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RESEARCH ARTICLE

The Effect of Carob Aqueous Extract on Oxidative Stress, Proinflammatory Cytokines, Glucose and Lipid Concentrations in the Blood of Diabetic Rats

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ABSTRACT

Herbal treatments offer potential benefits in controlling blood glucose levels and preventing diabetes-related complications. This study was conducted to determine the effects of application of an aqueous extract of carob fruit prepared by ultrasonic-assisted extraction (UAE) method on oxidative stress, glycemic level, pro-inflammatory cytokines and lipid profile levels in the blood of rats induced experimental diabetes with streptozotocin-nicotinamide (STZ-NA) model. Forty male Wistar Albino (200-250 g live weight) animals used in the study were divided into four groups as normal control, diabetic control, normal group given 200 mg.kg^{-1} carob aqueous extract and diabetic group given 200 mg.kg^{-1} carob aqueous extract. At the end of the 21-day study, plasma glucose, hemoglobin A1C (HbA1c), insulin, homeostatic model assessment of insulin resistance (HOMA-IR), malondialdehyde (MDA), reduced glutathione (GSH), proinflammatory cytokines interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), total cholesterol, triglyceride, leptin and 25-hydroxy vitamin D levels were measured in blood samples taken from all animals. Treatment of carob aqueous extract did not cause a significant decrease in high glucose, HbA1c and HOMA-IR values caused by diabetes. The blood insulin levels were not affected by the treatments. Diabetes increased MDA, IL-6 and triglyceride levels ($p<0.05$), while treatment of carob aqueous extract to diabetic animals did not affect these parameters. However, treatment of carob aqueous extract to diabetic animals increased GSH levels ($p<0.05$). The treatments had no effect on IL-1β, TNF-α, total cholesterol and leptin levels. We concluded that the carob fruit aqueous extract treatment had no effect on controlling hyperglycemia.

Keywords: Carob aqueous extract, Cytokines, Diabetes, Dyslipidemia, Hyperglycemia, Oxidative stress

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Diyabetik Sıçanların Kanında Keçiboynuzu Sulu Ekstraktının Oksidatif Stres, Proinflamatuar Sitokinler, Glikoz ve Lipid Düzeylerine Etkisi

ÖZ

Bitkisel tedaviler kan glikoz seviyelerinin kontrol altına alınması ve diyabetle ilişkili komplikasyonların önlenmesinde potansiyel faydalar sunmaktadır. Bu çalışma; streptozotosin-nikotinamid (STZ-NA) modelle deneysel diyabet oluşturulan sıçanlara ultrasonik-destekli ekstraksiyon (UAE) yöntemiyle hazırlanmış keçiboynuzu meyvesi sulu ekstraktı uygulamasının kan oksidatif stres, glisemik düzey, proinflamatuar sitokin ve lipit profil düzeylerine etkilerinin belirlenmesi amacıyla yapıldı. Çalışmada 40 adet erkek Wistar Albino (200-250g canlı ağırlık) hayvan kullanıldı. Deneydeki hayvanlar normal kontrol, diyabetik kontrol, 200 mg.kg^{-1} keçiboynuzu ekstraktı verilen normal ve 200 mg.kg^{-1} keçiboynuzu ekstraktı verilen diyabetik grup olmak üzere dört gruba ayrıldı. Toplam 21 gün süren çalışmanın sonunda tüm hayvanlardan alınan kan örneklerinde; plazma glikoz, hemoglobin A1C (HbA1c), insülin, insülin direncinin homeostatik model değerlendirmesi (HOMA-IR), malondialdehid (MDA), indirgenmiş glutatyon (GSH), proinflamatuar sitokinler interlökin-1beta (IL-1β), tümör nekroz faktörü-alfa (TNF-α), interlökin-6 (IL-6), total kolesterol, trigliserit, leptin ve 25-hidroksi vitamin D düzeyleri ölçüldü. Keçiboynuzu ekstraktı uygulaması, diyabetin yol açtığı yüksek glikoz, HbA1c ve HOMA-IR değerlerinde önemli bir düşüşe neden olmadı. Uygulamaların kan insülin düzeylerine etkisi olmadı. Diyabet; MDA, IL-6 ve trigliserit düzeylerini artırırken ($p<0.05$), keçiboynuzu sulu ekstrakt uygulamasının bu değerlere etkisi gözlenmedi. Bununla birlikte, diyabetli hayvanlara keçiboynuzu sulu ekstraktı verilmesi, GSH düzeylerini yükseltti ($p<0.05$). Uygulamaların IL-1β, TNF-α, total kolesterol ve leptin düzeylerine etkisi bulunmadı. Sonuç olarak, keçiboynuzu meyvesi sulu ekstraktı uygulamasının hiperglisemiyi kontrol altına almada etkili olmadığı kanaatine varıldı.

