Effects of crocin on experimental obesity and type-2 diabetes

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Background/aim: The aim of this study is to scrutinize the effects of crocin on obesity and type-2 diabetes, with an approach that takes oxidative stress and inflammatory parameters into account.

Materials and methods: The experimental obesity model was created by utilizing a 10-week-long high-fat diet (HFD). An experimental type-2 diabetes model was created by injecting multiple low-dose streptozocin (STZ) injections into rats that were fed with the HFD. The treatment groups were administered a daily crocin dose of 150 mg/kg for 6 weeks via gavage.

Results: Findings of the study demonstrated that crocin could be effective in relieving the symptoms of obesity and diabetes (hyperinsulinemia, hyperleptinemia, insulin resistance, and weight gain). It was determined that crocin lowered the plasma TNF-α and IL-1β levels and the pancreas tissue TNF-α and IFN-γ levels, which were increased due to diabetes, and reduced the inflammation in diabetic rats. Similarly, it was found that oxidative stress, which increased due to the progress of diabetes, was reduced in crocin treatment group.

Conclusion: Crocin could contribute to the development of phytotherapeutic approaches in the treatment of obesity, diabetes, and diabesity (obesity-induced diabetes), which is promising as the abovementioned incidences have increased considerably in today's world.

Key words: Crocin, diabetes, obesity, high-fat diet, inflammation, oxidative stress

1. Introduction

Today obesity and diabetes are among the most widespread social problems and are significant health problems both for governments and for society at large. Studies demonstrate that the frequency of obesity and diabetes (especially type-2 diabetes) have increased rapidly throughout the world (1–3).

Most individuals with type-2 diabetes display obesity or adiposity, and hence it is possible to assert that these two problems observed in society are interlinked. The reason for this assertion is straightforward since both conditions share the same characteristic, insulin resistance (4–6). Increasing adipose tissue and increasing activity of adipokines, which are secreted from tissues due to obesity, stimulate insulin resistance both in peripheral tissues and in the pancreas (6–8). Furthermore, the lipotoxicity that is induced due to the increase in adipose tissue increases the oxidative stress in the related tissues, thus contributing to the development of insulin resistance (9,10). Hyperglycemia develops over time as a result of insulin resistance and accordingly oxidative stress increases due

to glucotoxicity and lipotoxicity. Pancreatic beta (β)-cell dysfunction develops due to the increase in oxidative stress and/or as a result of adipose tissue-induced inflammation, and further β-cell damage could cause the development of type-2 diabetes (11–13).

Today several natural antioxidants that reduce the inflammation and oxidative stress induced by obesity and type-2 diabetes are the subjects of debate about their possible use in treatment and have increasingly appeared as the focus of recent studies. Crocin is one of these components, which is a natural carotenoid found in saffron (*Crocus sativus* L.) and gardenia (*Gardenia jasminoides* J.Ellis) flowers. It is a compound formed by a disaccharide called gentiobiose and a dicarboxylic acid called crocetin, which is soluble in water, and is in diester form with high thermal stability (14–16). Studies demonstrated that crocin could possess antioxidant (17), antiplatelet (18), neuroprotective (19), antihyperlipidemic (20,21), antidiabetic (22–24), and anticarcinogenic (25) properties due to its pharmacological effects. However, a literature review revealed no studies on the possible

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ways in which crocin affects obesity and type-2 diabetes-induced inflammation and oxidative stress. In this context, it was concluded that by exhibiting the effects of crocin on oxidative stress and inflammation in obesity and type-2 diabetes, this study might serve as a foundation for future studies on the treatment of these diseases.

2. Materials and methods

2.1. Materials

In this study, tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6, and interferon gamma (IFN-γ) were purchased from e-Bioscience in Vienna, Austria. The cytokine, leptin (BioVendor, Bratislava, Slovakia), insulin (DRG-Diagnostic, Marburg, Germany), and total protein (Fluka, St. Louis, MO, USA) levels were measured with an ELISA device (ELx800, BioTek Inc., Winooski, VT, USA). Total antioxidant status (TAS) and total oxidant status (TOS), which are among the oxidative stress parameters, were measured using kits (Rell Assay, Gaziantep, Turkey) that work with spectrophotometric methods. The streptozocin (STZ), crocin, and PBS were obtained from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used in the study were of the highest grade available.

2.2. Animals and high-fat diet preparation

A total of 40 male Wistar albino rats were used in this study. Rats were procured from the Afyon Kocatepe University Experimental Animal Research and Application Center. Rats were continuously cared for, fed, and provided with water daily. The study was conducted at the Afyon Kocatepe University Experimental Animal Research and Application Center by providing 55%–60% humidity and a 12:12-h light-dark cycle. All interventions on the animals during the study process were conducted in compliance with the permissions obtained from the Afyon Kocatepe University Experimental Animal Local Ethics Committee, Turkey (Approval Date/No: 2012/182).

