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Crocin Protects Mice Pancreatic Islets from Oxidative Stress Induced by Methylglyoxal and Increases Insulin Secretion

Krosin, Fare Pankreas Adacıklarını Metilglioksal Tarafından İndüklenen Oksidatif Stresten Korur ve İnsülin Sekresyonunu Arttırır

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ABSTRACT

Aim: Islets of Langerhans are more sensitive to oxidative damage because of their low antioxidant capacity. In diabetes, methylglyoxal (MG) accumulates in the pancreas. The present study examined the effect of crocin on oxidative stress induced by MG in isolated Langerhans islets from male mice.

Material and Methods: Twenty-four male mice weighing 20 to 25 g were prepared. The isolated Langerhans islets were transferred to the culture medium. Oxidative stress was induced through MG administration for 30 min, and then 10, 20, 30, and 40 µM of crocin was used for 2 h. Samples were divided into seven groups with 2.8, 5.6, and 16.7 mM glucose concentrations: control, MG 300 µM, MG+glibenclamide 10 µM, and MG+crocin in four doses of 10, 20, 30, and 40 µM. At the end, the islet's insulin, antioxidant levels, and lipid peroxidation were assessed by ELISA and calorimetry methods.

Results: Increased levels of malondialdehyde (MDA) in MG groups significantly decreased in 2.8 ($p=0.008$), 5.6 ($p=0.004$), and 16.7 ($p<0.001$) mM glucose concentrations, with administration of 30 and 40 µM crocin. Total antioxidant capacity (TAC) was reduced in MG groups ($p<0.001$) and significantly restored in all crocin-treated groups in 2.8, 5.6, and 16.7 mM glucose concentrations. Also, a significant decrease in insulin secretion and content was observed in MG groups of all three glucose concentrations ($p<0.001$). Crocin at high doses improved these alterations.

Conclusion: MG caused oxidative damage and reduced insulin secretion in isolated islets. Crocin improved the antioxidant defense system, diminished MDA, and increased insulin secretion.

Keywords: Beta-cell; crocin; diabetes; methylglyoxal; oxidative stress.

ÖZ

Amaç: Langerhans adacıkları, düşük antioksidan kapasiteleri nedeniyle oksidatif strese karşı daha duyarlıdır. Diyabette, pankreasta metilglioksal (MG) birikir. Bu çalışmada, erkek farelerden izole edilmiş olan Langerhans adacıklarında MG tarafından indüklenen oksidatif stres üzerinde krosinin etkisini araştırıldı.

Gereç ve Yöntemler: Ağırlıkları 20 ile 25 g arasında olan 24 adet erkek fare kullanıldı. İzole edilen Langerhans adacıkları kültür ortamına aktarıldı. 30 dakika boyunca MG uygulaması ile oksidatif stres indüklendi ve ardından 2 saat boyunca 10, 20, 30 ve 40 uM krosin kullanıldı. Örnekler, 2,8, 5,6 ve 16,7 mM glikoz konsantrasyonlarında yedi gruba ayrıldı: kontrol, MG 300 uM, MG+glibenclamide 10 uM ile 10, 20, 30 ve 40 µM'lik dört dozda MG+krosin. Son olarak adacığın insülin, antioksidan seviyeleri ve lipid peroksidasyonu ELISA ve kalorimetri yöntemleri ile değerlendirildi.

Bulgular: MG gruplarında artmış olan malondialdehit (MDA) düzeyleri, 30 ve 40 µM krosin uygulanmasıyla 2,8 ($p=0,008$), 5,6 ($p=0,004$) ve 16,7 ($p<0,001$) mM glikoz konsantrasyonlarında anlamlı olarak azaldı. Toplam antioksidan kapasite (TAC), MG gruplarında azalmıştı ($p<0,001$) ve krosinle tedavi edilen tüm gruplarda 2,8, 5,6 ve 16,7 mM glikoz konsantrasyonlarında önemli ölçüde düzeldi. Ayrıca MG gruplarında her üç glukoz konsantrasyonunda da insülin sekresyonu ve içeriğinde anlamlı azalma gözlendi ($p<0,001$). Yüksek dozlarda krosin bu değişiklikleri iyileştirdi.

Sonuç: MG, izole adacıklarda oksidatif hasara neden olmuş ve sonuç olarak insülin sekresyonunu azaltmıştır. Krosin antioksidan savunma sistemini iyileştirdi, MDA üretimini baskıladı ve insülin sekresyonunu artırdı.

