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Antidiabetic and Antioxidant Effect of *Origanum minutiflorum* O. Schwarz & P. H. Davis in Streptozotocin-Induced Diabetic Rats

Streptozotosin ile Indüklenmiş Diyabetik Sıçanlarda *Origanum minutiflorum* O. Schwarz & P. H. Davis'un Antidiyabetik ve Antioksidan Etkisi

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ABSTRACT

In this study, the antidiabetic potential of the aqueous extract of *Origanum minutiflorum* was tested using streptozotocin induced diabetic rats. Changes in body weight, blood glucose level, biochemical parameters, antioxidant enzyme activities and histopathological examination of kidney were evaluated. The administration of the aqueous extract and carvacrol to diabetic group rats caused a significant decrease in blood glucose, biochemical parameters of serum and significantly increased the concentration of high density lipoprotein cholesterol. The administration of the aqueous extract and carvacrol significantly increased the antioxidant enzyme activity and reduced the MDA level. The histopathological analysis of kidney showed that the aqueous extract and carvacrol had a possible ameliorative effect against kidney damage. The aqueous extract also showed α-amylase and α-glucosidase inhibitor activity with high antioxidant activity. These scientific results confirm the use of *O. minutiflorum* as adjuvant antidiabetic therapy.

Key Words

Antidiabetic activity, antioxidant activity, carvacrol, *Origanum minutiflorum*.

ÖZ

Bu çalışmada, O*riganum minutiflorum* sulu ekstresinin streptozotosin ile indüklenmiş sıçanlarda antidiyabetik potansiyeli test edilmiştir. Vücut ağırlığındaki değişim, kan glikoz seviyesi, biyokimyasal parametreler, antioksidan enzim aktiviteleri ve böbreğin histopatolojik incelenmesi değerlendirilmiştir. Sulu ekstre ve karvakrolün diyabetik grup sıçanlarına uygulanması kan glikoz seviyesi, serum biyokimyasal parametrelerde önemli derecede azalma ve yüksek yoğunluklu lipoprotein kolesterol seviyesinde önemli artışa sebep olmuştur. Sulu ekstre ve karvakrol uygulanması antioksidan enzim aktivitelerini önemli derecede artırırken, MDA seviyesini azaltmıştır. Böbreğin histopatolojik analizi, sulu ekstre ve karvakrolün böbrek hasarına karşı iyileştirici etkiye sahip olduğunu göstermektedir. Sulu ekstre aynı zamanda yüksek antioksidan aktivite ile α-amilaz *ve* α-glukosidaz inhibitor aktivite göstermiştir. Bu bilimsel sonuçlar *O. minutiflorum*'un antidiyabetik tedaviyi destekleyici olarak kullanımını doğrulamaktadır.

Anahtar Kelimeler

Antidiyabetik aktivite, antioksidan aktivite, karvakrol, Origanum minutiflorum.

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INTRODUCTION

Diabetes mellitus (DM) is defined as chronic hyperglycemia generated by defect of insulin action or insulin secretion or both. DM causes damage, dysfunctions and disabilities in various organs (eyes, kidneys, heart, brain, blood cells) in the longterm [1]. DM is a chronic metabolic disorder as well as an increased status of oxidative stress. [2]. The risk of cardiovascular diseases, retinopathy, nephropathy and neuropathy is higher in uncontrolled hyperglycemia patients. It has been suggested that free radical production increases and but radical binding systems decrease in DM. These developments have increased interest in free radicals in the pathogenesis of diabetes complications [3]. The prevalence of diabetes has increased dramatically all over the world at the last 20 years [4]. In our country, according to the Turkey Diabetes Epidemiology Project data, the prevalence of DM has been said as 7.2% [5]. However, if the diabetic patient number which is not recorded is considered, it can be estimated that this percentage will be more than that.