A high-fat diet (HFD) was administered to all study groups except for the control group to induce experimental obesity and to induce insulin resistance, which is one of the characteristics of type-2 diabetes. HFD implementation to induce weight gain and insulin resistance was designed in obese and diabetes study groups as follows: rat food obtained from the feed plant was pulverized and homogenized after adding 5% fatty soy and 5% eggs as a source of protein, and 50% tallow as an energy source, and then it was formed into a pellet and preserved in deep-freeze. During the study HFD with 4930 cal/kg of metabolic energy level was prepared fresh every week and given to the animals 1 h after being removed from deep-freeze.

2.3. Experimental design

HFD was administered to the rats for 10 weeks to induce experimental obesity. At the end of this period, only the rats in the HFD-administered obese group that displayed hyperinsulinemia and hyperleptinemia in parallel with the weight increase and insulin resistance were considered obese.

A literature review on inducing an experimental type-2 diabetes model demonstrated that low-dose STZ injection after HFD administration could successfully imitate type-2 diabetes physiopathology (26,27). It was stated that two doses of 30 mg/kg STZ injections administered a week apart was the most ideal application (28). Thus, initially insulin resistance was initiated in rats, which were planned to be induced with experimental diabetes. Subsequently, 30 mg/kg doses of STZ dissolved in citrate buffer (pH 4.5) were administered in the 3rd week (1 dose) and the 4th week (1 dose) of the study using i.p. injection (28). One week after the last injection (at the end of the 4th week), glucose levels of the fasting rats were measured in a drop of blood taken from the tail veins. Rats with a glucose level of ≥300 mg/dL were accepted as suffering from type-2 diabetes.

Crocin administration was initiated to two groups: one diabetic group after the induction of type-2 diabetes and one obesity group that was only administered the HFD. Crocin was prepared daily, just before its application to rats. It was dissolved in physiological saline water for 6 weeks with a daily dose of 150 mg/kg (22) before being administered to the experimental animals by gavage. A total of 40 male Wistar albino rats weighing 200–250 g (mean: 230 g) were randomly divided into five groups including 8 rats in all groups. The study groups were designed as follows:

Group 1: Control group (Control): Composed of healthy rats.

Group 2: Obesity group (Obesity): Composed of rats that were fed with the HFD during the study.

Group 3: Obesity-treatment group (Obesity-Crocin): Healthy rats that were fed with the HFD throughout the study and were administered crocin for 6 weeks.

Group 4: Diabetes group (DM): Composed of rats that were fed with a high-energy high-fat diet during the study and were induced with experimental type-2 diabetes.

Group 5: Diabetes-treatment group (DM-Crocin): Rats induced with type-2 diabetes that were treated with crocin for 6 weeks.

The changes in the weights and fasting glucose levels of rats were measured with an interval of 2 weeks during the study. Prior to measurements, rats were left hungry for a night. Fasting glucose levels were measured using a drop of blood obtained from the tail vein by a glucometer (Accu-Chek Performa Nano, Roche, Mannheim, Germany). At

the end of the 6-week-long crocin application (10th week of the study), rats were anesthetized using ketamine (65 mg/kg) and xylazine (7 mg/kg) to obtain the required blood and tissue specimens for the analysis, and consequently specimens were prepared for biochemical analyses.

Blood samples, which were used in analyses containing plasma heparin, were transferred into tubes and centrifuged (Hettich, Tuttlingen, Germany) for 10 min at 3000 rpm and 4 °C. Tissue homogenates used in analyses were prepared as follows: 0.5 g of incised pancreas tissue was transferred into a homogenizer (IKA T18, IKA, Staufen, Germany) and 5 mL of PBS (pH 7.4) solution that contained 8% protease inhibitor (Roche) was added and homogenized. In all stages of the study, the maintenance of a cold chain was ensured. Homogenates were centrifuged for 10 min at 15,000 rpm and 4 °C. Plasma and pancreas samples, which were prepared to be used in the analyses, were stored at –80 °C until they were analyzed in the laboratory.

2.4. Biochemical analysis

Pancreas and plasma TNF-α, IL-1β, IL-6, and IFN-γ levels; plasma insulin levels; plasma leptin levels; and pancreas tissue total protein levels were analyzed with an ELISA device in compliance with the rat-specific kit protocols. Oxidative stress parameters of TAS and TOS levels were measured with kits running on the spectrophotometric method (29,30). Resulting TAS and TOS data were used to calculate the oxidative stress index (OSI = [(TOS / TAS) × 100]). Furthermore, β-cell function (HOMA-β), insulin resistance (HOMA-IR), and quantitative insulin sensitivity check (QUICKI) indices for test groups were calculated with the help of the formulas shown below, using starvation insulin and glucose levels (31,32).