Anahtar kelimeler: Beta hücresi; krosin; diyabet; metilglioksal; oksidatif stress.

INTRODUCTION

Diabetes is a common metabolic disease marked by hyperglycemia due to the inability of the pancreas to produce sufficient insulin or peripheral insulin resistance (1). Prolonged hyperglycemia has been found to cause excessive reactive oxygen species (ROS) production, which leads to pancreatic beta-cell damage and, finally beta-cell death (2,3). Also, the overproduction of ROS is an essential factor in the progression of diabetes and related complications (4,5). In biological systems, antioxidants defend cells against free radicals, especially ROS (6). Conversely, because pancreatic beta-cells have minor amounts of antioxidant enzymes under physiological conditions, they are highly vulnerable to oxidative stress (7). Lipid peroxidation is caused by the reaction of ROS with lipids in oxidative damage and plays a role in several diseases, including diabetes (8). Malondialdehyde (MDA) is produced in this process, and its content is a key indicator for the determination of oxidative stress (9).

Methylglyoxal (MG) is a reactive metabolite of glucose metabolism that accumulates during prolonged hyperglycemia (10). MG is known to produce free radicals in tissues. However, the glyoxalase system detoxifies MG under physiological conditions (11). Numerous evidences show that MG changes insulin structure and function, modulates the pathogenesis of insulin resistance (12,13), and is involved in various complications of diabetes (14).

The management of diabetes is complex due to its heterogeneous pathophysiology and various complications. Therefore, some drugs are used to treat and manage diabetes, including sulfonylureas. Glibenclamide (GLY) is an oral sulfonylurea that stimulates insulin secretion in beta-cells. However, efforts are underway to find effective drugs in controlling diabetes, especially natural antioxidants. Studies have shown that supplementing with natural products reduces the effects of hyperglycemia and maintains pancreatic beta-cell function (15,16). Flavonoids are major metabolites with multiple physiological and biological processes and mainly act as antioxidants to prevent ROS-induced damage under oxidative stress conditions (17-19). Various documents have indicated the valuable effects of flavonoids in treating diabetes and its complications (20-22).

Crocus sativus L. has been known for its various medical properties. This plant grows in various countries, including Iran, Egypt, Turkey, and Morocco, and is commonly known as saffron (23). Due to flavonoid content, saffron possesses antioxidant properties (24). Crocin is the major bioactive component of *Crocus sativa* L. and has particular antioxidant, radical scavenging, and anti-inflammatory properties (24-26). Chemically, crocin is a diester derived from the disaccharide gentiubiose and the dicarboxylic acid that intensely scavenges free radicals such as superoxide anions (27). In addition to its beneficial antidepressant and anti-anxiety effects (28,29), studies have reported that crocin exhibits neuroprotective (30), antihypertensive, and cardioprotective properties (31). According to a previous study, crocin effectively reduces oxidative stress markers, and blood glucose, and increases insulin levels in diabetic rats (32). Treatment with crocin has also been shown to reduce diabetic complications in rats with nicotinamide streptozotocin-induced diabetes (33). The present study was conducted to determine the protective effect of crocin as a potent antioxidant on insulin secretion against MG-induced oxidative damage in pancreatic islets under in vitro conditions.

MATERIAL AND METHODS

Animals

In this study, twenty-four male Naval Medical Research Institute (NMRI) mice weighing 25 to 35 g were obtained from the Ahvaz Jundishapur University of Medical Sciences. Animals were kept in an air-conditioned room at a temperature of 20-24 °C under 12 hours of light/dark cycle, and humidity of 70-80%, with free access to water and food. All experimental procedures were approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (27.04.2021, IR.AJUMS.ABHC.REC.1400.009).

Sample Size

Minitab software was used for determining the number of animals by considering the values of α=0.05 and β=0.2. Assuming a 35% drop, 24 mice were used in this study.

Chemicals

Crocin and MG were provided from Sigma (St. Louis, MO, USA), glibenclamide from Solar, bio (South Korea), xylazine 2%, and ketamine 10% (Alfasan Co, The Netherlands). Assay kits for MDA and TAC levels were obtained from Zell Bio GmbH (Germany). Collagenase type P was obtained from Roch Company (Germany). Glucose and insulin were purchased from Pars Azmoon (Tehran, Iran), and (Monobind Inc., USA), respectively. Bovine serum albumin, potassium chloride (KCl), sodium chloride (NaCl), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), sodium bicarbonate ($NaHCO_3$), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and hydrochloric acid (HCL) all purchased from Merck, Germany.

