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# Investigation of the Anti-Inflammatory Effects of Safranal on High-Fat Diet and Multiple Low-Dose Streptozotocin Induced Type 2 Diabetes Rat Model

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*Abstract*—In the present study, it was aimed to investigate the effects of safranal, one of the components of saffron plant, on the inflammation in the rats in which experimental type 2 diabetes and obesity were formed. Type 2 diabetes is a disease characterized by insulin resistance and β-cell dysfunction. Therefore, in the present study, high-fat diet (HFD) and streptozotocin were used for being able to create experimental type 2 diabetes. In the first 6 weeks of the study, experimental groups were formed in five groups, after the stage of creating insulin resistance. The study groups were designed as control, HFD, HFD-Saf, DYB, and DYB-Saf groups. Safranal treatment was applied to the treatment groups for a period of 4 weeks. Throughout the study period (10 weeks), the weight gains and plasma glucose levels of the rats were determined each week and bi-weekly, respectively. At the end of the study, interferon gamma (IFN-γ), interleukin (IL)-1β, IL-6, tumor necrosis factor alpha (TNF-α), TAS and TOS levels in the pancreas and plasma were measured. In addition, the insulin and leptin levels in the plasma were determined. It was ascertained that, compared to the diabetic group, safranal decreased the inflammation both in the plasma and pancreas tissue, by reducing the TNF-α and IL-1β levels in particular. In addition, safranal was also found to decrease the oxidative stress increased due to type 2 diabetes in the plasma and pancreas tissue. It was concluded that safranal might be helpful in terms of reduction of diabetic complications, by means of its effects on both oxidative stress and inflammation, and that further studies should be carried out for this purpose.

**KEY WORDS:** safranal; type 2 diabetes; inflammation; oxidative stress.

## INTRODUCTION

Diabetes is one of the current health problems not only for Turkey but also for the global world and is a rapidly spreading metabolic disease that affects national economies. The diabetes prevalence increased dramatically in the world during the last three decades. According to the data from the International Diabetes Federation, there were approximately 382 million people with diabetes in 2013, and the total is expected to rise to 592 million by 2035 [1]. As the most prevalent one among diabetes types, type 2 diabetes (T2DM) is characterized by insulin resistance, β-cell dysfunction, and uncontrollable hepatic glucose production [2]. Today, many methods and medications are used in the treatment of diabetes. Such methods and medications used in treatment take effect with different mechanisms and help to balance impaired glucose homeostasis, by increasing the insulin production or reducing the glucose production or minimizing the insulin resistance formed in cell receptors in the relevant tissues [3].

Since the available treatment method intended for type 2 diabetes have dose-dependent limitations and side effects (weight gain, hypoglycemia, and gastrointestinal problems, *etc.*), new treatment options are needed. Therefore, today, possible effects of many natural and synthetic active agents continue to be the favorites of researchers, for the development of treatment methods intended for type 2 diabetes.

Safranal is the organic constituent primarily responsible for the aroma of saffron. Safranal is believed to be a

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degradation product of the carotenoid zeaxanthin *via* the intermediacy of picrocrocin [4–6]. In various studies, it has been stated that safranal has antidiabetic [7], antioxidant [8–10], anti-cancer [11], and hypotensive [12] effects because of its free radical scavenging activity. In the present study, it was aimed to investigate its inflammatory and oxidative stress effects on the rats, in which the model of type 2 diabetes was formed.

## MATERIAL AND METHOD

In the study, a total of 40 male Wistar-Albino rats (eight in each experimental group) were procured from Afyon Kocatepe University, Experimental Animal Research Center. During the study period, the care and feeding of the rats were provided under the condition of 55–60 % humidity and a light dark cycle of 12:12 h. According to the groups, high-fat diet (HFD) or feeding standard rat chow and daily supply of water were provided continuously. Throughout the study period, all the interventions in the animals were made at Afyon Kocatepe University, Experimental Animal Research and Application Center, in line with the rules announced by Afyon Kocatepe University, Local Experimental Animal Ethics Committee.