HOMA-β = [20 × fasting insulin levels (mU/L)] / [fasting glucose levels (mmol/L) – 3.5]

HOMA-IR = [fasting insulin levels (mU/L) × fasting glucose levels (mmol/L) / 22.5]

QUICKI = [1 / (log (fasting insulin µU / mL) + log (fasting glucose mg / dL)]

The concentrations, which are related to the parameters analyzed in pancreas tissue (TAS, TOS, TNF-α, IL-1β, IL-6, IFN-γ), were divided into the final total protein levels to obtain the results. Results were expressed in const./g protein or const./mg protein.

2.5. Statistical analysis

All numerical results are expressed as mean ± standard deviation of the mean for the indicated number of experiments. Statistical significance was calculated with the ANOVA test with the Duncan posttest and results were considered significant at P < 0.05. Data were analyzed with SPSS 18.0 (SPSS Inc., Chicago, IL, USA) for Windows.

3. Results

3.1. Weight change in the experimental groups during the study

Once the data collected in the last week of the study (10th week) were evaluated it was observed that average rat weight in the HFD group was statistically different and was heavier than in all the other groups (Table 1; Figure 1A). The findings demonstrated that average rat weight in the DM group and DM-Crocin group was significantly lower than in the other groups. The comparison of the last week's data for the Obesity group and Obesity-Crocin group displayed that average rat weight in the Obesity-Crocin group was significantly lower than in the Obesity group. However, there was no difference between the average rat

Table 1. Average weight gain observed in the experimental group during the study period.

	Study groups					
	Control (g)	Obesity (g)	Obesity-Crocin (g)	DM (g)	DM-Crocin (g)	P
Week 0	237.3 ± 17.6	234.3 ± 23.1	239.8 ± 19.4	235.8 ± 26.2	239.1 ± 22.7	0.989
Week 2	262.6 ± 18.9	266.7 ± 20.7	278.4 ± 19.3	272.7 ± 28.4	279.8 ± 24.7	0.569
Week 4	276.3 ± 16.1 [a,b]	300.4 ± 21.9 [b,c]	325.3 ± 39.3 [c]	251.6 ± 22.1 [a]	298.4 ± 19.8 [b,c]	0.000
Week 6	290.1 ± 20.5 [a]	324.8 ± 29.1 [b]	328.8 ± 36.8 [b]	261.3 ± 23.2 [a]	286.4 ± 22.4 [a]	0.000
Week 8	305.0 ± 16.0 [a,b]	361.3 ± 21.5 [c]	321.8 ± 33.7 [b]	276.4 ± 35.3 [a]	279.6 ± 25.3 [a]	0.000
Week 10	323.3 ± 14.4 [b]	387.8 ± 31.3 [c]	342.0 ± 28.6 [b]	280.0 ± 44.2 [a]	279.1 ± 24.5 [a]	0.000

Values are mean ± standard deviation; n = 8.
[a, b, c]: Different letters in the same line represent statistically significant differences (P < 0.05) among experimental groups. Obesity: The group that was given only high-fat diet. DM: The group that was fed a high-fat diet throughout the study and in which type-2 diabetes was generated. DM-Crocin: Diabetic group treated with crocin. Obesity-Crocin: Crocin was given for 6 weeks to rats that were given a high-fat diet during the study.
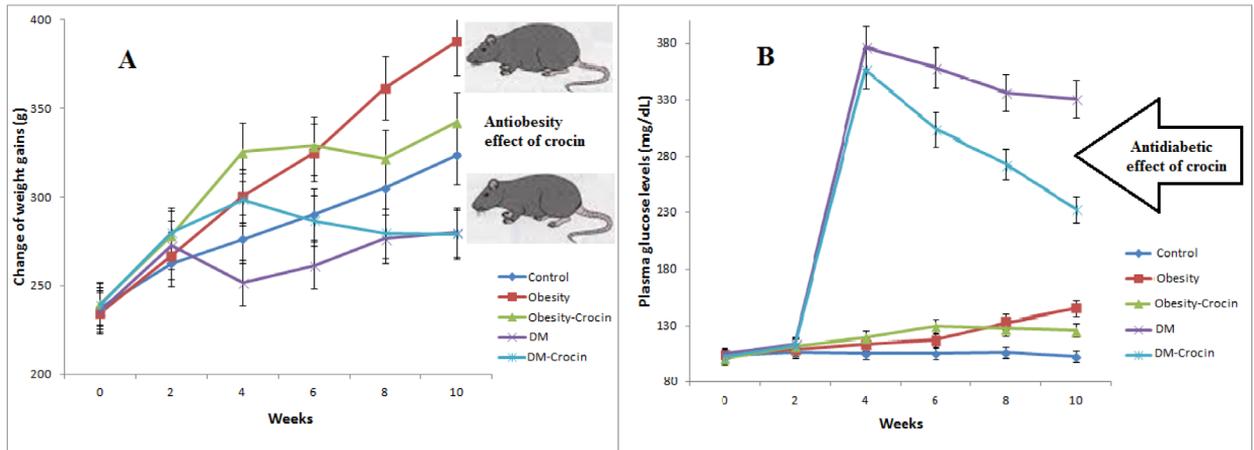
Figure 1. Antiobesity and antidiabetic effects of crocin. A) Antiobesity effect of crocin (the average weight gain observed in the experimental group throughout the study period). B) Antidiabetic effect of crocin (change in the plasma glucose levels of the experimental groups during the study period). Obesity: The group that was given only high-fat diet. DM: The group that was fed a high-fat diet throughout the study and in which type-2 diabetes was generated. DM-Crocin: Diabetic group treated with crocin. Obesity-Crocin: Crocin was given for 6 weeks to rats that were given a high-fat diet during the study.