Islet Isolation

After anesthetizing the animals with ketamine (70 mg/kg) and xylazine (10 mg/kg), the pancreas was excised and transferred to a Petri dish containing Krebs-bicarbonate buffer (KBB) (0.5% bovine serum albumin, KCl 5 mM, NaCl 115 mM, $CaCl_2$ 2.56 mM, $MgCl_2$ 1 mM, $NaHCO_3$ 10 mM, HEPES 15 mM, and stable with a mixture of 5% carbon dioxide, 95% oxygen, pH 7.4). The isolated pancreas was cut into minute pieces (1 mm) and then centrifuged for 5 min (at 100×g). To separate the islets, KBB plus P-type collagenase (12 mg per pancreas) was added to the deposition in a centrifuge conical tube. The conical tube was transferred to an 800 oscillating incubator for 5-10 min at 37 °C. To stop collagenase activity, cold KBB was added to the conical tube and centrifuged for 5 min (at 500×g). The islets were transferred to a petri and manually isolated under a stereo microscope (34). Eventually, the islets were cultured in the hanks' buffer and affected by different substances and solutions.

Experimental Design

This in vitro study was carried out in three different glucose concentrations, including 2.8, 5.6, and 16.7 mM, as hypoglycemic, normal, and hyperglycemic culture mediums, respectively. The grouping and islets treatment procedure was as follows:

MG: 10 isolated islets were incubated for 2 h and 30 min in 300 μM MG.

MG-GLY: 10 isolated islets were incubated for 30 min in 300 µM MG, and GLY was added to reach the final concentration of 10 µM and further incubated for 2 h.
MG-C10: 10 isolated islets were incubated for 30 min in 300 µM MG, and C was added to reach the final concentration of 10 µM and further incubated for 2 h.
MG-C20: 10 isolated islets were incubated for 30 min in 300 µM MG, and C was added to reach the final concentration of 20 µM and further incubated for 2 h.
MG-C30: 10 isolated islets were incubated for 30 min in 300 µM MG, and C was added to reach the final concentration of 30 µM and further incubated for 2 h.
MG-C40: 10 isolated islets were incubated for 30 min in 300 µM MG, and C was added to reach the final concentration of 40 µM and further incubated for 2 h.
At the same time, control groups for culture mediums were defined as 10 isolated islets incubated in 2.8, 5.6, and 16.7 mM glucose-containing culture medium for 2 hours and 30 min.

Insulin Secretion and Content Measurement

The isolated islets were placed in a microtube with a KBB (1 mL) in the mentioned groups. Briefly, after MG incubated for 30 min administration, in treated groups, samples were exposed to crocin or GLY for 2 h at 37 °C. The samples were centrifuged at 100×g for 5 min. Then, the supernatant was kept at -70 °C until insulin secretion was measured. The mentioned method was performed to assess the insulin content of the islets. Insulin extraction buffer was prepared [1 mL of HCL (0.18 M) in ethanol (96%)]. After the mentioned incubation steps, glass tubes re-pipet and vortex for 1 min and kept at 4 °C overnight. The samples were centrifuged at 2500 rpm for 5 min at 4 °C. Then, 900 µL of supernatant was kept at -70 °C until insulin content was measured using a colorimetric assay kit (35).

Antioxidant and Lipid Peroxidation Analysis

The level of lipid peroxidation (MDA content) and TAC levels were measured using a colorimetric method according to the manufacturer's instructions.

Statistical Analysis

Obtained data passed the normality assumption by using the Shapiro-Wilk test. Also, Levene's test was used for homogeneity of variances. GraphPad Prism Version 9 for Windows (GraphPad Software, San Diego, CA) was utilized to analyze the data. The results were presented as mean and standard deviation. The one-way analysis of variance followed by the post hoc Bonferroni test was used for the data analysis. A $p<0.05$ was considered significant.