HFD applied with intent to form weight gain and insulin resistance in the rats was prepared as follows. The standard rat diet procured from the chow factory (Bilyem, Ankara, Turkey) were ground into powder. And, they were made a homogeneous mixture with the addition of 5 % oily soy and 5 % egg, as a source of protein, and 50 % suet as a source of energy [13], and then they were turned into pallets and stored in a freezer. The diets prepared fresh every week were removed from the freezer, an hour before feeding the experimental animals. The ratio and energy value of the chows prepared are presented in Table 1.

The study was completed in a total of 10 weeks. The first 6 weeks were planned to be the stage of HFD feeding (the phase of forming insulin resistance) and the stage of forming experimental type 2 diabetes model. And, in the last 4 weeks, the rats in the relevant groups were treated with safranal.

**Table 1.** Contents of the Diets Used in This Study

| Ingredient (%) | Composition of the standard diet (%) | Composition of the HFD (%) |
|---|---|---|
| Fat | 4.1 | 57.3 |
| Protein | 17 | 13.6 |
| Carbohydrate | 76.4 | 30.1 |
| Other substances | 2.5 | 1.5 |
| Metabolizable energy (cal/kg) | 2600 | 4930 |

## FORMATION OF THE EXPERIMENTAL TYPE 2 DIABETES MODEL

First, the development of insulin resistance in the rats was provided, for a successful simulation of insulin resistance. After that, β-cell dysfunction was formed. With intent to ensure the development of insulin resistance in the experimental animals, HFD was applied to the rats other than the ones in the control group, for a period of 4 weeks. Afterward (in the fourth and fifth weeks), streptozotocin (STZ) (Sigma, St. Louis, USA) dissolved in citrate buffer (pH 4.5) was administered every other week by ip injection, in a dose of 30 mg/kg and two times. A week after the last injection (6 weeks), plasma glucose levels of rats were controlled with a glucometer (Accu-Chek Performa Nano, Roche, Germany), and the formation of type 2 diabetes was considered to occur in the rats with a plasma glucose level of 300 mg/dL and above [14]. The research groups were designed as follows:

Group 1 (CONT): Formed with healthy rats.
Group 2 (HFD): Formed with the rats, which were fed with high-fat diets having high energy during the study period.
Group 3 (DYB): T2DM model was formed in the rats, which were fed with high-fat diets having high energy during the study period.
Group 4 (DYB-Saf): Safranal treatment was applied to the rats, in which type 2 diabetes model was formed, for a period of 4 weeks.
Group 5 (HFD-Saf): Safranal treatment was applied to the healthy rats fed with HFD during the study.

During the study period, the changes in the weights and fasting glucose levels (FPG) of the rats were measured bi-weekly. Before the measurements, the rats were left without food all night long. The FPG levels were determined with one drop of blood taken from the tail vein and by means of a glucometer. At the end of the 4-week safranal application (tenth week of the study), the required blood and tissue samples were taken from the rats anesthetized with ketamine (65 mg/kg) and xylazine (7 mg/kg), which then were prepared biochemical analyses.

The plasma used in the analyses was obtained by centrifuging the blood samples taken into heparinized

tubes, at +4 °C and 3000 rpm for 10 min. The tissue homogenates used in the analyses were prepared as follows. The pancreatic tissue received in a weight of about 0.5 g was placed into the homogenizer (IKA T18, Germany), and then 5 mL of PBS (pH 7.4) solution containing 8 % protease inhibitor (Roche, Germany) was added and the mixture was homogenized. Ensuring the cold chain was attached importance at all stages of the experiment. The homogenates were centrifuged (Kubota, Japan) for 10 min at 15,000 rpm and +4 °C. The plasma and pancreatic samples prepared for use in the analyses were stored at −80 °C, until the performance of the laboratory analyses.

## BIOCHEMICAL ANALYSES

In the present study, tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6, and interferon gamma (IFN-γ) (e-Bioscience, Vienna, Austria) levels of the pancreas and plasma, levels of plasma insulin (Drg-Diagnostic, Germany), levels of plasma leptin (BioVendor, Bratislava, Slovak Republic), and total protein levels of pancreatic tissue were analyzed (Fluka, St. Louis, USA) in ELISA device, in accordance with the rat-specific kit protocols. Total antioxidant (TAS) and total oxidant (TOS) levels, which are among the oxidative stress parameters, were measured using kits (Rell Assay, Gaziantep, Turkey) that work with spectrophotometric methods [15, 16]. Oxidative stress index (OSI=[(TOS/TAS)×100]) was calculated by using the obtained TAS and TOS data. The results were calculated dividing the concentrations of the parameters analyzed in the pancreatic tissue (TAS, TOS, TNF-α, IL-1β, IL-6, IFN-γ) by the most recently obtained total protein levels. The results were shown as concentration per gram protein or concentration milligram protein.