weights of the DM group and DM-Crocin group (Table 1; Figure 1A).

3.2. Blood glucose levels determined during the study
The data for the 4th week of the study were analyzed and the analysis revealed that fasting blood glucose levels for groups with induced diabetes (DM and DM-Crocin) were statistically different and quite higher than those of the other groups (Table 2; Figure 1B). Correspondingly, HOMA-β values, which were calculated as an expression of pancreatic β-cell function, established that HOMA-β index data (Table 3) for the DM groups were very low. In the comparison between the Control group and Obesity group, no statistical difference was observed for the first 8 weeks of the study. Nevertheless, a slow and slight increase in the blood glucose levels of the Obesity group was evident for each passing week after week 8. Once the 10th week's fasting glucose levels were examined, it was observed that the Obesity group's average fasting blood glucose levels were higher with a statistically significant difference as compared to Control group findings (Table 2). The Obesity-Crocin group's blood glucose levels did not change significantly due to crocin administration to HFD-administered rats in comparison to the HFD group. These findings demonstrate that studies with longer durations are required to determine the effects of crocin on fasting glucose levels in obesity-induced rats with HFD, and through such studies it would be possible to obtain more concrete findings.

3.3. Results of the biochemical parameters analyzed in plasma
The findings indicated that Obesity group insulin levels were increased, specifying the case of hyperinsulinemia.

However, data obtained from the comparison of the DM and Obesity-Crocin groups showed that crocin treatment in diabetic rats did not affect the insulin levels (Table 3). Comparison between the Obesity group and other groups demonstrated that the HOMA-IR index findings were highest in Obesity group rats, while QUICKI findings were the lowest. Crocin decreased insulin resistance for the Obesity group that was treated with crocin, yet it did not reduce insulin resistance in the diabetic group. Another characteristic feature observed in obesity and type-2 diabetes is the development of hyperleptinemia. In this study, it could clearly be observed that hyperleptinemia developed in the Obesity group (Table 3). As the effect of crocin on Obesity and diabetic groups was scrutinized, it was observed that crocin did not significantly affect the leptin levels. However, crocin treatment increased insulin sensitivity (QUICKI) both in the Obesity-Crocin group (with respect to the Obesity group) and the DM-Crocin group (with respect to the DM group).

3.4. Plasma cytokine and oxidative stress levels
The findings based on IL-6 levels indicate that there was no statistically significant difference between the groups. However, when the TNF-α levels were analyzed, significant differences between the TNF-α levels of the groups were recognized. Data for the DM group demonstrated that there was a significant difference between the DM group and both the Control group and Obesity group based on the increase in TNF-α levels. TNF-α level for treatment groups showed that the 6-week-long crocin treatment statistically significantly lowered the TNF-α levels, which had increased to levels of 79.72 ± 17.88 pg/mL due to type-2 diabetes. However, this therapeutic effect of crocin was

Table 2. Change in glucose levels in study groups during the study.

	Study groups					
	Control (mg/dL)	Obesity (mg/dL)	Obesity-Crocin (mg/dL)	DM (mg/dL)	DM-Crocin (mg/dL)	P
Week 0	103.0 ± 9.6	104.1 ± 9.1	100.3 ± 8.6	105.1 ± 8.0	102.0 ± 9.5	0.871
Week 2	106.4 ± 4.6	108.4 ± 6.6	111.8 ± 8.7	113.8 ± 9.3	112.7 ± 5.2	0.280
Week 4	104.8 ± 8.8 [a]	113.3 ± 5.9 [a]	119.6 ± 8.1 [a]	376.6 ± 53.8 [b]	357.0 ± 44.5 [b]	0.000
Week 6	105.3 ± 6.5 [a]	117.4 ± 13.4 [a]	129.4 ± 10.3 [a]	358.4 ± 55.4 [c]	303.7 ± 43.3 [b]	0.000
Week 8	105.7 ± 7.1 [a]	132.6 ± 15.4 [a]	127.3 ± 12.0 [a]	336.3 ± 55.3 [c]	272.6 ± 41.8 [b]	0.000
Week 10	102.6 ± 11.2 [a]	144.8 ± 15.8 [b]	125.6 ± 10.6 [a,b]	330.4 ± 46.6 [d]	232.7 ± 33.7 [c]	0.000

Values are mean ± standard deviation; n = 8.
[a, b, c]: Different letters in the same line represent statistically significant differences (P < 0.05) among experimental groups. Obesity: The group that was given only high-fat diet. DM: The group that was fed a high-fat diet throughout the study and in which type-2 diabetes was generated. DM-Crocin: Diabetic group treated with crocin. Obesity-Crocin: Crocin was given for 6 weeks to rats that were given a high-fat diet during the study.

