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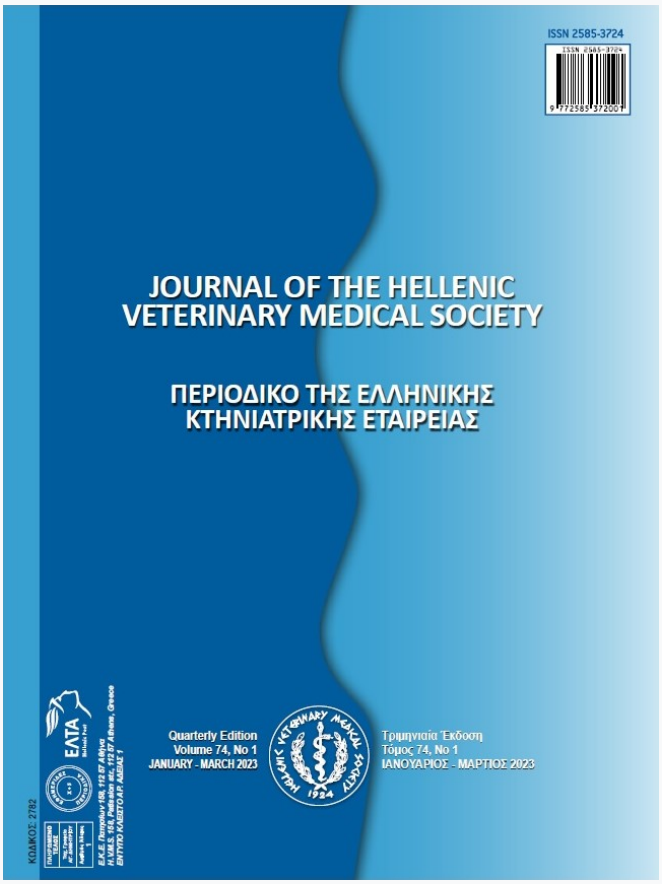

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Research article
Ερευνητικό άρθρο

Antidiabetic potential of chia (*Salvia hispanica* L.) seed oil in streptozotocin induced diabetic rat

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ABSTRACT: Chia seed oil (CSO) has biologically active components, such as vitamins, antioxidants, sterols, and others has been a subject of interest in recent years. The potential of chia seed oil to improve glucose levels and insulin tolerance, as well as regulate fasting serum insulin levels has been the focus of recent research.The aim this study was to investigate the hypoglycemic antioxidative/nitrosative, oxidative DNA damage and adenosine deaminase effects of chia seed oil on streptozotocin induced diabetes in rats. Sprague Dawley, adult, male rats were used in the study. Animals were divided into the following groups: Group I (Control, n=7), Group II (Chia Group, Chia seed oil (CSO) was applied by oral gavage at 1g/kg for 14 days, n=7), Group III ((Diabetes (DM) Group, Streptozotocin (STZ) was applied as 50 mg/kg i.p. single dose (n=7)), and Group IV (DM+CSO Group, STZ 50 mg/kg i.p. single dose+CSO was applied by oral gavage at 1g/kg for 14 days after diabetes was induced, (n=7)). Physiological parameters (body weight, blood glucose) were evaluated. STZ exposure increased serum TOS, OSI and NO levels, which are markers associated with oxidative damage, while PON enzyme activities decreased compared to the control group and did not change TAS levels. Moreover, 8-OHdG level and ADA activity were increased in the diabetic groups. In addition, it was found to regulate body weight and increased glucose in the diabetic group.

In line with our results, we think that chia treatment can regulate oxidative stress-mediated free radical damage caused by diabetes and may usefull as a therapeutic agent in reducing oxidative damage in diabetic subjects.

Keywords: chia; diabetes;oxidative/nitrosative stress; streptozotocin.

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INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder that is accepted with its most common feature, hyperglycemia (Ormazabal *et al*., 2018). Several physiological functions of the body are affected by DM. Rely on the type and course of diabetes, various complications may develop depending on the severity of carbohydrate, lipid and protein metabolism disorders (Sefi *et al*., 2012). The latest data published in the 9th edition of the International Diabetes Federation (IDF) Diabetes Atlas showed that 463 million adults worldwide are currently living with diabetes. It affects 14.6 % of the population in 1 out of 11 adults (20-79 years old). If adequate measures are not taken, it is estimated that 578 million people will have diabetes by 2030 and this number will increase to 700 million by 2045 (Saeedi *et al*., 2019). It is known that there is an increase in the production of free radicals due to glucose oxidation together with the decrease in antioxidant defense mechanisms due to the effect of hyperglycemia in diabetes. It is stated that this unstable condition triggered by diabetes and called oxidative stress causes cellular damage (Dalle-Donne*et al*., 2006).To be diagnosed with DM, blood sugar should be 126 mg / dL (7.0 mmol / L) and above after at least eight hours of fasting (World Health Organization, 2006).

Streptozotocin (STZ) is a glucosamine-nitrosourea antibiotic obtained from Streptomyces achromogenes, which is used in the induction of diabetes by chemical way. This diabetogen is widely used to experimentally generate a model of type 1 diabetes mellitus (T1DM) that causes pancreatic β-cell destruction (Furman, 2015; Akbarzadeh *et al*., 2007; Wu and Youming, 2008).