RESULTS

Effects of Crocin on Islet's Lipid Peroxidation and Antioxidant Activity

2.8 mM Glucose-Containing Medium

The MDA as an indicator for peroxidation of lipids, increased in MG (p=0.008), MG-GLY (p=0.020), and MG-C10 (p=0.020) groups compared to the control group. Also, MG had a remarkable difference when compared with the MG-C40 group ($p<0.001$). The levels of lipid peroxidation in the MG-GLY (p=0.002), MG-C10 (p=0.002), and MG-C20 (p=0.009) groups were higher than in the MG-C40 group (Table 1, Figure 1A).

Measurement of TAC levels significantly decreased in the MG and MG-GLY groups when compared to the control group. Administration of GLY and crocin at all doses effectively recovered TAC levels compared to the MG group ($p<0.001$). Moreover, 40 µM of crocin positively improved the TAC levels of pancreatic islets compared to the GLY ($p<0.001$), 10 (p=0.010), 20 (p=0.002), and 30 (p=0.009) µM of crocin (Table 1, Figure 1B).

5.6 mM Glucose-Containing Medium

The MDA levels increased significantly in MG (p=0.004) and MG-C10 (p=0.005) groups compared to the control group. Treatment with GLY (p=0.006), 30 (p=0.005) and 40 (p=0.004) µM of crocin reduced the peroxidation process compared to the MG group. Also, there was a significant difference between MG-GLY and MG-C10 groups (p=0.007). Also, 40 µM of crocin had a better effect (p=0.004) than the other doses (Table 1, Figure 2A). At this glucose concentration, the TAC levels decreased in MG and MG-GLY ($p<0.001$) groups, but it was increased in MG-C30 (p=0.007) and MG-C40 ($p<0.001$) groups when compared to the control group. Administration of crocin at all doses improved the TAC levels compared to the MG and MG-GLY groups ($p<0.001$). The differences between MG-C30 (p=0.003) and MG-C40 ($p<0.001$) with the MG-C10 were significant. Moreover, the TAC content of pancreatic islets in the presence of 30 and 40 µM of crocin was higher than 20 µM (Table 1, Figure 2B).

16.7 mM Glucose-Containing Medium

Our findings indicated an elevation of lipid peroxidation in the MG, MG-C10, and MG-C20 groups compared to the control group ($p<0.001$), as recognized in the MDA levels. Administration of GLY, and 30 and 40 µM of crocin significantly diminished it ($p<0.001$). Among the

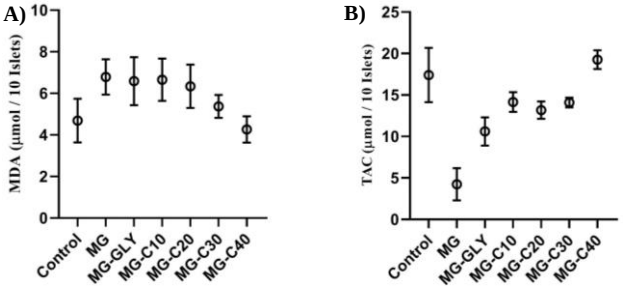

Figure 1. A) MDA and **B)** TAC levels of mice isolated islets incubated in 2.8 mM glucose-containing medium

MDA: malondialdehyde, TAC: total antioxidant capacity, MG: methylglyoxal, MG-GLY: methylglyoxal + glibenclamide, MG-C10: methylglyoxal + crocin 10 µM, MG-C20: methylglyoxal + crocin 20 µM, MG-C30: methylglyoxal + crocin 30 µM, MG-C40: methylglyoxal + crocin 40 µM

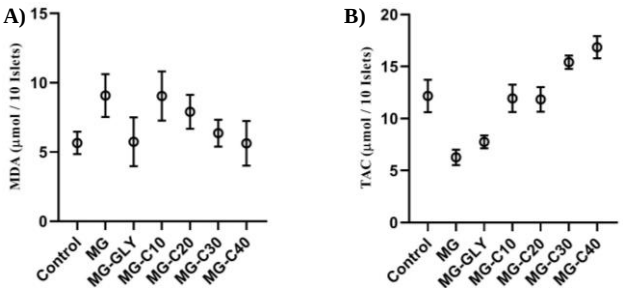

Figure 2. A) MDA and **B)** TAC levels of mice isolated islets incubated in 5.6 mM glucose-containing medium

MDA: malondialdehyde, TAC: total antioxidant capacity, MG: methylglyoxal, MG-GLY: methylglyoxal + glibenclamide, MG-C10: methylglyoxal + crocin 10 µM, MG-C20: methylglyoxal + crocin 20 µM, MG-C30: methylglyoxal + crocin 30 µM, MG-C40: methylglyoxal + crocin 40 µM

MG-treated islets, MG-C40, and MG-GLY had better effects than MG-10 and MG-C20 (p<0.001). Moreover, a significant difference was observed between the MG-C10 and MG-C30 (p=0.005) groups (Table 1, Figure 3A).