Table 3. Plasma levels of biochemical, inflammatory, and oxidative stress markers.

	Study groups					
	Control	Obesity	Obesity-Crocin	DM	DM-Crocin	P
Insulin (µg/L)	1.58 ± 0.26 [b]	2.11 ± 0.17 [c]	1.74 ± 0.25 [b]	0.42 ± 0.03 [a]	0.48 ± 0.07 [a]	0.000
Leptin (pg/mL)	276.0 ± 82.3 [a]	632.1 ± 149.9 [c,d]	742.9 ± 169.1 [d]	530.1 ± 114.3 [b,c]	456.2 ± 108.3 [b]	0.000
HOMA-β	346.4 ± 113.1 [c]	218.1 ± 51.8 [b]	230.6 ± 36.7 [b]	13.3 ± 3.5 [a]	24.2 ± 5.9 [a]	0.000
HOMA-IR	9.2 ± 1.6 [b]	17.4 ± 2.0 [d]	12.5 ± 2.5 [c]	7.8 ± 0.9 [a,b]	6.5 ± 1.7 [a]	0.000
QUICKI	0.2808 ± 0.0029 [c]	0.2602 ± 0.0013 [a]	0.2706 ± 0.0024 [b]	0.2856 ± 0.0017 [c]	0.2936 ± 0.0034 [d]	0.000
IL-6 (pg/mL)	31.9 ± 2.6	33.2 ± 2.2	30.3 ± 8.7	35.6 ± 3.2	32.1 ± 3.5	0.319
TNF-α (pg/mL)	74.3 ± 8.9 [a]	93.8 ± 10.5 [b]	91.4 ± 16.6 [b]	136.6 ± 16.8 [c]	79.7 ± 17.9 [a, b]	0.000
IFN-γ (pg/mL)	94.2 ± 4.3 [a]	112.2 ± 13.1 [b]	111.1 ± 14.1 [b]	119.8 ± 17.9 [b]	124.9 ± 13.4 [b]	0.002
IL-1β (pg/mL)	63.4 ± 16.8 [a]	91.7 ± 12.9 [b]	114.3 ± 13.8 [c]	113.7 ± 16.1 [c]	89.1 ± 19.9 [b]	0.000
TAS (mmol Trolox equiv./L)	1.60 ± 0.09 [b]	1.87 ± 0.27 [b]	1.69 ± 0.23 [b]	1.16 ± 0.20 [a]	1.71 ± 0.29 [b]	0.000
TOS (µmol H_2O_2 equiv./L)	4.63 ± 1.13 [a]	6.72 ± 1.85 [b]	9.29 ± 1.84 [c]	10.25 ± 2.56 [c]	5.32 ± 0.87 [a,b]	0.000
OSI [(TOS/TAS) × 100]	288.0 ± 65.3 [a]	362.5 ± 107.2 [a, b]	528.3 ± 113.6 [b]	915.8 ± 345.7 [c]	314.7 ± 62.2 [a]	0.000

Values are mean ± standard deviation; n = 8.
[a, b, c]: Different letters in the same line represent statistically significant differences (P < 0.05) among experimental groups. Obesity: The group that was given only high-fat diet. DM: The group that was fed a high-fat diet throughout the study and in which type-2 diabetes was generated. DM-Crocin: Diabetic group treated with crocin. Obesity-Crocin: Crocin was given for 6 weeks to rats that were given a high-fat diet during the study.

not observed in the Obesity group designed as the obese group (Table 3).

The comparison of IFN-γ levels for the Control group with the Obesity and DM groups revealed that both Obesity and DM group IFN-γ levels showed statistically significant differences and were higher than in the control group. However, the outcomes demonstrated that crocin treatment did not affect the IFN-γ levels in the Obesity or the DM group (Table 3). There was an increase in IL-1β levels for the DM and Obesity groups in comparison to the control group. IL-1β levels for treatment groups were analyzed and it was observed that crocin treatment

Table 4. Findings on pancreas tissue oxidative stress and inflammatory parameters.