Anahtar kelimeler: Dislipidemi, Diyabet, Hiperglisemi, Keçiboynuzu sulu ekstraktı, Oksidatif stres, Sitokinler

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INTRODUCTION

Diabetes mellitus (DM), a metabolic disorder, is increasingly common around the world and a growing public health problem (Kottaisamy et al. 2021; Cloete 2022). The two main types that constitute the majority of diabetics are type 1 diabetes mellitus (T1DM) and T2DM, which have unique pathophysiological features. Interventions aimed at controlling hyperglycemia, which is the primary line of treatment for diabetic patients have recently brought new insights into the understanding of DM. Current treatments for lowering blood glucose levels are only temporarily effective. However, they can not completely prevent the development of DM and its complications. Also, another burden for patients is side effects of most antidiabetic drugs such as gastrointestinal symptoms, weight gain, edema, heart failure, impaired renal function, pancreatitis and genital infections (Cloete 2022). Thus, natural plant products with multidimensional modes of action and various biologically active secondary metabolites are of interest for the management and treatment of diabetic patients due to their significant therapeutic potential and better safety profile. In numerous studies, bioactive compounds from herbal products, such as polyphenols, flavonoids and alkaloids have been shown to have beneficial effects on the prevention and treatment of T2DM through their effects on mechanisms related to glucose tolerance (Behl et al. 2022; Rodriguez et al. 2022; Hazra et al. 2023; Riaz et al. 2024; Shrivastav et al. 2024). In previous studies, bioactive compounds from plants have been reported to control blood glucose levels through various mechanisms such as modulation of antioxidant enzymes (Ighodora et al. 2017), stimulation of insulin secretion (Uehara et al. 2023) or alpha-glucosidase inhibition (Kumar et al. 2011).

Carob (*Ceratonia siliqua L.)* is also among the plants that have an effect on the lowering blood glucose concentrations (Rtibi et al. 2017; Qasem et al. 2018). Applications of carob fruit may have an antidiabetic effect due to their bioactive compounds, which may have glucose-lowering properties (Brassesco et al. 2021; Moumou et al. 2023). Previous studies have also shown that applications of carob fruit may have antioxidant, lipid-lowering and anti-inflammatory effects due to their bioactive compounds (Moumou et al. 2023; Laaraj et al. 2024). Because oxidative stress and low-grade inflammation are associated with diabetes, alleviating oxidative stress and preventing the elevation of chronic inflammation markers may benefit against diabetes-related complications (Dludla et al. 2023). Rtibi et al. (2017) evaluated the reduction of intestinal glucose absorption by carob aqueous extract, confirming that mainly polyphenols and flavonoids, as well as the high content of fiber and complex carbohydrates in carob pods may have hypoglycemic effects. We hypothesized that if carob aqueous extract has a hypoglycemic effect in diabetes, the oral administration of carob aqueous extract to experimental diabetic rats may alleviate oxidative stress, dyslipidemia and proinflammatory response caused by diabetes. Therefore, this study aimed to determine the effects of carob aqueous extract on glucose and insulin concentrations, proinflammatory cytokines, lipid peroxidation, glutathione levels, and lipid parameters in rats with experimental diabetes induced by streptozotocin (STZ)-nicotinamide (NA).