## 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 was considered significant at the $p<0.05$ level. Data was analyzed with the Statistical Package for the Social Sciences (SPSS) version 18.0 for Windows.

## RESULTS

In consequence of the HFD administration to the rats, intended to form insulin resistance in the experimental animals, the highest weight gain was observed in the HFD groups (HFD and HFD-Saf groups). And, the STZ injection made with intent to form β-cell dysfunction led to a decrease in the weight gain levels in the diabetic study groups (DYB and DYB-Saf groups). It has been ascertained that the weight gain of the safranal has no effect neither on HFD groups nor diabetic groups (Fig. 1, Table 2).

The FPG levels of the control group showed no significant change throughout the study period. The FPG levels increased in parallel to the increase in the HFD group, and at the end of the study, it was determined that the FPG levels of the rats in the HFD group increased averagely to 155 mg/dL. The FPG levels were reduced to 120 mg/dL as a result of the safranal treatment applied to the rats of the HFD group, which became pre-diabetic. Among the rats, in which type 2 diabetes was formed with STZ application, the diabetes groups were seen (fourth week and after) to have the highest glucose levels (365.6 ±44.3), and then the levels were balanced (332.4±37.9). It was seen that safranal reduced the FPG levels (233.2±25.7) in the diabetic rats (DYB-Saf group) (Fig. 2, Table 3).

Insulin levels in the control group was found to be 0.1841 μg/L, and the lowest (0.1685 μg/L) and highest (0.3096 μg/L) insulin levels were in the DYB group and HFD group, respectively. It was ascertained that safranal reduced the insulin levels in the groups, to which high-fat diets were applied, and that it raised the insulin levels in the

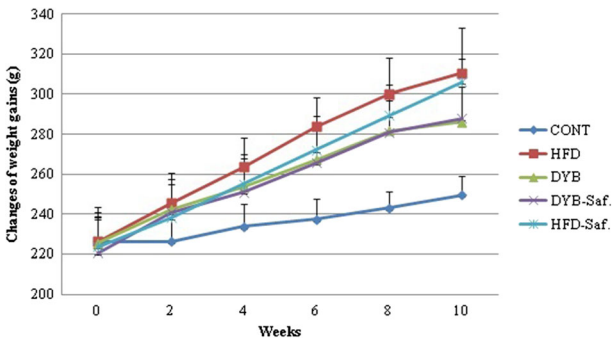


**Fig. 1.** The average weight gain observed in the experimental group throughout the study period. *CONT* control group, *HFD* the group that was given only high-fat diet, *DYB* the group that were fed a high-fat diet throughout the study and in which T2DM was generated, *DYB-Saf* diabetic group treated with safranal, *HFD-Saf* HFD group treated with safranal.

**Table 2.** The Average Weight Changes Observed in the Experimental Group Throughout the Study Period

| Study groups | The average weight changes observed in the experimental groups | | | | | |
|---|---|---|---|---|---|---|
| | Week 0 | Week 2 | Week 4 | Week 6 | Week 8 | Week 10 |
| CONT | 226.3±17.1 | 226.3±13.8$^{a}$ | 233.7±11.5$^{a}$ | 237.4±10.4$^{a}$ | 243.0±8.4$^{a}$ | 249.7±9.4$^{a}$ |
| HFD | 226.3±14.6 | 245.5±15.1$^{b}$ | 263.5±14.7$^{b}$ | 283.7±14.9$^{c}$ | 300.0±17.9$^{c}$ | 310.4±22.9$^{c}$ |
| DYB | 225.5±13.3 | 242.4±14.8$^{ab}$ | 253.5±14.5$^{b}$ | 267.1±15.8$^{bc}$ | 281.6±15.5$^{b}$ | 286.1±22.1$^{b}$ |
| DYB-Saf | 220.5±17.7 | 240.8±16.8$^{ab}$ | 251.0±12.7$^{b}$ | 265.6±18.6$^{b}$ | 280.8±17.4$^{b}$ | 288.0±15.8$^{b}$ |
| HFD-Saf | 223.6±13.8 | 238.12±16.6$^{a}$ | 254.9±14.9$^{b}$ | 272.0±17.1$^{bc}$ | 289.4±15.3$^{bc}$ | 306.1±11.5$^{c}$ |

Values are mean±standard deviation; $n=8$.