	Study groups					
	Control	Obesity	Obesity-Crocin	DM	DM-Crocin	P
IL-6 (pg/mg protein)	42.6 ± 3.2 [a,b]	45.9 ± 2.4 [b]	41.5 ± 7.7 [a,b]	44.0 ± 6.9 [a,b]	38.3 ± 4.6 [a]	0.127
TNF-α (pg/mg protein)	35.8 ± 4.1 [a]	54.3 ± 4.0 [c]	55.3 ± 6.8 [c]	65.6 ± 3.9 [d]	45.9 ± 5.9 [b]	0.000
IFN-γ (pg/mg protein)	40.3 ± 3.3 [a]	44.5 ± 5.2 [a]	44.3 ± 3.8 [a]	53.4 ± 4.6 [b]	40.0 ± 4.0 [a]	0.000
IL-1β (pg/mg protein)	49.9 ± 1.3 [a]	49.7 ± 3.1 [a]	52.0 ± 2.6 [a,b]	56.4 ± 4.7 [c]	54.6 ± 3.7 [b,c]	0.002
TAS (mmol Trolox equiv./g protein)	0.59 ± 0.08	0.65 ± 0.13	0.61 ± 0.13	0.73 ± 0.13	0.61 ± 0.19	0.327
TOS (μmol H_2O_2 equiv./g protein)	3.38 ± 0.56 [a]	4.16 ± 0.44 [b]	4.06 ± 0.42 [b]	4.70 ± 0.32 [c]	3.67 ± 0.41 [a,b]	0.000
OSI [(TOS/TAS) × 100]	595.1 ± 163.2	670.1 ± 186.8	686.9 ± 149.7	662.7 ± 156.8	651.5 ± 183.7	0.775

Values are mean ± standard deviation; n = 8.
[a, b, c]: Different letters in the same line represent statistically significant differences (P < 0.05) among experimental groups. Obesity: The group that was given only high-fat diet. DM: The group that was fed a high-fat diet throughout the study and in which type-2 diabetes was generated. DM-Crocin: Diabetic group treated with crocin. Obesity-Crocin: Crocin was given for 6 weeks to rats that were given a high-fat diet during the study.

decreased the IL-1β levels that were previously increased due to type-2 diabetes. However, this therapeutic effect was not observed in the Obesity-Crocin group.

Examination of DM group findings demonstrated that TAS levels decreased in comparison to both Control and Obesity group data. Inspection of TAS levels in treatment groups revealed that crocin significantly increased the TAS levels, which were decreased due to the occurrence of diabetes. However, comparison between TAS levels of the Obesity and Obesity-Crocin groups showed that there was no statistically significant difference. A review of TOS levels revealed that the TOS levels of the Obesity and DM groups were increased in comparison to the control group. An inspection of the TOS levels in treatment groups revealed that the 6-week-long crocin treatment decreased the TOS levels in a statistically significant manner. However, this therapeutic effect of crocin was not observed in HFD groups, which were designed as the obese groups. A review of OSI levels showed that the increase in OSI levels in the DM group was significantly higher than the results for all other groups. However, the oxidative stress-reducing effect of crocin that was observed in the Diabetic group was not repeated in Obesity groups.

3.5. Pancreas tissue cytokine and oxidative stress levels

Table 4 presents the levels of biochemical parameters analyzed in the pancreas tissue of the study groups. The comparison of TNF-α levels for Obesity and Control groups indicated an increase in TNF-α levels in the HFD group. On the other hand, the increase in TNF-α levels in the DM group presented a statistically significant difference and was higher than both the Control and Obesity group findings. An inspection of the TNF-α levels in treatment groups indicated that crocin treatment decreased the

previously increased TNF-α levels due to the type-2 diabetes in a statistically significant manner, to levels of 45.9 ± 5.9 pg/mL. However, this therapeutic effect was not observed in Obesity groups (Table 4). The examination of IFN-γ levels for study groups revealed that IFN-γ levels of the DM group were statistically different and higher than in all other study groups. Furthermore, crocin treatment did not affect the IL-1β levels in Obesity groups or diabetic rats. The examination of TAS, TOS, and OSI data for study groups revealed that Obesity group TOS levels were higher than those of the control group (Table 4). DM group TOS levels were higher than both Obesity and Control group levels. Crocin treatment lowered TOS levels only in the Diabetic group.

4. Discussion

This study investigated the possible effects of crocin on obesity and type-2 diabetes. In addition, the study attempted to determine the effects of crocin on obesity induced by HFD application and define the possible increase of oxidative stress and inflammation induced by an experimental type-2 diabetes model.

4.1. Effects of crocin on obesity symptoms

Certain clinical symptoms that are characterized by obesity, such as insulin resistance, hyperinsulinemia, and hyperlipidemia, could be easily determined in individuals in the first stages of obesity due to the increase of body weight and glucose levels, although these individuals experience no health problems (11,13,33,34). A literature review revealed that several studies demonstrate an increase in insulin and leptin levels due to weight increase in obesity. In a study on the development of obesity related to diet, hepatic lipidosis, hyperleptinemia,

hyperinsulinemia, and insulin resistance were observed in rats on a HFD, as well as weight increase (35). This study and several parallel studies (36,37) demonstrated that rats on HFDs developed differences in weight gains with respect to the control group, and consequently symptoms such as increase in insulin and leptin levels or development of insulin resistance developed due to obesity. Similarly, at the end of this study, the insulin, leptin, and starvation glucose levels of the Obesity group had increased with respect to the control group.