MATERIALS and METHODS

Plant Material and Preparation of Extract

The mature carob plant fruits were obtained fresh from a seller of medicinal herbs whose products can be used for scientific study purposes. This plant material (pulp and seed) was dried and ground into powder with a mesh size of 80-100 in a high-speed rotor mill (ZM 200, Retsch GmbH, Haan, Germany). Aqueous extract of carob fruit powder, consisting of 90% pulp and 10% seed by weight, was prepared by an ultrasonic bath (425 W, 40 kHz) (Daihan WUC-D06H, Wonju, South Korea) using 100 g carob powder and 500 ml ultrapure water, for 30 min at 30 °C (Roseiro et al. 2013). The extract was then filtered through white band filter paper with 6 micron pore size. The extract was given to the experimental animals immediately after preparation. The ultrasound-assisted extraction method used in this study can effectively preserve the antioxidant and anti-inflammatory properties of phytochemicals (Demesa et al. 2024).

Animals

A total of 40 male Wistar Albino rats (200-250g) used in the study were obtained from Afyon Kocatepe University Experimental Animal Application and Research Center. All rats were kept at 22±2 °C, 50±10% humidity, 12 hours:12 hours light/dark cycle and in a regularly ventilated environment. Rats were fed with standard pellet diet ad libitum and had free access to water. The study protocol was approved by Afyon Kocatepe University Animal Experiments Ethics Committee (protocol no: 49533702/150).

Induction of Diabetes

Diabetes was induced in animals with the streptozotocin-nicotinamide (STZ-NA) model according to the method of El-Beih et al. (2019) with some modifications. A single intraperitoneal (i.p.) injection of 80 $mg.kg^{-1}$ freshly dissolved STZ (AB352315, abcr GmbH, Karlsruhe, Germany) in ice-cold citrate buffer (0.1 M, pH 4.5) was administered to rats fasted from previous day, 30 min after i.p. administration of 100 $mg.kg^{-1}$ NA (Thermo Scientific Chemicals, Leicestershire, UK). 10% glucose was administered to the rats 6–24 h after STZ-

administration. Hyperglycemia was confirmed at 72 h and on day 10 after injection by Contour Plus Blood Glucometer (Ascensia Diabetes Care, Basel, Switzerland). Ten days after STZ-NA injection, almost all rats had non-fasting blood glucose levels >300 $mg.dl^{-1}$ and were considered diabetic.

Experimental Design

In the experiment, animals were divided into four groups: control group, diabetic control group (diabetes group), normal rats given carob aqueous extract (carob group) and diabetic group given carob aqueous extract (carob+diabetes group). 200 $mg.kg^{-1}$ carob aqueous extract was given to the carob and carob+diabetes groups and 0.5 ml physiological saline was given to other groups orally as a single dose for 21 days. As ensuring proper administration to animals by oral gavage, the extract was diluted to three times its volume with physiological saline and administered daily to animals.

