198.3 ± 13.65	193.65 ± 9.01	20.1 ± 2.06	19.7 ± 1.35
After	121.85 ± 7.93	43.2 ± 1.61	190.8 ± 11.47	199.4 ± 7.89	21.05 ± 1.92	18.8 ± 1.03
P value (95% CI)	.23 (119.76-129.45)	.09 (38.7-45.9)	.28 (132.05-202.94)	.16 (184.45-213.89)	.09 (16.6-25.44)	.08 (16.8-22.49)

ALT = alanine aminotransferase; AST = aspartate aminotransferase; Chol = cholesterol; HDL = high-density lipoprotein; LDL = low-density lipoprotein; TG = triglyceride.
P values represent differences within each group.

TABLE 4. Results of Blood Chemical and Element Profiles in Patients Before and After Trial

Group	BUN	Cr	Na	K	Ca	Phosphorus
Placebo (Mean ± SEM)						
Before	23.95 ± 1.05	1.39 ± 0.11	136.9 ± 1.17	4.22 ± 0.07	9.29 ± 0.05	4.22 ± 0.08
After	22.5 ± 0.84	1.21 ± 0.07	134.5 ± 1.37	4.02 ± 0.05	9.33 ± 0.05	4.31 ± 0.08
P value (95% CI)	.21 (18.2-23.98)	.09 (1.07-1.49)	.43 (132.35-138.44)	1.0 (4 -4.66)	.66 (9.2-9.87)	.44 (3.69-4.37)
Crocin 15 mg (Mean ± SEM)						
Before	27 ± 1.56	1.23 ± 0.13	134.3 ± 1.39	4.39 ± 0.09	9.36 ± 0.08	4.14 ± 0.11
After	25.7 ± 1.19	1.22 ± 0.12	134.05 ± 1.81	4.23 ± 0.08	9.34 ± 0.07	3.97 ± 0.07
P value (95% CI)	.19 (23.4-30)	.1 (0.99-1.51)	.36 (130.85-137.79)	.88 (4.08-4.55)	.75 (9.21-9.51)	.35 (3.85-4.37)
Crocin 5 mg (Mean ± SEM)						
Before	21.4 ± 1.38	1.23 ± 0.09	134.05 ± 0.84	4.37 ± 0.07	9.63 ± 0.11	3.99 ± 0.15
After	20.2 ± 1.04	1.33 ± 0.08	136.85 ± 0.82	4.54 ± 0.06	9.66 ± 1.05	4.02 ± 0.11
P value (95% CI)	.32 (20.12-26.39)	.16 (1.07-1.63)	.54 (131.75-139.09)	1.0 (3.89-4.88)	.8 (9.18-9.7)	.69 (3.88-4.47)

BUN = blood urea nitrogen; Ca = calcium; Cr = creatine; K = potassium; Na = sodium.
P values represent differences within each group.

DISCUSSION

IN THIS CLINICAL TRIAL, THE THERAPEUTIC EFFECTS OF crocin on refractory DME were evaluated at 2 different doses (5 and 15 mg). Our findings demonstrated that vision and the thickness of macula improved in the crocin treatment groups. Consistent with the reports by Piccardi and associates[22] and Lashay and associates,[23] where vision was improved by saffron in AMD patients, the mean BCVA improved even in the 19 excluded eyes (excluded owing to the CMT value < 296 μm) with no DME. This finding indicated that crocin can be used to improve vision in eyes with no DME. Also, the results revealed that the retinal protective effects of crocin were dose-dependent. Also, crocin could effectively lower both HbA1c and FBS in diabetic patients. Our data demonstrated the dose-dependency of the hypoglycemic effects of crocin. We evaluated lipid profiles, serum electrolytes, and liver enzymes. All the parameters were within the normal range in both the crocin 5 mg and crocin 15 mg groups.