Antioxidants are molecules that contain phenolic function in the structure and prevent cell damage caused by free radicals [6]. Phenolics are known as plant secondary metabolites that are found widespread in plants [7]. Phenolic compounds have antioxidant, hydrogen donor and metal ions chelating properties [8]. Medicinal plants and extract, drugs etc. products which is extracted from plants are widely used for therapeutic purpose in our country and the world. Scientific data about the biological activities and action mechanisms of many extracts obtained from medicinal plants are still insufficient. Therefore, there has been a lot of interest in the scientific investigation of the pharmaceutical effects of products obtained from medicinal plants [9]. Nowadays, the pharmaceutical industry produced expensive drugs and the emergence of some side effects of synthetic products have caused people to seek remedy from natural herbal products again [10]. *Origanum* (Lamiaceae) includes 27 taxa and 24 species (endemic number 16) in Turkish flora. It is commonly consumed as spices and tea in Anatolia. It has antimicrobial, antioxidant, antiparasitic, antihyperglycemic and anti-diabetic activities. *O. minutiflorum* is an endemic plant. They are consumed as herbal tea, spice and medicinal herb in Turkey. The main compound of *Origanum* genus and also *O. minutiflorum* essential oils was reported as carvacrol in many studies [11]. No study has been found on its antidiabetic property in the literature. So, the objective of this study was to investigate the antidiabetic and antioxidant property of *O. minutiflorum* aqueous extract.

MATERIALS and METHODS

Plant material

Origanum minutiflorum O. Schwarz & P. H. Davis was collected from Antalya, Turkey (Konyaaltı-next to Saklıkent rock resort) during flowering season in July 2015 (39°50'03''K-30°20'41''D, 1815 m). The voucher specimen (Voucher number: Aksoy 2521) has been saved at Herbarium of Biology Department, Erciyes University.

Extraction (OM)

Decoction that is a traditional method used in Turkey was prepared. The underground part of the plants was dried at about at 25 °C and ground with a grinder. The powdered plant (1 g) was mixed with dH_2O (100 ml) and boiled for 10 min. The aqueous extract was filtered (Millipore 0.2 mm) after cooling at room temperature [12]. The extracts were evaporated to dryness (38-39 °C, 20-60 m bar, 90-108 rpm) with a rotary evaporator. The solvent was completely removed using a lyophilizer. The extract (OM) yield was measured and then stored at 4 °C.

HPLC assay

Phenolic components of extract were detected by HPLC/DAD according to assay detailed in our previous work [13]. Protocatechic, gallic, sinapinic, caffeic, *p*-hydroxy benzoic, syringic, *p*-coumaric, chlorogenic, ferulic, rosmarinic acid, cinnamic acid, *o*- coumaric, benzoic acids and catechin, epicatechin, vanilin, rutin, hesperidin, eriodictiol, quercetin, apigenin, kamferol and luteolin were used as standards. For identification and quantitative determination of phenolics in the tested sample, the obtained peaks were compared with corresponding peaks of the standards.

Total phenolic and flavonoid amounts

Folin-Ciocalteau and $AlCl_3$ colorimetric assays were used to examine total phenolic and flavonoid amounts of OM as detailed in our previous work [13]. Results were expressed as milligrams of gallic acid (GAE) and quercetin (QE) equivalents /g extract.

Antioxidant activity methods

The antioxidant activity of OM was spectrophotometrically determined by many assays which of procedures

were given reported in our earlier studies, including phosphomolybdenum, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and H_2O_2 scavenging activity, prevention of β-carotene bleaching, ferrous (Fe^{2+}) ions chelating, reducing power, ferric ions (FRAP) and cupric ions reducing (CUPRAC) activity. Total antioxidant activity was expressed as mg of ascorbic acid equivalents (AAE) /g extract. IC_{50} rates were detected [11].

Antidiabetic Activity

α-Amylase inhibitor activity was examined by Caraway-Somogyi iodine / potassium iodide (IKI) assay. Sample solution (1-16 mg/ml, 25 µl) was mixed with α-amylase solution (50 µl) in phosphate buffer (pH 6.9 with 6 mM sodium chloride) in a 96-well microplate and incubated for 10 min at 37 ◦C. After pre-incubation, the reaction was initiated with the addition of starch solution (50 µl, 0.05%). Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme (α -amylase) solution. The reaction mixture was incubated 10 min at 37 ◦C. The reaction was then stopped with the addition of HCl (25 µl, 1 M). This was followed by addition of the iodine-potassium iodide solution (100 µl). The sample and blank absorbances were read at 630 nm with Micro-plate Reader. The α-amylase inhibitory activity was expressed as % inhibition [14].