Group 1: Control group: Normal+0.5 ml physiological saline

Group 2: Diabetes group: Diabetic+0.5 ml physiological şaline

Group 3: Carob group: Normal+200 mg.kg-1 carob aqueous extract

Group 4: Carob+Diabetes group: Diabetic+200 mg.kg-1 carob aqueous extract

Blood Collection for Parameter Determination

The next day of the 21-day period, blood were collected from rats fasted from previous day under ketamine (87 mg.kg-1) and xylazine (13 mg.kg-1) anesthesia. Blood was taken from the hearts of rats using syringes and placed in tubes containing dipotassium ethylenediaminetetraacetic acid (K2EDTA) and heparinised plasma. Blood samples collected in K2EDTA tubes were used to measure hemoglobin A1C (HbA1c) levels. Blood samples taken in plasma tubes were immediately separated in a Heraeus Megafuge 8 R refrigerated centrifuge (Thermo Fisher Scientific, MA, USA) at 4 °C and 4000 rpm for 10 minutes. The separated plasma was collected in Eppendorf tubes. Glucose, total cholesterol and triglyceride levels were determined by Cobas c-702, HbA1c levels by Cobas c-502 and 25-hydroxyvitamin D levels by Cobas e-801 analysers (Roche Diagnostics, Basel, Switzerland) in the Afyonkarahisar Health Sciences University Health Application and Research Center Medical Biochemistry Laboratory. Immediately afterwards, cytokines; interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), insulin and leptin hormone levels were determined by Enzyme-Linked Immunosorbent Assay (ELISA) method (BT LAB, China). Determination of plasma malondialdehyde (MDA) and reduced glutathione (GSH) levels were performed using the methods described by Draper and Hadley (1990) and Beutler et al. (1963), respectively, and the results were obtained on a Shimadzu 1601 UV-vis spectrophotometer (Tokyo, Japan). Calculation of homeostatic model assessment of insulin resistance (HOMA-IR) was performed using the formula; fasting glucose (mg.dl-1) x fasting insulin (mIU.l-1) / 405 (Matthews et al. 1985).

Statistical Analysis

Statistical analysis of the data was performed using the Statistical Package for Social Sciences (SPSS) 20.0 (IBM Corp., Armonk, NY, USA). Because the data obtained from the study did not show normal distribution, Kruskal Wallis test was used to determine whether there was a difference between the groups, and Dunn's test was used for pairwise comparisons when there was a difference. Data were expressed as mean±standard deviation. A p value of <0.05 was considered statistically significant.

RESULTS

As a result of the loss of animals during the experiment, the experiment was completed with 7, 8, 7 and 7 animals in the Control, Diabetes, Carob and Carob+Diabetes groups, respectively.

The effects of treatment of carob aqueous extract to experimentally diabetic animals on plasma fasting blood glucose, HbA1c, plasma insulin and HOMA-IR levels are shown in Table 1. Fasting blood glucose, HbA1c and HOMA-IR values in the Diabetes and Carob+Diabetes groups increased significantly compared to the rats in the Control group ($p<0.05$). There was no improvement in these parameters in diabetic rats with the treatment of carob aqueous extract ($p>0.05$). In insulin levels, there was not significant difference between the groups.

The effects of treatment of carob aqueous extract to experimentally diabetic animals on plasma MDA, GSH, IL-1β, TNF-α and IL-6 cytokine levels are shown in Table 2. While MDA levels increased significantly in the diabetic groups compared to the non diabetic groups ($p<0.05$), the oral administration of carob aqueous extract to diabetic animals did not decrease this increase ($p>0.05$). Plasma GSH levels increased significantly with treatment of carob aqueous extract in diabetic rats compared to the other groups ($p<0.05$). There was not significant difference in IL-1β levels between the diabetic groups and the Control group ($p>0.05$). However, the IL-1β levels of the Diabetes and Carob+Diabetes groups were found to be significantly higher than in the Carob group receiving carob aqueous extract ($p<0.05$). There was no statistically difference in TNF-α levels between the groups. While there was a significant increase in IL-6 levels in the Diabetes and Carob+Diabetes groups compared to the Control and Carob groups ($p<0.05$), the oral administration of carob aqueous extract to diabetic rats did not improve these levels ($p>0.05$).

Table 3 shows the effects of oral carob aqueous extract administration to diabetic animals on plasma total cholesterol, triglyceride, leptin and plasma 25-hydroxy

vitamin D levels. Diabetes significantly increased triglyceride levels ($p<0.05$), while the oral administration of carob aqueous extract to diabetic rats did not improve this increase ($p>0.05$). Total cholesterol and leptin levels did not differ significantly between the groups. Vitamin D levels decreased significantly in the Carob+Diabetes group compared to the Control and Carob groups ($p<0.05$).

Table 1. The effects of carob aqueus extract on plasma fasting blood glucose, HbA1c, insulin and HOMA-IR levels in diabetic animals (mean±SD).