Chia seeds have become important for human health in terms of antioxidant component content such as phenolic compounds, α-linolenic acid, tocopherol, carotenoids, vitamins and some peptides (Ayerza and Coates, 2011; Marineli *et al*., 2014; Silva *et al*., 2018).These phenolic compounds, which can act as antioxidants, are shown to be associated with effective protection against oxidative stress related diseases by supporting the maintenance of oxidative balance in the cell (Marineli*et al*., 2015).It is stated that the high dietary fiber content of chia seeds reduces postprandial glycemia and exerts the hypoglycemic effect in diabetes by reducing carbohydrate release and blood glucose level (Oliveira-Alves *et al*., 2017; Vuksan *et al*., 2017).In addition, it was emphasized that it regulates glucose transport and impaired oxidative and non-oxidative glucose metabolism in the skeletal muscle of rats and reduces intracellular lipid content (Oliva*et al*., 2013). In recent years, chia seeds and oil have been investigated for their potential role and benefits on glucose metabolism (Enes *et al*., 2020; Fonte-Faria *et al*., 2019). Besides being an important metabolic and vascular regulator of nitric oxide (NO), it is stated that its production is related to insülin (Tessari *et al*., 2010).Adenosine deaminase (ADA) is suggested to be an important enzyme for modulating the bioactivity of insulin, but its clinical significance in diabetes mellitus (DM) is not yet characterized (Kurtul *et al*., 2004). DNA damage is one of the most common complications in diabetes mellitus; urinary 8-hydroxyguanosine (8-OHdG) as a marker for oxidative stress that reflects mitochondrial oxidative damage (Farrag *et al*., 2019). Studies evaluating the antioxidant capacity of chia *in vivo* are very limited.

In the study, the antidiabetic and body weight effects of Chia oil in a rat model with STZ-induced diabetes were evaluated. Besides, chia supplements; It was to aimed how to effects biochemical parameters such as total oxidant status (TOS), total antioxidant status (TAS), nitric oxide (NO), 8-hydroxydeoxyguanosine (8-OHdG) levels, and adenosine deaminase (ADA) and paraoxonase activity.

MATERIAL AND METHODS

Chemicals

The STZ and other chemicals used in the study were of analytically pure and were purchased from Sigma-Aldrich (St Louis, MO).For the induction of experimental diabetes, STZ 0.1M (pH 4.5) was dissolved in citrate buffer and administered at a dose of 50 mg/kg as described in previous studies (Furman, 2015; Wang-Fischer and Garyantes, 2018). For the treatment of experimental diabetes, Chia seed oil (100% cold press, 250 mL, ISO 9001naturoil, Turkey (80% of the CSO content is composed of α-linolenic acid (55-60% omega-3; n-3) and linoleic acid (18-20% omega-6; n-6)) was administered at a dose of 1g/kg/day as modified of Nieman *et al*., (2015) method.Glucose levels were measured after 72 h from the administration of STZ, fasting glucose levels in blood samples taken from the tail vein of rats fasted for 8 hours at night were determined by PlusMED Accuro brand biosensor glucose meter and strips. Those with a blood glucose level of 250 mg/dl and above were considered diabetic.Fasting glucose levels in tail vein blood samples and body weight were measured be-

Table 1. Effect of CSO on the serum physiological parameters (body weight, blood glucose) of STZ-induced diabetic rats

Parameters		Control (GrupI)	CSO (GrupII)	DM (GrupIII)	DM+CSO (GrupIV)	P (Anova. Kruskall)
Body Weight (g)	Initial	276.00±7.44	278.85±6.727	276.42±7.65	276.00±4.90	NS P=0.989
	Finish	277.28±7.70	276.42±6.61	254.00±8.98	278.57±4.19	P<0.05 X^2 (sd=3.n=28).P=0.099
Blood Glucose (mg/dl)	Initial	79.42±1,65	79.71±1.74	80±1.75	79.57±1.41	NS
	Finish	79.5714^{c}	77.0000^{c}	332.7143^{a}*	296.8571^{b}	P<0.001 X^2 (sd=3.n=28).P<0.001

Values are expressed as mean ± SEM of seven rats in each group. *: Differences between groups are statistically significant.Ns: There is no statistical difference between the groups. (Ns: Non-significant). a-b-c: The difference between groups in columns with different character is significant (*P<0.001)

Initial: weighing and measurement at 8 in the morning on the 1st day and every week. Finish: weighing and measurement at 8 in the morning on the 14th. day

fore (in case of fastingat 8 in the morning on the 1st day and every week (initial)) and at the end of the experiment (in case of fasting in the morning before at the end of the 14th day (finish) (Table 1).

Animals

In this study, 28 male Albino Sprague Dawley rats weighing 200-305 g were used as materials.Prior to the trial, permission was obtained from the Local Ethics Committee of the Animal Experiments of the Kafkas University (Decision no: KAU-HADYEK: 2019/079).The animals used in the experiment were obtained from the Experimental Animal Unit of Kafkas University. In addition, housing, care and experimental procedures were carried out at the Experimental Animal Unit of Kafkas University. The animals were kept in conventional rooms with controlled photoperiod (12:12 light:dark, lights on at 06:00 h, approximately 470 lx at 1 m above the floor), temperature (23 ± 2 °C), relative humidity (50-70%) and ventilation (18 air changes h^{-1}). The rats were housed per strain in groups of two animals in wire topped Macrolon type LF-3H cages (800 cm^2, Kobay Experimental Animals Laboratory, Ankara, Turkey) provided with sawdust bedding ((Tapvei 2H (2x2x1mm) Kobay Experimental Animals Laboratory, Ankara, Turkey), (low dust content in order not to harm the respiratory system of animals easy to remove from cages and it provide a large surface area that is absorbent and capable of taking up and binding both urine and faecal moisture for hygienic reasons)). Tap water and food-pellets (Bayramoglu Feed and Flour Industry and Trade Inc., Erzurum, Turkey) were provided ad libitum. The physical and chemical properties of feed are collectively presented in Table 2. The location of the objects remained the same throughout the experimental period without any contact between the groups. The tissues were renewed with daily cage cleaning. Five days before the start of a test the mice were brought in their cages to the test room to acclimatise. The same conditions were applied to all groups during the experiment.