The decreased levels of TAC in the MG group were enhanced in the MG-GLY (p<0.001), MG-C10 (p=0.001), and the other crocin-treated groups (p<0.001). Furthermore, a high dose of crocin had a better effect than the MG-GLY (p=0.020), MG-C10 (p=0.020), and MG-C20 (p=0.030) groups (Table 1, Figure 3B).

Effects of Crocin on Insulin Secretion and Insulin Content in Isolated Pancreatic Islets

2.8 mM Glucose-Containing Medium

Treatment with MG reduced the insulin secretion in the MG (p<0.001), MG-C10 (p<0.001), MG-C20 (p<0.001), and MG-C30 (p=0.003) groups compared to the control group. Treatment with GLY (p<0.001), and 30 (p=0.030), and 40 (p<0.001) μM of crocin effectively recovered the insulin levels when compared to the MG group. Also, the differences in insulin secretion between the MG-GLY group and the MG-C10 (p<0.001), MG-C20 (p<0.001), and MG-C30 (p=0.020) groups were significant. In addition, our results demonstrated that the high dose of crocin treatment (p<0.001) was more beneficial than the other doses (Table 2, Figure 4A). Insulin content decreased in the MG, MG-C10, and MG-C20 groups compared to the control group (p<0.001). Among the treatments, only the high crocin dose improved the insulin content compared to the MG group (p<0.001). Moreover, insulin contents in the

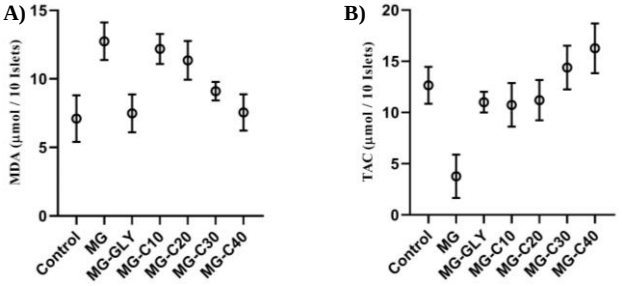

Figure 3. A) MDA and **B)** TAC levels of mice isolated islets incubated in 16.7 mM glucose-containing medium

MDA: malondialdehyde, TAC: total antioxidant capacity, MG: methylglyoxal, MG-GLY: methylglyoxal + glibenclamide, MG-C10: methylglyoxal + crocin 10 μM, MG-C20: methylglyoxal + crocin 20 μM, MG-C30: methylglyoxal + crocin 30 μM, MG-C40: methylglyoxal + crocin 40 μM

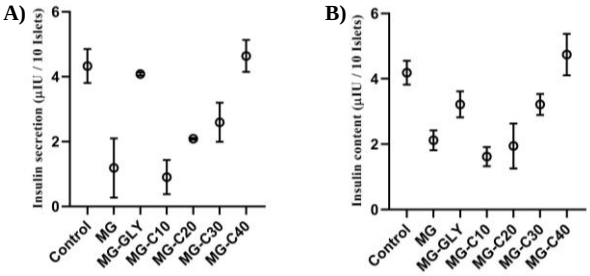

Figure 4. A) Insulin secretion and **B)** content of isolated islets incubated in 2.8 mM glucose-containing medium

MDA: malondialdehyde, TAC: total antioxidant capacity, MG: methylglyoxal, MG-GLY: methylglyoxal + glibenclamide, MG-C10: methylglyoxal + crocin 10 μM, MG-C20: methylglyoxal + crocin 20 μM, MG-C30: methylglyoxal + crocin 30 μM, MG-C40: methylglyoxal + crocin 40 μM

Table 1. Effect of crocin and glibenclamaide on islet's lipid peroxidation and antioxidant activity