In this study, 1 patient in the crocin 5 mg group and 1 patient in the placebo group complained of swollen feet. The results of the current study demonstrated that crocin was safe, since no serious side effects were observed in the crocin-administered groups during the trial. Several studies have shown that crocin has no serious side effects[20,21]; in addition, all the biochemical variables and lipid profile were within the normal range after crocin treatment.

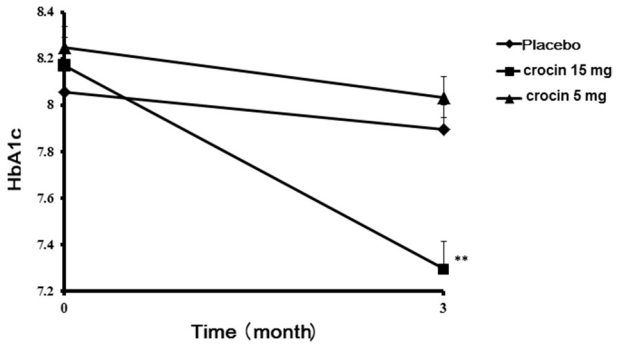

FIGURE 2. Glycated hemoglobin A1c (HbA1c) values before and after intervention. Data represent mean ± standard error of the mean. **$P < .01$ compared to placebo.

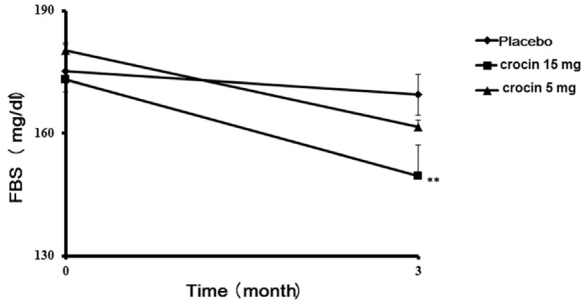

FIGURE 3. Fasting blood sugar (FBS) level before and after intervention. Data represent mean ± standard error of the mean. **$P < .01$ compared to placebo.

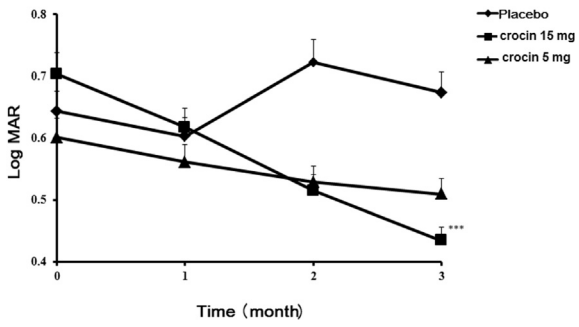

FIGURE 4. Logarithm of minimum angle of resolution (logMAR) values in the 3 groups before and during the trial. Total number of eyes in this trial was 101: crocin 5 mg, n = 34; crocin 15 mg, n = 33; and placebo, n = 34. Data represent mean ± standard error of the mean. ***$P < .001$ compared to placebo.

Hence, as mentioned before, crocin can be considered a safe supplement for the treatment of various diseases, including diabetes.[24,25]

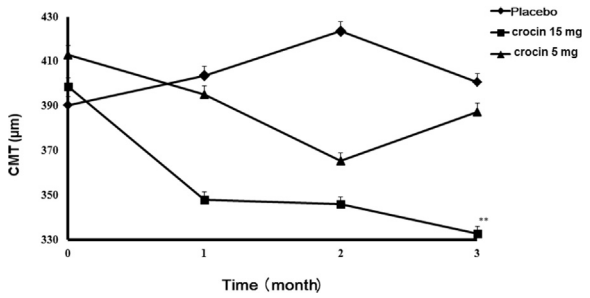

FIGURE 5. Central macular thickness (CMT) in the 3 groups before and during the trial. Total number of eyes in this trial was 101: crocin 5 mg, n = 34; crocin 15 mg, n = 33; and placebo, n = 34. Data represent mean ± standard error of the mean. **$P < .01$ compared to placebo.