Table 2. Feed content

Feed content	
Humidity	12,80%
Crude protein	23.00 %
Crude cellulose	3.70 %
Crude ash	8.30 %
Crude oil	1.70%
Sodium	0.50%
Manganese	96.00 mg/kg
Iron	31.00 mg/kg
Zinc	95.00 mg/kg
Cobalt	0.50 mg/kg
Selenium	0.30 mg/kg
Iodine	2.28 mg/kg
Vitamin A	12.000.000 IU/kg

Food-pellets (Bayramoglu Feed and Flour Industry and Trade Inc., Erzurum, Turkey)

Experimental design

Twenty-eight male AlbinoSprague-Dawley rats were randomly divided into four groups as seven rats in each group in the experiment.Groups were created as follows;

Group I (Control group): Non-diabetic control group.

Group II (Chia group): Chia seed oil (CSO) was administered orally at a dose of in at 1g/kg/day for14 days.

Group III (DM): STZ dissolved in citrate buffer was administered intraperitoneally (i.p) to rats at a single dose of 50 mg/kg.

Group IV (DM+CSO): Rats were administered a single dose of 50 mg/kg intraperitoneally (i.p) followed by oral administration of 1g/kg/day CSO to diabetic rats for 14 days.

At the end of the experiment, after intraperitoneal administration of xylazine-ketamine combination (15mg/kg-50mg/kg), blood samples were taken from the hearts of rats into heparin tubes. Serum samples were obtained by centrifuging the blood samples for 15 minutes at 3000 rpm and +4°C. All the samples were stored at -45°C until analyses. Serum samples taken were used to measure total oxidant status (TOS), total antioxidant status (TAS), 8-hydroxy-2'-deoxyguanosine (8-OHdG), nitric oxide (NO) levels andadenosine deaminase (ADA),paraoxonase activity (PON).

Biochemical analysis

Determination of total antioxidant and oxidant status: Total antioxidant status (TAS) were determined colorimetrically using commercial kit (Rel Assay®, Gaziantep, Turkey) in plasma samples. Antioxidants in the sample reduce dark blue-green colored 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical to colorless reduced ABTS form. The change of absorbance at 660 nm is related with total antioxidant level of the sample (Erel O,2004). Plasma total oxidant status (TOS) were determined with commercial kit (Rel Assay®, Gaziantep, Turkey). Oxidants present in the sample oxidize the ferrous ion-chelator complex to ferric ion. The oxidation reaction is prolonged by enhancer molecules, which are present in reaction medium. The ferric ion makes a colored complex with chromogen in an acidic medium. The color intensity is related to the total oxidant molecules present in the sample at 530 nm. Trolox and hydrogen peroxide standards were used for total antioxidant and total oxidant capacities **(**Erel O,2005).

Determination of oxidative stress Index (OSI): The OSI, which is an indicator of the degree of oxidative stress, was calculated according to the formula below, from the TAS and TOS kit protocol of Rell Assay.

OSI (arbitrary unit) =TOS (μmol H_2O_2 Eq/L) /TAS (mmolTrolox Eq/L).

Determination of nitric oxide levels: Nitric oxide concentrations were determined with chemical method in plasma samples. Plasma samples were deproteinized with 10% zinc sulphate. Total NO (nitrate and nitrite) concentrations were determined colorimetrically by the acidic Griess reaction (Miranda *et al*., 2001).

Adenosine deaminase (ADA) activity assay: ADA activity in serum samples was performed according to the method of Giusti and Galanti (1984). Adenosine, used as a substrate, was incubated with the sample at 37°C for 30 min. The ammonia formed forms blue indophenol in the presence of sodium hypochlorite and phenol in alkaline medium. In this experiment where sodium nitroprusside has a catalyst effect, the ammonia concentration is directly proportional to the absorbance of indophenol. Briefly, 1 mL of phosphate buffer and 50 μL of distilled water into the reagent blind tube, 1 mL ammonium sulfate and 50 μL distilled water into the standard tube, 1 mL adenosine solution and 50 μL sample into the sample tube, 1 mL of adenosine solution was added to the sample blind tube. After the test tubes were closed, they were incubated in a 37°C incubator for 1 h and 3 mL of phenol/nitroprusside solution was added. After adding 0.05 mL of sample to the sample blind tube, 3 mL of alkaline hypochlorite solution was added to all tubes. The tubes were closed again and incubated at 37°C for 30 min. After incubation, the absorbance of the tubes against the blind was read at 625 nm. One unit of ADA activity was defined as the amount of enzyme that releases 1 μmol of ammonia from adenosine per minute.

Paraoxonase (PON) activity assay:PON activity was determined according to the method specified by Furlong *et al*. (1988). In the measurement of PON activity, paraoxon was used as substrate. Absorbance of the colour, which emerges as the result of the hydrolysis of paraoxon at 37°C, was determined at 412 nm.

8-hydroxy-2'-deoxyguanosine (8-OHdG) : Levels of 8-OHdG were determined using enzyme-linked immunosorbent assay commercial kits according to manufacturer's procedures (SunLong and SunRed Bio, Shangai, China).All spectrophotometric analyzes were performed on the microplate reader (Bio-Tek Eon, USA).