In this study, in compliance with the literature, the rats that had an increase in body weight, leptin, and insulin levels and had developed insulin resistance (36,38,39) were administered a HFD and were accepted as obese. The main reason behind the increase in insulin levels in the Obesity group could be the secretion and release of more insulin from the pancreas to tolerate the increasing glucose levels, due to the insulin resistance developed parallel to development of obesity. The leptin levels could be due to the adipose tissue, which increased as a result of HFD applications (11,40). In a study that examined energy balance and leptin sensitivity in rats that developed diet-induced obesity, leptin levels in parallel to weight gains were found to be almost doubled in obese rats in comparison to the control group (41).

It was determined that crocin treatment decreased hyperleptinemia and hyperinsulinemia in the DM-Crocin and Obesity-Crocin groups. The reason for the decrease in secreted insulin levels in Obesity groups due to crocin treatment could be the decrease in insulin secretion from the pancreas as a result of crocin-increased sensitivity of insulin (by reducing insulin resistance). Thus, it was determined that at the end of the 6-week crocin treatment, insulin resistance decreased while insulin sensitivity increased in the Obesity-Crocin group. Decreased leptin levels due to crocin could be explained by the negative effect of crocin on the increase of body weight, since it has been reported that leptin levels increased as a result of the stimulation of adipogenesis together with the increase in body weight due to HFD applications (11,42).

In summary, it is possible to assert that crocin could contribute to the development of alternative treatment protocols in the fight against obesity, since crocin treatment, which was reported to reduce hyperleptinemia and hyperinsulinemia in obesity, is considered to have reduced insulin resistance (HOMA-IR) and body weight increase in the Obesity group.

4.2. Effects of crocin on inflammation and oxidative stress induced by obesity

In clinical studies and obesity models induced in experimental animals using HFDs, it was determined that obesity increased low-grade oxidative stress and inflammation (11,33,43,44). The findings of this study

also pointed out that the administered fatty diet increased oxidative stress and inflammation levels in the Obesity group. It could be stated that inflammation occurred in plasma due to the increase in the levels of TNF-α, IL-1β, and IFN-γ and in pancreas tissue due to the increase in the levels of TNF-α and IFN-γ (Tables 3 and 4). One of the rare studies on the effects of crocin on obesity reported that hyperlipidemia was induced by fatty diet implementation and crocin reduced the serum triglyceride, total cholesterol, ALP, AST, MDA, GPx, and GSH levels increased by the HFD application (21). However, this study, in contradiction with the findings of the above study, determined that crocin treatment did not affect oxidative stress parameters (TAS-TOS and OSI) in plasma or pancreas tissue in the Obesity group (Tables 3 and 4). Relatedly, it was determined that crocin did not affect inflammation in plasma or pancreas tissue, which was increased as a result of obesity. The reason for the nonprevalence of the antiinflammatory (19) and antioxidant (17,21) efficiency of crocin noted in the literature for obese rats could be related to the application length, methods (gavage or i.p), and the dose of crocin given to obese rats. The literature review demonstrated that most of the crocin is found in feces or in intestinal content 24 h after oral administration. It was reported that crocetin derived from crocin was found in plasma in low concentrations (44). The reason for that could be summarized as the break-up of the crocin into crocetin in the small intestine 2 h after oral administration (45). Thus, it could be stated that the determined effects of the crocin in this study were in fact due to crocetin (46).

4.3. Antidiabetic efficiency of crocin in type-2 diabetes

HFD is usually not used in studies that aim to determine the effects of crocin on diabetes (22–24); contrarily these studies were conducted using high-dose STZ/alloxan injection. It was stated in the literature that high-dose STZ injections that were administered in experimental animals without insulin resistance formation induced an experimental type-1 diabetes model (26–28). In this study, rats were initially administered HFD followed by low-dose STZ injection to simulate type-2 diabetes physiopathology.