	Control	MG	MG-GLY	MG-C10	MG-C20	MG-C30	MG-C40	p
MDA								
a	4.69±1.05	6.78±0.85*	6.58±1.15*	6.65±1.02*	6.34±1.04	5.37±0.55	4.26±0.63$^{\#\$\&@}$	**<0.001**
b	5.66±0.80	9.07±1.54*	5.74±1.76$^{\#}$	9.04±1.77$^{*\$}$	7.91±1.22	6.36±0.96$^{\#}$	5.63±1.61$^{\#\&}$	**<0.001**
c	7.10±1.70	12.75±1.37*	7.49±1.38$^{\#}$	12.19±1.09$^{*\$}$	11.36±1.41$^{*\$}$	9.10±0.67$^{\#}$	7.55±1.32$^{\#}$	**<0.001**
TAC								
a	17.4±3.27	4.24±1.93*	10.60±1.70$^{*\#}$	14.16±1.18$^{\#}$	13.18±1.04$^{\#}$	14.09±0.58$^{\#}$	19.27±1.13$^{\#\$\&@¥}$	**<0.001**
b	12.17±1.56	6.27±0.74*	7.75±0.62*	11.94±1.31$^{\#\$}$	11.85±1.18$^{\#\$}$	15.42±0.64$^{*\#\$\&}$	16.86±1.06$^{*\#\$\&@}$	**<0.001**
c	12.66±1.80	3.77±2.10*	11.01±1.01$^{\#}$	10.74±2.12$^{\#}$	11.21±1.96$^{\#}$	14.39±2.13$^{\#\&}$	16.27±2.42$^{\#\$\&@}$	**<0.001**

MG: methylglyoxal, MG-GLY: methylglyoxal + glibenclamide, MG-C10: methylglyoxal + crocin 10 μM, MG-C20: methylglyoxal + crocin 20 μM, MG-C30: methylglyoxal + crocin 30 μM, MG-C40: methylglyoxal + crocin 40 μM, MDA: malondialdehyde, TAC: total antioxidant capacity, a: 2.8 mM, b: 5.6 mM, and c: 16.7 mM glucose-containing medium, superscript symbols denote significant differences of the group when compared with the *: control group, $^{\#}$: MG group, $^{\$}$: MG-GLY group, $^{\&}$: MG-C10 group, $^{@}$: MG-C20 group, $^{¥}$: MG-C30 group

Table 2. Effect of crocin and glibenclamaide on insulin secretion and content in MG-induced oxidative stress of islets

	Control	MG	MG-GLY	MG-C10	MG-C20	MG-C30	MG-C40	p
Insulin secretion								
a	4.33±0.53	1.18±0.91*	4.08±0.04	0.9±0.53$^{*\$}$	2.08±0.03$^{*\$}$	2.6±0.6$^{\&@}$	4.64±0.5$^{\#\$\&@¥}$	**<0.001**
b	9.99±0.58	1.15±0.66*	7.3±0.42$^{*\#}$	2.21±0.19$^{*\$}$	3.65±0.22$^{*\#\$}$	8.13±0.7$^{*\#\&@}$	9.45±1.15$^{\#\$\&@}$	**<0.001**
c	10.33±1.63	1.92±1.43*	8.73±1.02$^{\#}$	4.7±0.64$^{*\#\$}$	6.47±0.52$^{*\#}$	9.9±0.75$^{\#\&@}$	10.88±1.18$^{\#\&@}$	**<0.001**
Insulin content								
a	4.18±0.37	2.12±0.3*	3.22±0.4	1.62±0.3$^{*\$}$	1.94±0.7$^{*\$}$	3.21±0.32$^{\&@}$	4.74±0.63$^{\#\$\&@¥}$	**<0.001**
b	6.82±0.52	0.97±0.8*	4.13±0.53$^{*\#}$	2.94±0.73$^{*\#}$	2.9±0.66$^{*\#}$	6.08±0.83$^{\#\$\&@}$	8.02±0.75$^{\#\$\&@¥}$	**<0.001**
c	5.97±0.55	1.5±0.6*	4.17±0.22$^{*\#}$	1.98±0.4$^{*\$}$	2.47±0.29$^{*\$}$	4.76±0.54$^{*\#\$\&@}$	5.41±0.63$^{\#\&@}$	**<0.001**