	Control (n=7)	Diabetes (n=8)	Carob (n=7)	Carob+Diabetes (n=7)
FBG (mg.dl^{-1})	167±35^{b}	364±46^{a}	190±26^{b}	370±98^{a}
HbA1c (%)	3.88±0.14^{b}	7.70±0.45^{a}	3.92±0.10^{b}	7.98±0.55^{a}
Insulin (mIU.l^{-1})	2.34±0.29	2.62±0.45	2.11±0.27	2.65±0.65
HOMA-IR	**0.98±0.24^{b}**	**2.34±0.42^{a}**	**0.98±0.11^{b}**	**2.55±0.46^{a}**

FBG: Fasting blood glucose, HbA1c: Hemoglobin A1C, HOMA-IR: Homeostasis model assessment of insulin resistance, SD: standart deviation.
^{a,b}:The difference between groups with different letters on the same line is statistically significant ($p<0.05$).

Table 2. The effects of carob aqueus extract on plasma oxidative stress, proinflammatory cytokine parameters in diabetic animals (mean±SD).

	Control (n=7)	Diabetes (n=8)	Carob (n=7)	Carob+Diabetes (n=7)
MDA (nmol.ml^{-1})	5.96±1.40^{b}	9.37±0.83^{a}	4.84±1.11^{b}	8.72±1.30^{a}
GSH (mg.dl^{-1})	3.38±0.61^{b}	4.48±0.97^{b}	4.80±1.15^{b}	15.29±4.93^{a}
IL-1β (ng.ml^{-1})	4.94±0.42^{ab}	6.23±1.01^{a}	3.91±0.44^{b}	6.41±2.22^{a}
TNF-α (ng.l^{-1})	109±18	158±51	112±23	135±44
IL-6 (ng.l^{-1})	1.43±0.32^{b}	3.03±0.68^{a}	1.37±0.37^{b}	3.02±1.23^{a}

MDA: Malondialdehyde, GSH: Reduced Glutathione, IL-1β: Interleukin-1beta, TNF-α: Tumor necrosis factor-alpha, IL-6: Interleukin-6, SD: standart deviation.
^{a,b}:The difference between groups with different letters on the same line is statistically significant ($p<0.05$).

Table 3. The effects of carob aqueus extract on plasma total cholesterol, triglyceride, leptin and 25-hydroxy vitamin D levels in diabetic animals (mean±SD).

	Control (n=7)	Diabetes (n=8)	Carob (n=7)	Carob+Diabetes (n=7)
Total Cholesterol (mg.dl^{-1})	52±10	53±8	45±6	51±6
Triglyceride (mg.dl^{-1})	51±9^{b}	85±12^{a}	46±7^{b}	83±14^{a}
Leptin (ng.ml^{-1})	0.99±0.44	1.19±0.48	1.03±0.54	1.15±0.36
Vitamin D (µg.l^{-1})	13.08±2.06^{a}	11.11±1.32^{ab}	14.05±2.47^{a}	9.31±2.11^{b}

^{a,b}: The difference between groups with different letters on the same line is statistically significant ($p<0.05$).

DISCUSSION

The experimental diabetes animal models play an important role for studying the pathophysiological mechanisms and treatment methods of DM (Akinlade et al. 2021). Due to the high genetic similarity between mice, rats and humans (especially in terms of pancreatic structure and function), these animals are among the most widely used animal models in DM disease research (Kottaisamy et al. 2021). Carob pulp and seeds are reported to have natural biologically active compounds with beneficial biological actions

against diabetic conditions (Rtibi et al. 2017; Brassesco et al. 2021; Laaraj et al. 2023). Therefore, this study was conducted to evaluate the efficacy of an aqueous extract from carob pulp and seeds on oxidative stress, insulin and glucose levels. Since Rtibi et al. (2017) showed that 200 mg.kg-1 carob aqueous extract has hypoglycemic effects in the alloxan-induced diabetic rats, this dose level of carob aqueous extract was used in this study. Streptozotocin (STZ) is widely used to induce experimental diabetes in rats and mice because it produces a diabetes model with changes similar to those in human diabetes (Eleazu et al. 2013; Akinlade et al. 2021). Because nicotinamide has been shown to be protective against STZ damage to beta cells (Kuchmerovska et al. 2012), STZ and nicotinamide were used together in this study.