$^{a, b, c}$ Different letters in the same column represent statistically significant differences ($P<0.05$) among experimental groups for each parameter. Statistical differences were determined by comparing the experimental groups with each other. The differences were represented by letters. Among the data related to the experimental groups at the same column, only two groups were compared with each other as follows. It was considered that whereas there was no difference between the two groups bearing the same letter(s), statistical difference emerged between the groups bearing different letter(s).

*FPG* fasting plasma glucose level, *CONT* control group, *HFD* the group that was given only high-fat diet, *DYB* the group that were fed a high-fat diet throughout the study and in which T2DM was generated, *DYB-Saf* diabetic group treated with safranal, *HFD-Saf* safranal was given for 4 weeks to rats that were given a high-fat diet during the study

diabetic rats. Similar results were obtained with the examination of the leptin levels. The leptin levels were measured to be 315.2 and 655.9 pg/mL in the control and DYB groups, respectively. It was observed that the leptin levels raised (1039.9 pg/mL) in consequence of the HFD application. It was ascertained that safrana reduced the leptin levels (551.1 pg/mL) in the groups to which high-fat diets were applied, and that it raised the leptin levels (999.7 pg/mL) in the diabetic rats (Table 4).

IFN-γ, IL-β, IL-6, and TNF-α levels among the inflammatory cytokines analyzed in plasma are given in Table 4. When the table given was analyzed, an increase was observed in the IFN-γ, IL-β, and TNF-α levels in both the HFD and DYB groups, compared to the control group. This situation is a clear indication of the fact that the HFD feeding as well as diabetes inflammation increased. In terms of the treatment groups, safranal was observed to decrease inflammation by reducing IL-β and TNF-α levels, especially in the diabetic treatment groups. Similarly, it was seen that safranal significantly reduced the OSI index, *i.e.*, oxidative stress, by raising the TAS levels and reducing the TOS levels (Tables 4 and 5).

The results of the inflammatory cytokine and oxidative stress parameters analyzed in pancreatic tissue were considerably similar to the findings obtained in the plasma. Creation of T2DM in experimental animals leads to inflammation by increasing IFN-γ, IL-β, and TNF-α levels in pancreatic tissue. TAS levels decreased and TOS levels increased in the DYB group, in which T2DM was formed by means of HFD and STZ. In other words, the OSI was found to be statistically different and low-level in both the DYB and HFD groups, compared to the control group (Table 5).

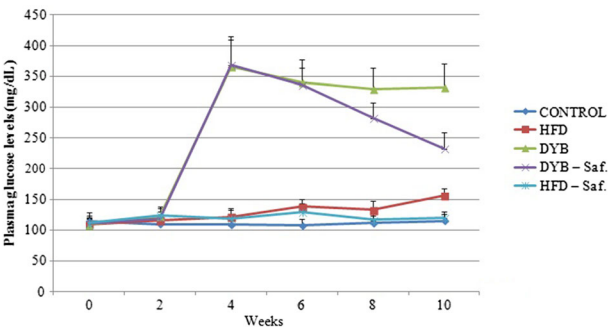


**Fig. 2.** Change in the FPG levels of the experimental groups during the study period. *CONT* control group, *HFD* the group that was given only high-fat diet, *DYB* the group that were fed a high-fat diet throughout the study and in which T2DM was generated, *DYB-Saf* diabetic group treated with safranal, *HFD-Saf* HFD group treated with safranal.