Statistical analysis

Statistical analysis of the data obtained in the study was evaluated using the SPSS 20.0 software package

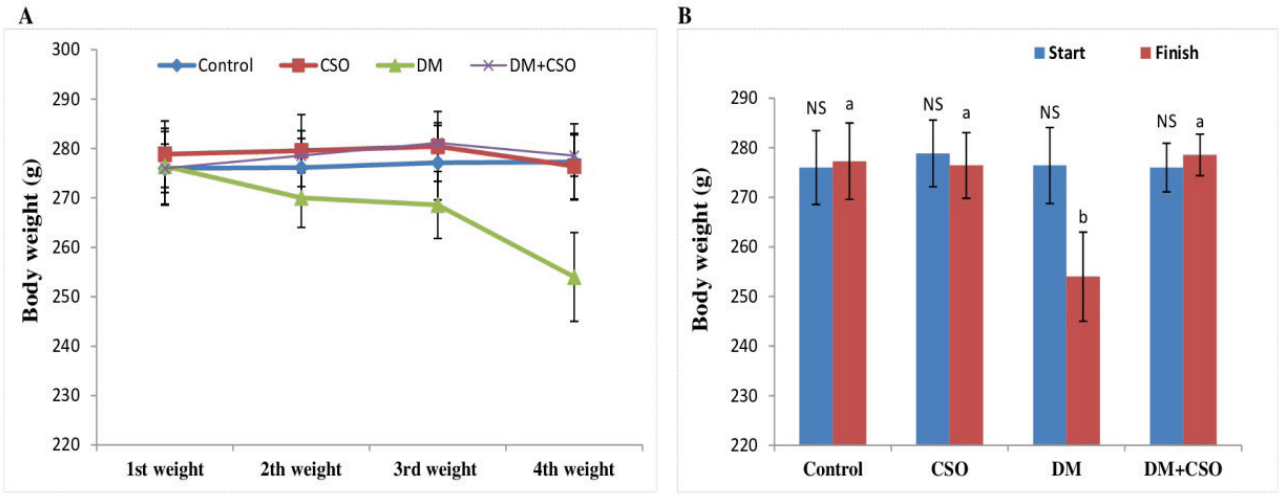

Figure 1. A) Effect of CSO on body weight of STZ-induced diabetic rats (P<0.05). B) Effect of CSO on body weight of STZ-induced diabetic rats. Start: weighing at 8 in the morning on the 1st day and every week. Finish: weighing at 8 in the morning at the end of the 14th day
The dissimilar fonts on the bar graph indicates the significant difference at P < 0.05.
[b]P<0.05 when Group III is compared with Group IV.
P=0.097 when Group I is compared with Group III.
P=0.053 when Group II is compared with Group III.

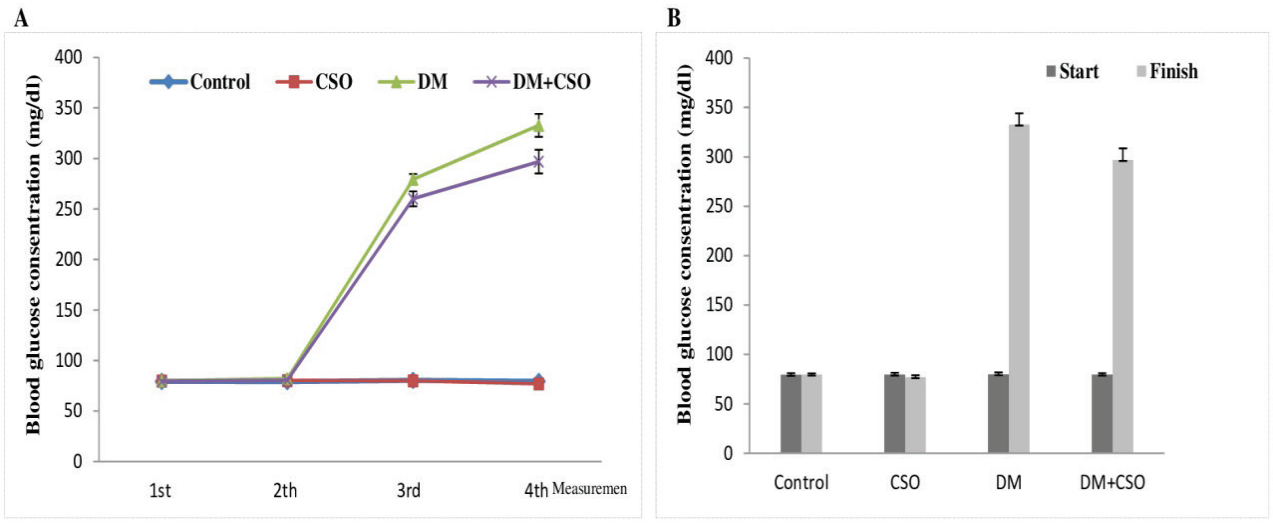

Figure2. Start: measurement at 8 in the morning on the 1st day and every week. Finish: weighing at 8 in the morning at the end of the 14th dayA) Effect of CSO fasting blood glucose level of STZ-induced diabetic rat.The dissimilar fonts on the bar graph indicates the significant difference at P < 0.001.B) Effect of CSO fasting blood glucose level of STZ-induced diabetic rat.
The dissimilar fonts on the bar graph indicates the significant difference at P < 0.05.
[c]P<0.001 when Group I and Group II is compared with Group III and IV.
[a]P<0.050 when Group III is compared with Group IIV.

(SPSS ver. 20.0 for windows professional edition). The mean and standard errors were used in data analyses. Kruskall-Wallis H analysis was conducted to determine the differences between the groups. The Mann-Whitney U-test was conducted to determine the source of significant differences among groups. The results of this study were presented as the mean ± standard error of means (SEM). Level of significance was accepted as p<0.05.