The findings demonstrated that leptin levels in the DM group were very high, while insulin and HOMA-β levels decreased (Table 3). These findings indicated that leptin secretion from the adipose tissue was stimulated by HFD application in diabetic rats, and with the STZ injection β-cell dysfunction was induced. Crocin did not affect insulin, leptin, HOMA-β, and HOMA-IR levels when the crocin treatment was administered to diabetic rats; however, it decreased glucose levels in comparison to the DM group (Figure 1B). Certain findings among these differ from the findings in the literature. In a study examining STZ-induced diabetes (24) it was determined that crocin administered to diabetic rats using

intraperitoneal injections in daily doses of 50 and 100 mg/kg lowered insulin levels and insulin resistance. On the contrary, in this study, insulin levels and insulin resistance did not change as a result of crocin treatment. The reason for different postcrocin treatment results in diabetic rats could be explained by the difference between experimental diabetes models in different studies or between the application methods or the doses of crocin administration. For instance, in a study (24) on STZ-induced diabetes, a type-1 diabetes model was induced with a high dose (90 mg/kg) STZ injection without HFD application (without inducing insulin resistance). Hence, in this study a type-2 diabetes model was created. Different experimental diabetes models could yield different results even though they use the same treatment agent such as crocin.

This study determined that crocin increased insulin sensitivity (QUICKI) in diabetic rats although it had no effects on insulin levels and insulin resistance. Conceivably, this could be the reason why crocin decreased the glucose levels in diabetic rats without affecting the insulin levels and insulin resistance. The fact that crocin lowered glucose levels without affecting insulin and insulin resistance suggested that crocin might have suppressed hepatic glucose production. However, further studies are required to verify this.

4.4. Antiinflammatory and antioxidant efficiency of crocin in type-2 diabetes

With the progress of type-2 diabetes, lipotoxicity and glucotoxicity induced in patients debilitate the antioxidant system and the oxidative stress increases. Oxidative stress causes an increase in inflammation, especially by stimulating the production of cytokines (8,11,47,48). Thus, increased oxidative stress and inflammation with diabetes play a significant role both in the further progress of diabetes and the development of other diabetic complications (7,49). This study determined that crocin decreased plasma and pancreas oxidative stress levels. These findings were in parallel with other studies cited in the literature. It has been reported that this antioxidant effect of crocin was due to its decreasing effect on advanced glycation end products formation via the reduction in glucose levels, suppression of lipid peroxidation, and its support for the antioxidant system (23,24).

Although there are studies in the literature that attempted to determine the effect of crocin on oxidative stress, there are none that scrutinized the effects of crocin on diabetes-induced inflammation. In this respect, the findings of this study would contribute to explaining the effects of crocin on inflammation developed due to diabetes. The antiinflammatory efficiency of crocin was significant when its effects on inflammation developed in the diabetic group were examined. Thus, it was determined that plasma TNF-α and IL-1β and pancreas tissue TNF-α

and IFN-γ levels, which were increased due to diabetes (Tables 3 and 4), were reduced by crocin. Several studies demonstrated that a diabetes-induced increase in inflammation activated apoptotic signal paths, causing an increase in β-cell dysfunction and damage. Crocin, due to its antiinflammatory efficiency as mentioned, could be effective in reducing the pancreatic β-cell damage. Accordingly, in a study conducted in a microglial cell line with inflammation, crocin decreased cytokine release and suppressed NF-κB activation (50). Another study demonstrated that, due to its antiinflammatory characteristics, crocin could activate/inhibit one or several metabolic pathways. It was reported that crocin, due to its antiinflammatory effects, inhibited the cytochrome-c that activates caspase-3 by splitting off the mitochondria, and thus decreased neuronal cell damage in the neuronal PC-12 cell path (19). This study also suggested that crocin inhibiting/activating internal and external apoptotic stimulations, especially in β cells, could be significant in diabetic treatment. However, further studies are needed to determine concrete results.

This study further scrutinized the effects of crocin on the increased inflammation and oxidative stress in rats, parallel to the progress of experimental obesity and type-2 diabetes. It was determined that crocin had limited effects on obesity-induced oxidative stress and inflammation. However, it was specified that crocin had antioxidative and antiinflammatory effects against oxidative stress and inflammation that has increased as a result of glucotoxicity and lipotoxicity in type-2 diabetes. While low-grade oxidative stress and inflammation are induced as a result of the development of obesity, higher levels of oxidative stress and inflammation are observed due to the development of diabetes. In the present study, antiinflammatory and antioxidant effects of crocin on diabetic rats were observed more clearly than in obese rats. This could be due to the possibility that crocin might not be very effective in pathologies with low levels of oxidative stress and inflammation. Furthermore, an analysis of the data presented in Table 3 would demonstrate that IL-1β and TOS levels increased when crocin was applied to the obesity group. These findings might be due to the possible increasing effects of crocin on inflammation and oxidative stress. However, the data available in the present study are limited for drawing concrete conclusions.

When the significant roles of oxidative stress and inflammation on the development of diabetic complications (48) and insulin resistance (11,33) are considered, the reducing effect of crocin on oxidative stress and inflammation could be beneficial in diabetic treatment. In addition, it was determined that crocin helped reduce the symptoms of obesity. Concerning its

antiobesity effects, crocin could contribute to developing alternative treatment protocols in the fight against obesity. However, for crocin to be defined as an antiobesity agent, further research is necessary to investigate its effects on adipogenesis and energy metabolism.

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