## DISCUSSION

Inflammation and oxidative stress that occurs in T2DM and obesity may cause formation of many complications. Especially in obese T2DM patients, it increases the risk of complications such as inflammation and oxidative stress, coronary heart diseases, hypertension, endothelial inflammation, neuropathy, and nephropathy [17–19]. Therefore, the possible effects of the agents with antioxidant and anti-inflammatory effects, on the treatment of diabetic and diabetic complications, are among the popular

**Table 3.** Change of FPG Levels During the Study Period

| Study groups | The average glucose level (mg/dL) changes of the study groups | | | | | |
|---|---|---|---|---|---|---|
| | Week 0 | Week 2 | Week 4 | Week 6 | Week 8 | Week 10 |
| CONT | 114.1±15.7 | 109.7±9.56$^{a}$ | 108.4±9.6$^{a}$ | 107.4±10.7$^{a}$ | 111.4±12.1$^{a}$ | 114.6±11.5$^{a}$ |
| HFD | 109.4±10.3 | 115.3±7.65$^{ab}$ | 121.2±11.4$^{a}$ | 138.1±11.7$^{b}$ | 132.6±15.1$^{a}$ | 155.6±12.4$^{b}$ |
| DYB | 108.4±8.8 | 122.8±12.0$^{b}$ | 365.6±44.3$^{b}$ | 340.7±36.8$^{c}$ | 329.9±34.2$^{c}$ | 332.4±37.9$^{d}$ |
| DYB-Saf | 111.0±9.7 | 119.1±10.7$^{ab}$ | 368.3±46.6$^{b}$ | 336.6±27.3$^{c}$ | 283.1±24.6$^{b}$ | 233.2±25.7$^{c}$ |
| HFD-Saf | 111.4±11.5 | 124.4±13.1$^{b}$ | 119.1±16.5$^{a}$ | 129.1±13.8$^{ab}$ | 116.6±12.4$^{a}$ | 120.1±9.5$^{a}$ |

Values are mean±standard deviation; $n=8$.
$^{a, b, c}$ Different letters in the same column represent statistically significant differences ($P<0.05$) among experimental groups for each parameter. Statistical differences were determined by comparing the experimental groups with each other. The differences were represented by letters. Among the data related to the experimental groups at the same column, only two groups were compared with each other as follows. It was considered that whereas there was no difference between the two groups bearing the same letter(s), statistical difference emerged between the groups bearing different letter(s)
*FPG* fasting plasma glucose level, *CONT* control group, *HFD* the group that was given only high-fat diet, *DYB* the group that were fed a high-fat diet throughout the study and in which T2DM was generated, *DYB-Saf* diabetic group treated with safranal, *HFD-Saf* safranal was given for 4 weeks to rats that were given a high-fat diet during the study

research topics of today's science world. It is because T2DM is a particular concern to the general population, due to the complications it causes. In the study presented in this context, the effects of safranal on inflammation and oxidative stress occurring both in obesity and stress were investigated. HFD was used to create obesity in experimental animals, and both HFD and STZ were used to create T2DM.

In the studies, where diet-induced obesity was developed, weight gain was observed to be greater in the rats subjected to HFD application, compared to the control group, and on the other hand, it was reported that liver steatosis, hyperleptinemia, hyperinsulinemia, and insulin resistance occurred in the HFD groups as well, during the formation of obesity, and that such increases were resulted from the induction of genes involved in obesity, such as