MG: methylglyoxal, MG-GLY: methylglyoxal + glibenclamide, MG-C10: methylglyoxal + crocin 10 μM, MG-C20: methylglyoxal + crocin 20 μM, MG-C30: methylglyoxal + crocin 30 μM, MG-C40: methylglyoxal + crocin 40 μM, MDA: malondialdehyde, TAC: total antioxidant capacity, a: 2.8 mM, b: 5.6 mM, and c: 16.7 mM glucose-containing medium, superscript symbols denote significant differences of the group when compared with the *: control group, $^{\#}$: MG group, $^{\$}$: MG-GLY group, $^{\&}$: MG-C10 group, $^{@}$: MG-C20 group, $^{¥}$: MG-C30 group

MG-C10 (p=0.001) and MG-C20 (p=0.020) groups were significantly lower than in the MG-GLY group. Treatment with a crocin dose of 40 μM had improving effects when compared to the MG-GLY (p=0.002) group, and other doses of MG-C10 (p<0.001), MG-C20 (p<0.001), and MG-C30 (p=0.002) groups (Table 2, Figure 4B).

5.6 mM Glucose-Containing Medium

MG significantly reduced insulin secretion and insulin content (p<0.001). Administration of GLY (p<0.001), and 20 (p=0.002), 30 (p<0.001), and 40 (p<0.001) μM of crocin increased insulin secretion and content when compared to the MG. But, crocin 10 μM just improved the content of insulin in this condition (p=0.010). The difference in the levels of insulin secretion between the MG-GLY and MG-C10 (p<0.001), MG-C20 (p<0.001), and MG-C40 (p=0.002) groups were significant. The positive effects of 30 (p=0.020) and 40 (p<0.001) μM of crocin on insulin secretion were higher than the GLY. Moreover, significant differences between MG-C10 and MG-C20 groups with MG-C30 and MG-C40 groups were observed (p<0.001) in both insulin secretion and insulin content (Table 2, Figure 5A and 5B).

16.7 mM Glucose-Containing Medium

The insulin secretion decreased in the MG (p<0.001), MG-C10 (p<0.001), and MG-C20 (p=0.001) groups when compared to the control group. Administration of GLY, 20, 30, 40 (p<0.001) and 10 (p=0.040) μM of crocin improved it. Between the crocin-treated groups, the efficacy of the dose of 40 μM crocin was better than the other doses (p<0.001). There were significant differences between the MG-GLY and MG-C10, and also MG-C20 and MG-C30 (p<0.001) groups (Table 2, Figure 6A). On the other side, the content of insulin remarkably reduced in the MG (p<0.001), MG-GLY (p<0.001), MG-C10 (p<0.001), MG-C20 (p<0.001), and MG-C30 (p=0.040) groups compared to the control group. The decreased levels of insulin content in the MG group recovered in the GLY, and 30 and 40 μM crocin-treated islets (p<0.001). Among the crocin-treated islets, the MG-C40 group had a better effect than the MG-GLY group (p=0.030). Furthermore, significant differences between the MG-C10 and MG-C20 groups and the MG-C30 and MG-C40 groups (p<0.001) were observed (Table 2, Figure 6B).

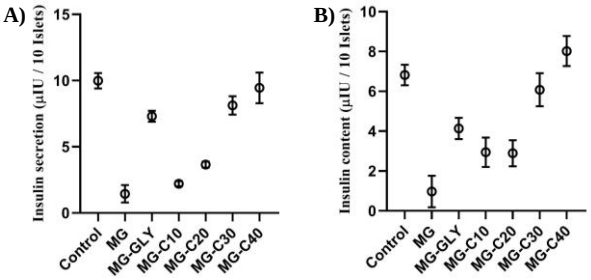

Figure 5. A) Insulin secretion and **B)** content of isolated islets incubated in 5.6 mM glucose-containing medium

MDA: malondialdehyde, TAC: total antioxidant capacity, MG: methylglyoxal, MG-GLY: methylglyoxal + glibenclamide, MG-C10: methylglyoxal + crocin 10 μM, MG-C20: methylglyoxal + crocin 20 μM, MG-C30: methylglyoxal + crocin 30 μM, MG-C40: methylglyoxal + crocin 40 μM

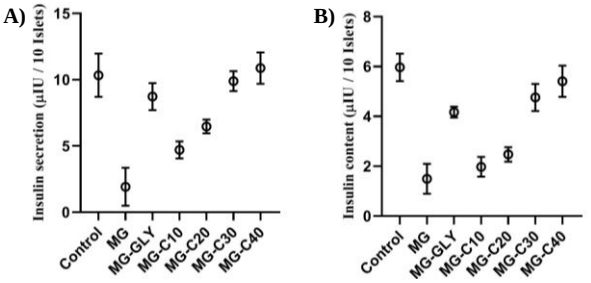

Figure 6. A) Insulin secretion and **B)** content of isolated islets incubated in 16.7 mM glucose-containing medium