RESULTS

In the study, it was found that the initial weight was significantly decreased in the group in which diabetes was induced by STZ injection compared to the other groups (P<0.05) (figure 1a).When the difference between the groups was examined, it was found that the starting weight of the DM group decreased significantly compared to the DM+CSO group (P<0.05), but it was not significant compared to the control and chia

groups (P=0.097, P=0.053, respectively) (figure 1b).

Blood glucose levels in animals were found to be significantly increased in DM and DM+CSO groups compared to the experimental baseline level. (P<0.001) (figure 2a). When the difference between all groups was examined, it was found that the glucose level in the DM group increased significantly compared to the control and CSO groups compared to the baseline level, but it was not significant compared to the DM+CSO group (Table 1) (figure 2b), (P<0.001, P<0.001, P<0.05) (P<0.001).

Serum TOS levels were higher in the DM group than the other groups (P<0.05) (figure 3). It was observed that this level decreased in the DM+CSO group compared to the DM group, but on the contrary, there was a significant increase compared to both the control and chia groups (P<0.05).There was no statistically significant difference in TOS levels in the CSO group compared to the control and DM+CSO groups, respectively (P=0.548, P=0.151, respectively) (figure 3). However, it was low compared to the diabetes group and a statistically significant difference was found (P<0.05). There was no statistically significant difference between DM and DM+CSO groups (P=0.222). When TAS levels were examined, there was no statistically significant difference within and between groups (P=0.830) (P = 0.751).

When the OSI values of the groups were evaluated, it was determined that there was no significant difference between the control and chia groups, but there was a significant difference between the DM and DM+CSO groups of the control group. (P=0.310, P<0.05, P<0.05, respectively) (figure 4). It was determined that there was a significant difference between the CSO group and the DM group (P<0.05), and there was no significant difference between the DM+CSO groups (P=0.151) (Figure 4). Similarly, no significant difference was observed between DM and DM+CSO (P=0.548).

Serum PON activities was significantly lower in the DM group compared to the control and CSO groups, but there was no significant difference compared to the DM+CSO group. (P<0.05, P<0.05, P=0.095, respectively) (Table 3). (figure 5).

NO levels were statistically significant differences in all groups (P<0.001) (Table 3). NO levels in the control, CSO and DM+CSO groups were lower than in the DM group (P<0.005, P<0.05 respectively) (figure 6). Serum NO levels in the DM+CSO group were significantly higher than in the control group, and higher than in the chia group, but there was no statisti-

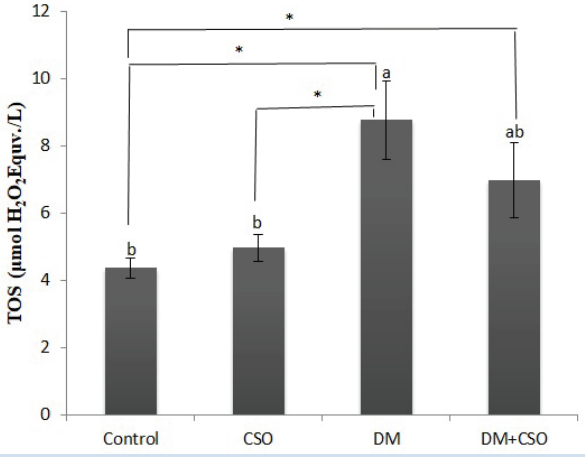

Figure 3. Effect of CSO on the serum TOS level of STZ-induced diabetic rats.
(a and b) : Different superscripts in thesame row show a statistical difference. (*P<0.05)
Group I compared with other groups P =0.548, P<0.05, P<0.05,respectively.
Group II compared with other groups P<0.05, P=0.151,respectively.
Group III compared with other groups P<0.05, P=0.151,respectively.
P=0.222 when Group III is compare with Group IV.

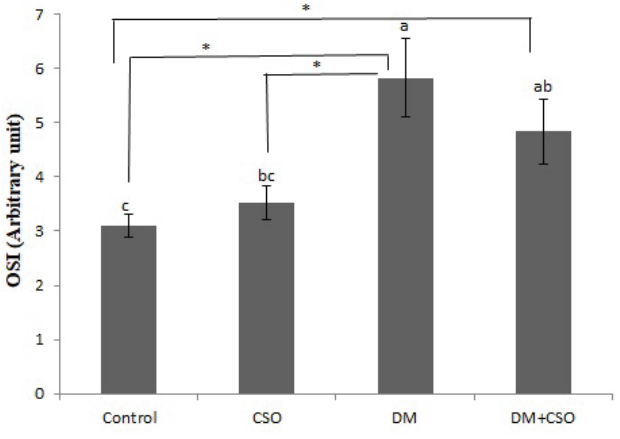

Figure 4. Effect of CSO on the serum OSI level of STZ-induced diabetic rats.
(a and b) : Different superscripts in thesame row show a statistical difference. (*P<0.05)
Group I compared with other groups P =0.310, P<0.05, P<0.05,respectively.
Group II compared with other groups P<0.05, P=0.151,respectively.
P=0.548 when Group III is compare with Group IV.