**Table 4.** Results of the Biochemical Parameters Analyzed in Plasma

| | Study groups | | | | |
|---|---|---|---|---|---|
| | CONT | HFD | DYB | DYB-Saf | HFD-Saf |
| FPG (mg/dL) | 114.6±11.5$^{a}$ | 155.6±12.4$^{b}$ | 332.4±37.9$^{d}$ | 232.2±25.7$^{c}$ | 120.1±9.5$^{a}$ |
| Insulin (ng/L) | 184.1±18$^{ab}$ | 309.6±5.5$^{c}$ | 168.5±18$^{a}$ | 219.7±36$^{b}$ | 215.7±24$^{b}$ |
| Leptin (pg/mL) | 315.2±73.5$^{a}$ | 1039.9±143.4$^{c}$ | 655.9±115.8$^{b}$ | 999.7±173.5$^{c}$ | 551.1±95.9$^{b}$ |
| TNF-α (pg/mL) | 54.6±6.9$^{a}$ | 70.4±12.9$^{b}$ | 100.9±14.2$^{c}$ | 70.3±9.7$^{b}$ | 73.6±6.5$^{b}$ |
| IL-1β (pg/mL) | 79.7±24.5$^{a}$ | 123.7±19.8$^{bc}$ | 153.4±27.5$^{c}$ | 97.9±33.6$^{ab}$ | 133.6±36.2$^{c}$ |
| IFN-γ (pg/mL) | 124.9±13.7$^{a}$ | 141.3±22.9$^{ab}$ | 153.4±22.7$^{bc}$ | 163.2±9.1$^{c}$ | 165.61±19.1$^{c}$ |
| IL-6 (pg/mL) | 24.55±3.90$^{a}$ | 26.27±2.19$^{a}$ | 27.76±3.07$^{a}$ | 27.92±1.50$^{a}$ | 33.91±7.26$^{b}$ |
| TAS (mmol torolox equiv./L) | 1.33±0.11$^{bc}$ | 1.46±0.22$^{c}$ | 0.95±0.17$^{a}$ | 1.38±0.28$^{bc}$ | 1.23±0.26$^{b}$ |
| TOS (μmol $H_2O_2$ equiv./L) | 3.28±0.31$^{a}$ | 4.71±0.64$^{b}$ | 10.56±0.95$^{d}$ | 3.87±0.52$^{a}$ | 5.59±0.76$^{c}$ |
| OSI (TOS/TAS)×100 | 248.7±31.2$^{a}$ | 325.8±56.6$^{a}$ | 1135.4±228.9$^{c}$ | 293±64.2$^{a}$ | 481.3±138.6$^{b}$ |

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 for each parameter. Statistical differences were determined by comparing the experimental groups with each other. The differences were represented by letters. Among the data related to the experimental groups at the same line, only two groups were compared with each other as follows. It was considered that whereas there was no difference between the two groups bearing the same letter(s), statistical difference emerged between the groups bearing different letter(s)
*FPG* fasting plasma glucose level, *CONT* control group, *HFD* the group that was given only high-fat diet, *DYB* the group that were fed a high-fat diet throughout the study and in which T2DM was generated, *DYB-Saf* diabetic group treated with safranal, *HFD-Saf* safranal was given for 4 weeks to rats that were given a high-fat diet during the study

**Table 5.** Results of the Biochemical Parameters Analyzed in Pancreas

| | Study groups | | | | |
|---|---|---|---|---|---|
| | CONT | HFD | DYB | DYB-Saf | HFD-Saf |
| TNF-α (pg/mg-protein) | $24.3 \pm 3.6^{a}$ | $38.8 \pm 4.1^{ca}$ | $47.9 \pm 3.5^{d}$ | $28.8 \pm 3.4^{b}$ | $39.4 \pm 4.7^{c}$ |
| IL-1β (pg/ mg-protein) | $25.5 \pm 2.7^{a}$ | $24.9 \pm 1.8^{a}$ | $29.2 \pm 1.7^{b}$ | $25.0 \pm 3.7^{a}$ | $28.4 \pm 1.6^{a}$ |
| IFN-γ (pg/ mg-protein) | $49.4 \pm 4.2^{a}$ | $53.2 \pm 5.6^{a}$ | $65.1 \pm 6.7^{b}$ | $50.4 \pm 3.8^{a}$ | $48.6 \pm 7.7^{a}$ |
| IL-6 (pg/ mg-protein) | $34.8 \pm 3.9^{a}$ | $37.5 \pm 1.1^{a}$ | $38.8 \pm 3.9^{a}$ | $43.3 \pm 4.3^{b}$ | $55.7 \pm 5.7^{c}$ |
| TAS (mmol torolox equiv./g protein) | $1.33 \pm 0.11^{bc}$ | $1.46 \pm 0.22^{c}$ | $0.95 \pm 0.17^{a}$ | $1.38 \pm 0.28^{bc}$ | $1.20 \pm 0.19^{b}$ |
| TOS (μmol $H_2O_2$ equiv./g-protein) | $2.09 \pm 0.30^{a}$ | $2.88 \pm 0.24^{c}$ | $3.28 \pm 0.19^{d}$ | $2.47 \pm 0.24^{b}$ | $2.98 \pm 0.23^{c}$ |
| OSI (TOS/TAS) × 100 | $584.9 \pm 141.8^{a}$ | $1004.9 \pm 265.7^{c}$ | $1346.6 \pm 166.9^{d}$ | $780.8 \pm 101.7^{b}$ | $868.5 \pm 79.3^{bc}$ |

Values are mean ± standard deviation; $n = 8$.