MDA: malondialdehyde, TAC: total antioxidant capacity, MG: methylglyoxal, MG-GLY: methylglyoxal + glibenclamide, MG-C10: methylglyoxal + crocin 10 μM, MG-C20: methylglyoxal + crocin 20 μM, MG-C30: methylglyoxal + crocin 30 μM, MG-C40: methylglyoxal + crocin 40 μM

DISCUSSION

Several studies have been conducted on the anti-diabetic effect of flavonoids, including crocin (active constituent of *Crocus sativus*). Flavonoids are known as improving compounds for insulin production and secretion (36). In the pancreas, flavonoids mainly reduce oxidative stress, improve cell viability, and enhance insulin secretion (20). This in vitro study examined the effects of crocin on pancreatic beta-cells under MG-induced oxidative damage conditions. Our findings demonstrated that incubation with 300 μM MG for 30 min impaired insulin secretion and insulin content in islets in low, normal, or high glucose concentrations. Based on a previous result, MG can induce toxicity and decrease insulin secretion in pancreatic beta cells (37). In oxidative conditions, MG accumulates in cells and leads to oxidative damage. It is well known that oxidative stress plays an essential role in the development and onset of diabetes. One of the consequences of oxidative stress is beta-cell dysfunction, which leads to decreased insulin secretion and, eventually diabetes (38). Therefore, these results further supported the noxious effects of MG on insulin secretion.

We evaluated insulin secretion in beta-cells after MG exposure, followed by crocin treatment. According to our results, crocin improved insulin secretion in hypo, normal, and hyperglycemic conditions. Also, crocin effectively increased insulin content in three glucose concentrations. Consequently, crocin improved the secretion of pancreatic beta-cells by inhibiting the harmful effects of MG and possibly regenerating pancreatic beta-cells. A previous report showed that *Crocus sativus* extract increased plasma insulin levels in alloxan-induced diabetic rats (39). Based on the study by Samaha et al. (40), crocin enhances insulin secretion in beta islets in STZ-induced diabetic rats. These reports are similar to the results of our study.

The MDA levels as a lipid peroxidation indicator of the beta-cells were measured to prove the ameliorating effect of crocin on oxidative stress. We revealed that MG increased the formation of MDA in glucose-containing mediums at three different concentrations. Hyperglycemia can increase oxidative stress markers such as lipid peroxidation (41). Lipid peroxidation is defined as the oxidation of lipid compounds in the presence of excess amounts of free radicals producing toxic byproducts such as aldehydes. In diabetes, lipid peroxidation occurs due to the excessive production of free radicals and, so exacerbates oxidative stress (42). The production of MDA was attenuated when the islets were treated with crocin, 30 and 40 μM. This finding is in agreement with Yaribeygi et al. (43), which mentioned that treatment of STZ-induced diabetic rats with crocin significantly diminished the MDA levels.

According to a previous study, increased levels of ROS along with decreased levels of antioxidants have been observed in diabetics (38). Due to the low levels of antioxidants, pancreatic islets are more vulnerable to oxidative damage than other tissues. In conditions of defects in the antioxidant defense system, the balance tends to favor the increase of free radicals and oxidative stress (44). Our results showed that MG induced a significant oxidative load with decreased TAC levels. Besides, crocin improved the antioxidant defense system by increasing the TAC levels in MG-exposed pancreatic beta-cells. This is in agreement with a previous study reported that crocin increased the pancreatic TAC in STZ-induced diabetic rats (40). In addition, the protective effect of crocin has been revealed through an increase in TAC levels of liver tissue in diabetic rats (33).

CONCLUSION

The present study suggests that MG negatively affects beta-cell function. MG induces lipid peroxidation and oxidative stress in pancreatic beta islets and reduces insulin secretion. Crocin administration improves these alterations by increasing the TAC levels and decreasing the MDA levels.

Ethics Committee Approval: The study was approved by the Ethics Committee of Research Center & Experimental Animal House - Ahvaz Jundishapur University of Medical Sciences (27.04.2021, IR.AJUMS.ABHC.REC.1400.009).

Conflict of Interest: None declared by the authors.

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