Table 3. The levels of CSO on biochemical parameters inSTZ-induced diabetes rats

Parameters	Control (GrupI)	CSO (GrupII)	DM (GrupIII)	DM+ CSO (GrupIV)	P (Anova. Kruskal)
TOS (μmol H_2O_2 Equv./L)	4.358±0.29^{b}	4.965±0.40^{b}	8.751±1.17^{a} *	6.972±1.11^{ab}	P<0.05 X^2 (sd=3.n=28).P<0.05
TAS (mmol Trolox Equv/L)	1.422±0.10	1.413±0.03	1.497±0.05	1.420±0.07	NS=0.830 X^2 (sd=3.n=28).P=0.751
OSI (arbitrary unit)	3.101±0.22^{c}	3.522±0.30^{bc}	5.832±0.73^{a} *	4.842±0.59^{ab}	P<0.05 X^2 (sd=3.n=28).P<0.05
PON (U/L)	40.278±2.083^{a}	42.042±2.25^{a}	30.77±2.427^{b} *	39.82±2.67^{ab}	P<0.05 X^2 (sd=3.n=28).P<0.05
8-OHdG (ng/mL)	0.975±0.05^{c}	1.055±0.02^{b}	1.740±0.06^{a} **	1.160±0.01^{b}	P<0.001 X^2 (sd=3.n=28).P<0.001
NO (μmol/L)	9.67±0.77^{c}	10.98±0.51^{bc}	15.52±0.96^{a} **	12.12±0.31^{b}	P<0.001 X^2 (sd=3.n=28).P<0.001
ADA (U/mL)	816.66±47.72^{b}	875.00±58.80^{b}	1233.33±176.85^{a} *	950.00±61.91^{ab}	(P<0.05) X^2 (sd=3.n=28).P<0.05

TAS. total antioxidant status; TOS.total oxidant status; OSI. oxidative stress ındex; PON. paraoxanase; NO. nitric oxide; 8-OHdG. 8-hydroxy-2'-deoxyguanosine; ADA. adenosine deaminase;
Values are expressed as mean ± SEM of nine rats in each group.*: Differences between groups are statistically significant.Ns: There is no statistical difference between the groups. (Ns: Non-significant). a-b-c: The difference between groups in columns with different character is significant. (*P<0.05;**P<0.001).

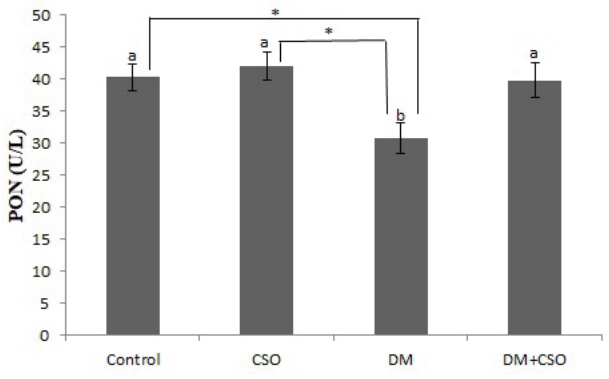

Figure 5. Effect of CSO on the serum PON activity level of STZ-induced diabetic rats. (a and b) : Different superscripts in thesame row show a statistical difference. (*P<0.05)
^{b}P<0.05 when Group III is compare with Group I and Group II.
P=0.095 when Group III is compare with Group IV.

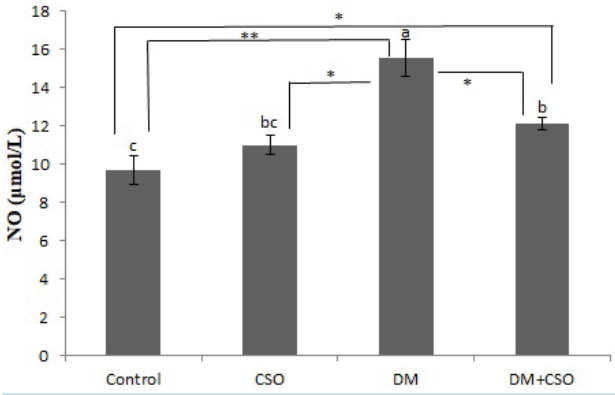

Figure 6. Effect of CSO on the serum NO level of STZ-induced diabetic rats.
(a, b and c) : Different superscripts in thesame row show a statistical difference. (*P<0.05;**P<0.005).
Group I compared with other groups P =0.589, P<0.005, P<0.05,respectively.
P<0.05 when Group III is compare with Group II and IV.
P=0.065 when Group IV is compare with Group II.

cal difference (P<0.05, P<0.05, P=0.065 respectively) (figure 6). There was no statistically significant difference between the control and CSO groups (P=0.589).

8-OHdG levels were higher statistically in the DM group compared to the other groups (P<0.001) (figure 7). When the differences between the groups were examined, it was found that there was no significant difference between the control and chia groups (P=0.240). However, significant differences were found between the control group and the DM and DM+CSO groups (P<0.005, P<0.05, respectively) (figure 7). Significant differences were found between the chia group and diabetes and DM+CSO groups (P<0.005, P<0.005, respectively) (figure 7). When the DM group was compared with DM+CSO group, it was found that there were significant differences (P<0.005) (figure 7).

Serum ADA activities were higher in the diabetes

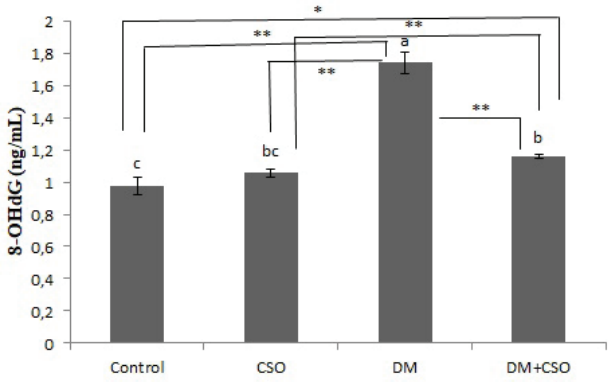

Figure 7. Effect of CSO on the serum 8-OHdG level of STZ-induced diabetic rats.
(a, b and c) : Different superscripts in thesame row show a statistical difference. (*P<0.05;**P<0.005).
Group I compared with other groups P =0.240, P<0.005, P<0.05,respectively.
P<0.05 when Group III is compare with Group II and IV.
P<0.05 when Group IV is compare with Group II.