Although Rtibi et al. (2017) showed the hypoglycemic effect of 200 mg.kg-1 carob aqueous extract at 2 weeks in alloxan-induced diabetes, we did not observe the hypoglycemic effect of 200 mg.kg-1 carob aqueous extract at 3 weeks in STZ+NA-induced diabetes. In the present study, the blood glucose levels in the diabetic animals were significantly higher than in the non-diabetic animals ($p<0.05$), which is consistent with the report that blood glucose levels increased in rats with experimental diabetes induced with the STZ-NA model (Szkudelski 2012). In the present study, the treatment of carob aqueous extract did not decrease the blood glucose levels in the diabetic animals. This result is not consistent with the report that aqueous extract of immature carob pulp showed control of glucose concentration in alloxan-induced diabetic rats (Rtibi et al. 2017). The different results between studies may be due to the use of different chemicals (alloxan or streptozotocin) to induce experimental diabetes (Rtibi et al. 2017) or different extraction methods and dose levels of the carob (Qasem et al. 2018). Alloxan and streptozotocin as a diabetogenic agent are the most commonly used chemicals to induce an experimental diabetes (Ighodaro et al. 2017). However, they have different effects in terms of their ability to cause insulin deficiency and type 1 diabetes, and type 2 diabetes with insulin resistance (Singh et al. 2024). Alloxan-induced diabetes produces a situation similar to human T1DM (Federiuk et al. 2004). However, co-administration of STZ and NA causes insulin resistance and induces partial depletion of pancreatic insulin, inducing a condition reflecting T2DM (Nakamura et al. 2006). In this study, while fasting blood glucose increased in STZ-nicotinamide-treated animals compared to non-diabetic animals, there was no significant difference in insulin levels of the groups, indicating that T2DM developed in diabetic animals. In fact, Szkudelski (2012) reported that co-administration of NA with STZ may protect from insulin deficiency caused by only STZ administration. The lack of glucose-lowering effect of carob aqueous extract in diabetic animals in the present study is also not consistent with the report that the high dose of carob methanolic extract reduced glucose levels in STZ-NA-induced rats (Qasem et al. 2018). These results suggest that extraction type (aqueous or methanolic) may also be one of the factors influencing the results of glucose levels with the carob extract treatment to diabetic animals.

In the present study, HOMA-IR levels, an indicator of insulin resistance (Mughni et al. 2023), increased significantly in the diabetes and carob+diabetes groups induced with STZ-NA compared to animals in the control group. This result showed that insulin resistance developed in animals administered STZ-NA. However, the carob aqueous extract treatment did not affect HOMA-IR values in the diabetic animals. This result may also suggest that the administration of carob aqueous extract to the diabetic animals did not improve the beta cell function.

In this study, increasing MDA levels, a product of lipid peroxidation, in the diabetic rats is consistent with the report that MDA, as a marker of oxidative stress, increased in diabetes (Fatani et al. 2016). The oral carob aqueous extract administration to diabetic rats did not decrease the MDA levels. This result indicates that the aqueous extract of carob fruit was ineffective in reducing oxidative stress in diabetes. However, this is not consistent with the report that biologically active natural compounds such as flavonoids found in plants reduce the level of lipid peroxidation in blood plasma and tissues (Aloud et al. 2018). The increase of GSH levels in the carob+diabetes group in this study may support the result of Laaraj et al. (2024) reported that the carob fruit has valuable antioxidant properties. This shows that oral administration of carob aqueous extract to diabetic rats may increase GSH synthesis and enhance cellular defense against oxidative stress. This result supported the report of Nzekwe et al. (2020) found that a plant extract containing phenolic compounds increased GSH levels in the liver of diabetic rats, while GSH levels were not affected in diabetic control. In addition, polyphenols can show antioxidant effects by stimulating antioxidant enzymes and producing a synergistic effect with other antioxidant compounds (Lv et al. 2021). Therefore, the components in the carob aqueous extract used in this study may have stimulated GSH synthesis against oxidative stress caused by diabetes or reduced its use in diabetic rats through such mechanisms. Further studies to determine the underlying mechanisms of the increase in GSH levels with carob aqueous extract in diabetes are needed.