In patients with uncontrolled diabetes, ocular complications appear over time. Some of these complications include diabetic retinopathy and diabetic maculopathy, which are commonly manifested with swelling of the macula. The CMT increases over time in patients with diabetic maculopathy, which is associated with decreased vision.[26] Studies have shown that the treatment of diabetic maculopathy with the intravitreal injection of triamcinolone increased BCVA from 0.1 to 0.4 in 5 months, while intraocular pressure remained normal.[27] Also, the intravitreal injection of bevacizumab, as an anti-VEGF drug, significantly diminished macular thickness relative to the control group. Bevacizumab and triamcinolone, as the routine medications in the treatment of macular edema, have some limitations, such as frequency and number of injections, which are painful and difficult for patients.[28,29] Thus, finding new effective agents for the treatment of DME is essential.

Since the treatment of DME in patients with diabetes requires controlling blood sugar, drugs with antioxidant effects can improve injuries resulting from increased blood glucose. The therapeutic effects of antioxidants can be considered for the management of DME. It is assumed that traditional medicine and herbal therapy may have a few adverse effects, but medicinal plants are widely accepted by patients. Saffron, owing to its numerous medicinal properties, is now extensively used in Iran for different therapeutic purposes and as a food additive.[30–33]

Previous works indicated that saffron and its active components such as crocin increase blood flow and oxygen supply in muscle cells. Crocin enhances the biogenesis of mitochondria in muscle cells and improves cellular respiration. It also has beneficial effects on the brain cortex. In general, these effects of crocin can elevate energy and assuage fatigue in daily activities.[34] Study on the effects of crocin in the treatment of metabolic syndrome has shown that crocin can be effective in losing body weight and reducing blood cholesterol level; hence crocin might be

TABLE 5. Minimum Angle of Resolution, Central Macular Thickness, Glycated Hemoglobin A1c, and Fasting Blood Sugar Before and After Trial in 3 Groups

Group	LogMAR	CMT	HbA1c	FBS
Placebo (Mean ± SEM)				
Before	0.64 ± 0.07	390 ± 34	8.15 ± 0.22	175.15 ± 7.388
After	0.67 ± 0.07	406 ± 29	8.03 ± 0.14	169.45 ± 7.61
P value	.42	.51	.09	.21
Crocin 15 mg (Mean ± SEM)				
Before	0.70 ± 0.09	398 ± 28	8.17 ± 0.11	173.1 ± 10.85
After	0.43 ± 0.06	332 ± 16	7.29 ± 0.12	149.55 ± 7.42
P value	.001	.01	.01	.01
Crocin 5 mg (Mean ± SEM)				
Before	0.60 ± 0.08	412 ± 32	8.25 ± 0.16	180.25 ± 8.4
After	0.50 ± 0.07	387 ± 24	8.03 ± 0.2	161.5 ± 9.2
P value	.06	.06	.07	.04

CMT = central macular thickness; FBS = fasting blood sugar; HbA1c = glycated hemoglobin A1c, LogMAR = logarithm of minimum angle of resolution.

P values represent differences within each group.