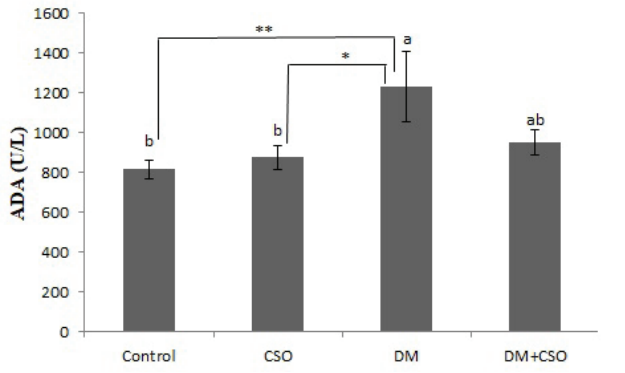

Figure 8. Effect of CSO on the serum ADA activity of STZ-induced diabetic rats.
(a and b) : Different superscripts in the same row show a statistical difference. (*P<0.05;**P<0.005).
Group I compared with other groups P =0.485, P<0.005, P=0.132,respectively.
P<0.05 when Group III is compare with Group II.
P=0.132 when Group III is compare with Group IV.
P=0.394 when Group II is compare with Group IV.

group compared to the other groups (P<0.05) (figure 8). There was no statistically significant difference between the control and chia groups (P=0.485). Besides, the ADA activities was lower in DM+CSO group compared to the DM group, but there was no statistically significant difference (P=0.132). Similarly, serum ADA activities were not statistically different in DM+CSO group compared to the control and chia groups (P=0.132, P=0.394, P=0.065, respectively) (figure 8) (Table 3).

DISCUSSION

The experimental model can be created based on the differences in the dose and frequency of STZ-induced diabetes model in the rat (Furman, 2015; Wu and Youming, 2008; Lee *et al*., 2003; Mohammed et al., 2021; Renitta et al., 2020).In this study, diabetes was induced in rats with STZ (50 mg/kg i.p.). CSO supplementation (1g/kg/day oral gavage) was then applied for 14 days. The experiment was terminated and blood was taken from animals.The effect of chia seed oil on serum 8-OHdG, NO, TOS, TAS, total protein levels,ADAand PON activityin experimental STZ-induced diabetic modelsin Sprague Dawley rats was investigated.

Because diabetes induced by STZ injection is a catabolic process, it leads to weight loss.The effect of chia seed oil on body weight in STZ-induced diabetes is shown in figure 1a and 1b.Similar to the results obtained with 50 mg/kg STZ administration in our current study, various researchers have shown that there is weight loss in diabetic animals (Akbarzadeh *et al*., 2007; Lee *et al*., 2003; Mohammed*et al*., 2021; Zhao et al., 2020).Also, our study showed that CSO treatment is beneficial in preventing weight loss in diabetic animals.There are data showing that it does not reduce or alter weight loss similar to that seen in animals we treated with 1 mg/kg of chia (Vuksan *et al*., 2017; Miranda *et al*., 2019).Although preventing of weight loss can be considered as a positive finding for stimulation of insulin secretion or breaking insulin resistance, diabetes and it does not appear to be a reliable criterion for the prognosis of its complications. However, it may be useful in evaluating the general well-being of animals.

β-cytotoxic drugs such as STZ are used experimentally as first-line options for induction of diabetes (Ward *et al*., 2001;Lenzen, 2008; Arison *et al*., 1967). In our study, there are different studies suitable for high glucose levels obtained in diabetic groups with 50 mg/mL STZ administration (Akbarzadeh *et al*., 2007; Zhao *et al*., 2020; Ly *et al*., 2019; McLaughlin *et al*., 2020).Treatment is an important process in hyperglycemia, which is the main consequence of diabetes. For this purpose, we observed that 1mg/kg CSO administration decreased the glucose level increased with STZ treatment. Vuksan et al., (2017) found that chia seeds reduce carbohydrate release while maintaining good glycemic control. Likewise, Enes *et al*., (2020) observed that chia oil improves glucose tolerance in in vivo and in vitro

studies. However, Miranda *et al*., (2019) found no improvement in glucose tolerance with chia flour administration.This situation may be related to the treatment duration of studies. Also, as mentioned earlier, these different results can be attributed to the composition of chia seed oil content, variety, and growing conditions (temperature, climate, and soil type) (Silva *et al*., 2017).