Considering the fact that diabetic patients usually exhibit irregular blood glucose levels, which leads to long-term hyperglycemia and low-grade tissue cytokine production (King 2008), in this study, IL-6 levels were observed to increase in the diabetes and carob+diabetes groups. IL-6, a pivotal cytokine in innate immunity (Ridker et al. 2021), was observed to be elevated in the blood of diabetic patients (Kado et al. 1999). Thus, the determination of blood IL-6 concentrations in diabetic patients is considered a valuable indicator of the low-grade inflammation of

diabetes at the systemic level (Pellegrini et al. 2024). However, the carob aqueous extract in the diabetic rats did not affect the blood IL-6 levels. This result may suggest that the carob aqueous extract can not alleviate the low-grade inflammation caused by diabetes at the systemic level.

In addition to hyperglycemia in DM, lipid metabolism disorders are often accompanied (Kane et al. 2021). In the study, diabetes increased blood triglyceride levels while total cholesterol levels were unchanged in the blood. This result is consistent with the report that total cholesterol levels can remain normal while blood triglyceride levels are elevated in diabetic dyslipidemia (Sugden and Holness 2011). The administration of carob aqueous extract to the diabetic rats did not affect triglyceride and total cholesterol levels. These results are not consistent with the results reported by Macho-Gonzalez et al. (2019). The discrepancy between studies may be due to differences in the diets and doses of carob fruit or extracts used in the studies. In fact, Nemet et al. (2022) reported that the efficacy of carob extracts in preventing diabetes-related dyslipidaemia varies depending on the extraction method and dose. In this study, levels of the leptin hormone released from the fat tissue and provided information about the size of the fat tissue (Schwartz and Porte 2005), did not differ significantly between the groups. This suggests that the change in blood lipid levels caused by diabetes did not affect fat tissue. In fact, the decrease in body fat stores in insulin-deficient diabetes causes a significant reduction in plasma leptin levels (German et al. 2010). In this study, plasma insulin and leptin levels in diabetic animals is consistent with the report of Soliman (2001) showed that there was a positive correlation in serum leptin and insulin levels of diabetic animals.

In the study, diabetes decreased the plazma 25-hydroxy vitamin D levels. This result is consistent with the results of Aly et al. (2016) reported that diabetes decreased the plasma vitamin D levels. Although the carob fruit is rich source of vitamin D (Laaraj et al. 2023), the oral administration of carob aqueous extract to diabetic animals did not improve the vitamin D concentration. This result may indicate that the use of carob fruit aqueous extract does not have a beneficial effect on vitamin D deficiency caused by diabetes.

CONCLUSION

The results of the study indicate that oral administration of the carob aqueous extract to experimentally diabetic rats does not have the ability to restore glycemic balance in DM. Also, the oral administration of carob aqueous extract to the rats with experimental diabetes did not exert activity to alleviate diabetes-induced oxidative stress, dyslipidemia, and pro-inflammatory response. However, the application method, extraction method, and dose factors may affect the effectiveness of carob fruit on diabetes. The results of the study will allow new research on medicinal plants to examine in depth the selection of chemical substances used to induce experimental diabetes, the metabolic pathways that cause diabetes, the underlying mechanisms of these metabolic pathways, and the evaluation of therapeutic interventions.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: The data of this study were taken of MTA's Phd thesis. AE contributed to the study as a supervisor.

Ethical approval: This study was carried out at Afyon Kocatepe University Reserch Animals Application Center. This research was approved by The Ethics Committee of the Faculty of Veterinary Medicine, Afyon Kocatepe University (AKUHADYEK, Ref No: 49533702/150, Date: 11/26/2021)

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