$^{a, b, c}$ Different letters in the same line represent statistically significant differences ($P < 0.05$). Statistical differences were determined by comparing the experimental groups with each other. The differences were represented by letters. Among the data related to the experimental groups at the same line, only two groups were compared with each other as follows. It was considered that whereas there was no difference between the two groups bearing the same letter(s), statistical difference emerged between the groups bearing different letter(s)

*FPG* fasting plasma glucose level, *CONT* control group, *HFD* the group that was given only high-fat diet, *DYB* the group that were fed a high-fat diet throughout the study and in which T2DM was generated, *DYB-Saf* diabetic group treated with safranal, *HFD-Saf* safranal was given for 4 weeks to rats that were given a high-fat diet during the study

leptin, TNF-α, and resistin, in the rats fed with HFD [20]. This study and many other similar studies show that the differences in the weight gains in the HFD-fed rats, compared to the control group, started from the second week, and obesity occurred in the following weeks, as a result of the increase in the insulin and leptin levels as well as the formation of insulin resistance [21, 22]. In consequence of the present study, as in the literature, obesity formation characterized by weight gain, hyperglycemia, hyperinsulinemia, and hyperleptinemia was observed in the rats of the HFD group.

In a study performed with intent to create T2DM model in the rats, by means of HFD and STZ injection, it is reported that the pre-STZ injection glucose levels of the rats fed with a diet providing 40 % of the 2-week-long energy value were similar to that of the control group, that the insulin levels were two times higher in the HFD group, and that an experimental diabetes was formed by performing STZ injection after the development of insulin resistance in the rats [23]. In another study, it is stated that low-dose STZ (30 mg/kg) injection applied every other week, after HFD application, forms a diabetes model that better imitates T2DM pathophysiology [14, 24]. If the data in Table 1 is analyzed, it is ascertained that first insulin resistance was formed in the rats by applying HFD, and then β-cell dysfunction was formed by means of low-dose STZ injection, in the present study. It is because when insulin levels were high in the HFD group, depending on the insulin resistance development, insulin levels were found to be the lowest in the diabetic group, due to the decrease in the insulin secretion resulted from β-cell damage in the diabetic group (Table 4). In the present study, these data show that the experimental T2DM model was successfully formed.

When safranal treatment was applied to the diabetic group (DYB-Saf) for a period of 4 weeks, the fasting glucose levels were observed to decrease from 368 to 233.2 mg/dL. These data have the quality to verify the antidiabetic effects of safranal stated in the literature [7]. However, although safranal was applied to the diabetic rats in doses close to each other (0.2 mL/kg-day for a period of 4 weeks), it did not reduce the fasting glucose levels to the extent specified in the study of Kainbakht and Hajiaghaee study [7]. This difference may be caused by the diabetes models formed in the studies. It is because in their studies, Kainbakht and Hajiaghaee (2011) state that they formed hyperglycemia by developing β-cell dysfunction with alloxan, without forming insulin resistance (without HFD application). Therefore, the findings of Kainbakht and Hajiaghaee (2011) are considered to be safranal's effect on diabetes, while the findings obtained with the present study can be interpreted as safranal's effect on type 2 diabetes.

In recent years, safranal's effect on oxidative stress were began to be investigated with experimental models simulating both diabetes and other disease physiopathologies. In one of such studies, it is stated that a 4-week safrana treatment was applied to the rats in which diabetes formed with STZ, in three different doses (0.25–0.50 and 0.75 mg/kg-day). It is reported that in the rats made diabetic with STZ injection, blood glucose, MDA, NO, total lipids, triacylglyceride, and cholesterol levels increased

while GSH levels, as well as CAT and SOD activities decreased; however, in the rats subjected to safranal treatment with different doses, these effects developed positively (recovered) in a dose-dependent manner. Thus, it is stated that safranal heals hyperglycemia, hyperlipidemia, and oxidative stress formed in rats made diabetic with STZ [9]; therefore, safranal may be protective against the complications of diabetes. Thus, also in the present study, safranal was found to decrease oxidative stress in both the plasma and pancreatic tissue (Tables 4 and 5). When the data on oxidative stress of pancreatic tissue are analyzed, safranal is observed to act as an antioxidant, especially by rising TAS levels and reducing TOS levels in diabetic rats. With the consideration of the fact that the antioxidant defense system in pancreatic β-cells are weak compared to other tissues, it is ascertained that safranal becomes useful in terms of diabetic treatment, by reducing β-cell that develops depending on any positive oxidative stress induced by STZ injection or glucotoxicity. However, it has been ascertained that although safranal reduces TAS levels, it does not affect oxidative stress in pancreatic tissues of HFD group rats made obese, and therefore, in terms of obesity treatment, it does not show its benefits that it shows in diabetic therapy.