Diabetes causes excessive ROS production as a marker of oxidative damage that develops as a result of hyperglycemia (Brownlee, 2001). Previous studies show that an association between hyperglycemia, oxidative stress, and diabetic complications (Damasceno *et al*., 2014; Raza *et al*., 2011; Simmons, 2006; Raza and John 2004; Fernandes *et al*., 2016). With that context; we analyzed TAS, TOS and OSI levels as markers of oxidative stress. Our study confirms that increased glucose in rats showed increased TOS levels associated with decreased antioxidant barriers. We observed that TOS level plays a critical role in cases that develop diabetes and increase in hyperglycemic conditions with STZ treatment (figure 3). In addition, we determined that the increased TOS level in animals that developed diabetic with STZ application decreased with CSO application. However, this decrease was not significant when compared to the diabetes group. Also, there was no change between groups at the TAS level. In addition, we found that PON enzyme activity, which is considered an antioxidant enzyme because it prevents the oxidation of lipid peroxides, is low in the diabetic group, PON activity increased with chia application and approached the control group ($P<0.05$). Namitha *et al*., (2015), Suvarna *et al*., (2011) and Elsaid et al., (2015) support this decrease in the PON activity we have achieved. OSI is another marker of oxidative stress that increases with the application of STZ in our study. When we evaluate the relationship between oxidative stress levels and diabetes in our study; In the diabetic group, TOS and OSI levels were higher than the healthy control group, while TAS level did not change, while PON activity was lower than the healthy control group. While there was a positive relationship between TOS and OSI, there was no positive relationship between PON and TAS. This situation indicates that in the deterioration of oxidant and antioxidant balance, unchanged or decreased antioxidants occur before the increase in oxidants. In a previous study, found that the serum TOS and OSI values were significantly higher in diabetic cataracts than in senile cataracts (Saygili *et al*., 2010). Besides, in two different studies, the OSI values were significantly greater in the diabetic nephropathy patients compared to controls (Inci et al., 2016; Tabur et al., 2015). Although there is no report regarding OSI in diabetes in the literature, previous reports support our results. Moreover, as we mentioned above, although the total antioxidant status level did not change, PON activity increased in the DM+CSO group with chia treatment. Decreased PON activity may be associated with glycation or a circulating inhibitor due to increased oxidant agents. This can be explained by the fact that chia prevents the inhibition effect on PON caused by STZ or increases its activity. In addition, some researchers have discovered that unlike us, there is a change in the level of different antioxidant parameters other than PON (Fernandes *et al*., 2016; Ghosh *et al*., 2015; Matsunami *et al*., 2011; Kahya *et al*., 2016; Raza *et al*., 2011)

NO level was also increased significantly in STZ exposed animals. Indeed, increased NO may have contributed to cell damage in diabetes and down regulated glucose entry into cells. Supporting, the results obtained by Raza *et al*., (2011) are in line with our results. In addition to these, it is stated in previous studies that STZ is a NO donor and contributes to cell damage with the cytotoxic effect of increased NO (Morgan*et al*.,1994; Turk *et al*.,1993). Likewise, Ghosh *et al*., (2015) found that exposure to STZ increased NO production due to liver damage and curcumin treatment had a reducing effect on this damage. The decreased NO level in the rats to which we applied chia can be associated with the chia regulating the entry of glucose into the cell.

Furthermore, some research in recent years suggests that streptozotocin has genotoxicity and contributes to DNA damage (Pettepher *et al*.,1991; Kroncke*et al*., 1995). Oxidative stress resulting from increased ROS production in hyperglycemia developing in diabetes causes oxidative DNA damage (Pan *et al*., 2010). As a biological marker for studying oxidative DNA damage, 8-OHdG is known as a sensitive indicator (Shigenaga*et al*.,1989; Loft *et al*., 1992;Kart*et al*., 2016). Park *et al*., (2001) concluded in their study on STZ-induced diabetic rats that plasma 8-OHdG is a good indicator for oxidative DNA damaged in tissue. As a result of our study, we found that the increased 8-OHdG level in the diabetic group undergoing STZ decreased with CSO treatment. Similar to us, Karahan et al 2018 found that the 8-OHdG level was high in the diabetes group, and this level decreased in the treatment group and approached control (Karahan *et*

al.,2018).In addition, the study examining the anti-diabetic effect of Heteroxenia ghardaqensis extract on streptozotocin (STZ) -induced diabetes showed that it was accompanied by increased DNA damage, which reduced the effectiveness of DNA repair (Farrag *et al*., 2019). CSO was considered as data supporting the positive effects of reducing DNA damage. Consequently, the decrease in glucose levels after CSO treatment may be evidence for healing islet cells damaged by STZ, and the decreased 8-OHdG levels with CSO treatment may be an indicator for diabetes prevention.

In addition to the increased oxidative DNA damage in diabetes mentioned above, it is stated that adenosine signaling affects insulin secretion (Ohtani *et al*.,2013). It also emphasizes that the adenosine system plays a role in the regulation of glucose homeostasis and is an important factor in the pathophysiology of diabetes (Antonioli *et al*.,2015). In the results we obtained, we found higher ADA levels in the hyperglycemic diabetes groups with STZ exposure. We found that chia application reduced this increase. One study also shows that in serum ADA activity is increase significantly in diabetes mellitus (Niraula *et al*., 2018). Another study showed higher serum ADA activity and fasting plasma glucose (FPG) levels in nonobese T2DM patients, and a strong correlation between ADA and FPG which suggests an association between ADA and nonobese T2DM subjects (Khemka *et al*., 2013).On the other hand, it has been stated that increased ADA activity in type 2 DM may be a sign for insulin indication (Kurtul *et al*., 2004). According to the results we obtained in line with these data, it may have triggered an increase in ADA as an immune response in diabetic rats damaged by STZ. It also supports the evidence that the increase in plasma glucose of diabetic rats is responsible for the increase in ADA activity.

CONCLUSIONS

In this study, we showed that an association between serum ADA activity,8-OHG and oxidant/antioxidant parameters in diabetes. This suggests that serum 8-OHG and ADA may be a useful biomarker for the assessment of oxidative DNA damage in diabetes. In addition, our data suggests that combined treatment of chia would be helpful for the reduction of oxidative stress in diabetes. Chia treatment can balanced oxidative stress-mediated free radical damage related to diabetes and may usefull as a therapeutic agent in reducing oxidative damage in diabetic subjects.

ETHICS STATEMENT

All procedures involving rats were operated under strict criteria based on the Guide for Care and Use of Laboratory Animals of Animal Experiments Center, Kafkas University, and the protocols were approved by Kafkas University, Animal Experiments Local Ethics Committee (Decision no: KAÜ-HADYEK: 2019/079).

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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