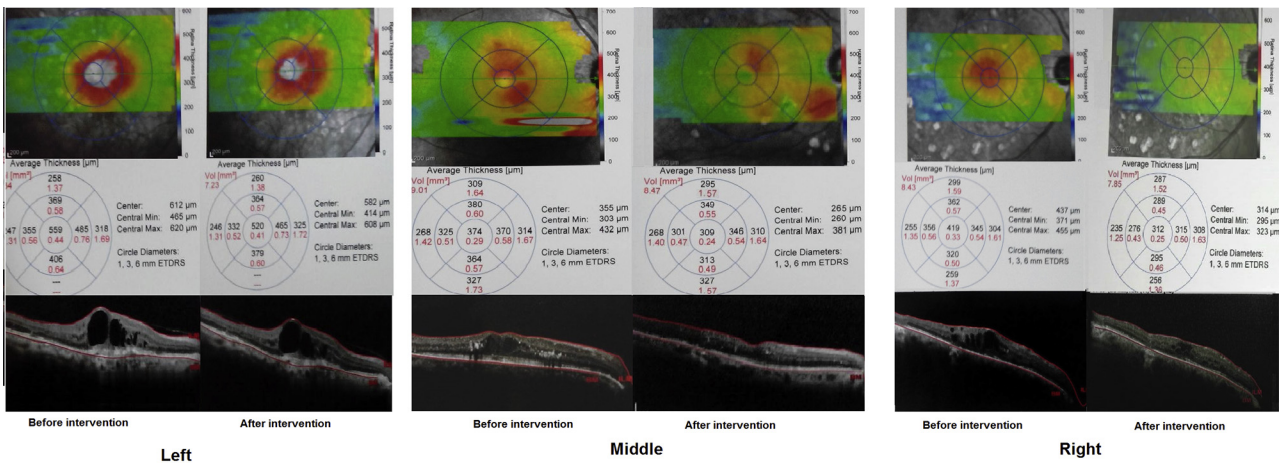

FIGURE 6. Optical coherence tomography (OCT) results of 3 patients in clinical trial before (first column) and 3 months after intervention (second column). (Left) Patient from placebo group. (Middle) Patient from crocin 5 mg group. (Right) Patient from crocin 15 mg group.

effective in reducing the risk of cardiovascular diseases. In addition, saffron and crocin are known to prevent the glycation of serum proteins, inflammatory damage, and oxidative stress induced by hyperglycemia. In other words, crocin can reduce the complications of diabetes.[24,25]

In some studies, the beneficial effects of saffron and crocin, such as its antioxidant, hypolipidemic, hypotensive, antidepressant, and hypoglycemic properties, have been proven and discussed.[35,36] In diabetic animal models, crocin reduced the blood glucose level (HbA1c). It was also shown that crocin increased the neuroprotective effect of insulin, reduced cellular glutathione reduction, and improved memory and learning. Crocin has a modulatory effect on the gene expression of the Bcl-2 protein family and can block cytochrome c–induced caspase-3 activity. Therefore, crocin can inhibit TNF-á and cell death in the PC-12 cell line.[37–41]

In addition, studies showed that crocin protects retinal cells and photoreceptors against the damage caused by white and blue light.[42] The study on retinal ganglion cells revealed that crocin inhibits apoptosis through the phosphatidylinositol 3-kinase/AKT pathway.[43]

The results of 3 studies on the therapeutic effects of saffron in patients with AMD indicated that saffron extract enhanced macular performance and visual function in the

treatment group compared to the placebo group. The *Y402H* polymorphism in the complement factor H (CFH) gene has been shown to be the strongest genetic factor to confer AMD susceptibility. Also, CFH could act as a protective factor against retinal oxidative stress with anti-inflammatory activity. Saffron, with neuroprotective properties, may protect retinal cells against damage. Findings have also shown that crocin has antiapoptotic properties in retinal cells.[22,44,45]

The results suggested that crocin, as an antioxidant supplement, may reduce the sensitivity of retinal cells to the inflammatory damage caused by oxidative stress. Limited sample size is the major limitation of this study, wherein the therapeutic potency of crocin was demonstrated in reducing inflammation and subsequent reduction in the thickness of the macula caused by oxidative stress. The underlying mechanistic pathway of crocin effects in human retinal cells is currently under investigation in our future studies.

In conclusion, this clinical trial showed that daily consumption of crocin 15 mg in addition to the intravitreal injection of bevacizumab could be effective in treatment of DME and significantly decreased macula thickness and improved BCVA. These findings suggested that crocin (a constituent of saffron stigma) plays an important role as an antioxidant agent in decreasing CMT in DME patients.

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