No data about safranal's effect on inflammation was found in the literature. The data obtained in this context may help us to understand safranal's effect on inflammation. When analyzing the pancreatic tissues of the rats, in which type 2 diabetes was formed, any statistical difference is not observed between the cytokine levels (TNF-α, IL-1β, IL-6, IFN-γ) of the HFD-fed rats (HFD group) and the data on the control group. In other words, the 10-week HFD seems to have no effect on inflammation. Safranal administration to the HFD-fed rats did not affect the inflammatory cytokine levels (except for IL-6). And, when diabetes is formed in the rats, the levels of cytokines (TNF-α, IL-1β, IFN-γ) are observed to increase in both the HFD and control groups, *i.e.*, the formed type 2 diabetes caused inflammation. If safranal's effects in the diabetic group are evaluated, it can be said that safranal reduces inflammation, by reducing the cytokine levels. It is because the TNF-α, IL-1β, and IFN-γ levels of the DYB-Saf group subjected to safranal treatment in the fourth week are observed to be reduced, compared to the DYB group. And, when the plasma data are analyzed, it is ascertained that in diabetic rats, safranal treatment does not affect IFN-γ and IL-6 levels but reduces TNF-α and IL-1β levels (Tables 4 and 5).

If safranal's effect on pancreas and plasma inflammation in type 2 diabetic rats is summarized, it can be said that safranal reduces inflammation by reducing especially TNF-α and IL-1β levels both in pancreas and plasma. If the fact is considered that TNF-α and IL-1 receptors (IL-1R and TNFR) are among the activators of nuclear factor kappa B (NFκB) [25], the importance of safranal's ability to reduce TNF-α and IL-1β levels can be better understood. It is because it is also in inactive form in NFκB cell cytoplasms, and it proceeds to the core only when it is active, and on the other hand, it affects more than 200 genes controlling the immune system, growth, and inflammation responsible for cell growth, proliferation, regulation of apoptosis, cytokine production, and neoplastic transformation [25, 26]. Therefore, safranal's ability to reduce TNF-α and IL-1β levels that increased in diabetic rats may contribute to diabetic treatment.

## CONCLUSION

NFκB's specific activation in β-cells as a result of oxidative stress or inflammation in diabetes plays a key role in the formation of β-cell depletions. Nuclear translocation of Rel proteins, which are proteins providing NFκB's activation can be provided by free oxygen radicals and nitric oxide (NO) formed in the organism due to bacterial and viral pathogens, immune and inflammatory cytokines (TNF-α, IL-1β, INF-γ, *etc.*), radiation, or a variety of reasons [24, 27, 28]. If the fact considered that the safranal used in the present study reduced oxidative stress as well as TNF-α and IL-1B levels, among the important inflammatory cytokines, it may be thought to have some effects reducing β-cell damage levels, by relatively suppressing NFκB activation.

As mentioned in the present study, safranal's effect on insulin levels and insulin resistance is another subject for which it can be useful in terms of diabetic treatment. It has been ascertained that safranal provides insulin resistance by inhibiting the tyrosine phosphatase 1B and provides glucotoxicity by increasing the levels of glucose transporter-4 protein [29], and therefore, determination of how safranal increases insulin synthesis may be useful in terms of diabetic treatment. It is because many active substances increasing insulin secretion in today's diabetic treatment are used as antidiabetic drug [3].

Safranal's ability to decrease inflammation and oxidative stress resulted from diabetes may contribute to diabetic treatment, for also prevention of diabetic complications. For this reason, further studies may contribute to the development of new treatment protocols, for the determination of how safranal reduces especially TNF-α- and IL-1β-mediated inflammation.

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