For α-Glucosidase inhibitor activity, sample solution (0.2-12.5 mg/ml, 50 µl) was mixed with glutathione (50 µl), α-glucosidase solution (50 µl, 0.2 units / ml) in phosphate buffer (pH 6.8) and PNPG (4-p-nitrophenyl -α-D-glucopyranoside, 10 mM, 50 µl) in a 96-well microplate and incubated for 10 min at 37 ◦C. Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme (α-glucosidase) solution. The reaction was then stopped with the addition of sodium carbonate (50 µl, 0.2 M). The sample and blank absorbances were read at 400 nm with Micro-plate Reader. The α-glucosidase inhibitory activity was expressed as % inhibition. As a positive control, acarbose was used. [14].

Animals

In present study, healthy adult male, Wistar albino rats (240±40 gr) were utilized. Animal experiments were conducted at Experimental Research Application and Research Center (DEKAM), Erciyes University (Kayseri, Turkey) after having approval from Erciyes University, Animal Experiments Local Ethic Board (ERUHADYEK) (Ethic decision no: 16/050/09.03.2016). Rats were placed in standard environmental conditions (25 ± 3 °C, 12 h dark /12 h light, 35-60 humidity) in polypropylene cages lined with husk. Rats were allowed to eat a standard pellet diet ad libitum and to have free access to water.

Induction of DM

DM was induced by intravenous injection of streptozotocin (STZ) at 40 mg/kg body weight. STZ was dispersed in cold sodium citrate buffer (pH: 4.5, 0.1 M). Buffer was administrated to control rats and non-diabetic extract groups (Group I and Group II). Glucose level of blood from tail vein was determined with glucometer (Glucometre, True result, USA) after 72h. Animal which had over 220 mg/dl blood glucose level was assessed diabetic and then distributed in groups [15].

Groups

The rats were distributed into eight groups. Each group included ten rats. Control Group: Normal rats applied only water, I and II: Non-diabetic rats were applied 20 and 40 mg/kg of OM, respectively, III: Diabetic rats were applied only water, IV and V: Diabetic rats were applied 20 and 40 mg/kg of OM, VI: Diabetic rats were applied 10 mg/kg of carvacrol, VII: Diabetic rats were applied the reference drug glibenclamide (10 mg/kg).

OM, carvacrol and glibenclamide solutions were prepared daily and orally administered during 21 days [12].

Body Weight

Body weights of rats were measured four times: on the day when rats were induced by injection of STZ, 5-6th days (diabetes was occurred), 12th day and 21th day (on the day the experiment was terminated).

Blood glucose levels

Blood glucose level was determined two times from tail vein blood on the day of first administration of extract, carvacrol and glibenclamide (1st day) and on the day the experiment was terminated (21th day) with glucometer (Glucometer, True result, USA).

Biochemical Analysis

Animals were sacrificed under anesthesia (500 µl ketamine + 200 µl xylazine) after 21th day. Obtained bloods were centrifuged at 2000xg for 20 min. The serums were stored at -20 °C [16]. Biochemical analyzes were performed in Erciyes University, Medical Faculty Central Laboratory. In the serum samples, the amounts of

triglycerides (TG), cholesterol, high density lipoprotein (HDL), creatinine, urea nitrogen (BUN), Ckd-Epi, AST (aspartate amino transferase) and ALT (alanine amino transferase) were detected [17].

Tissue preparation

Kidney tissues were removed from the sacrificed rats, washed in ice-cold saline and saved at -80 °C [16]. Kidneys were finely ground and homogenized in phosphate buffer (pH 7,4; 50 mM) and then centrifuged for 15 min at 9500×g at 4 °C. The supernatants were received for analysis [15].

Antioxidant Enzyme Analysis

The amount of MDA (Malondialdehyde), the activities of Superoxidase dismutase (SOD), Catalase (CAT), and Glutathione peroxidase (GSH-Px) in serum and kidney tissue were measured using commercial diagnostic kits (LZ ELIZA, Shanghai LZ Biotech Co, Ltd, China) [16].

Histopathological assessment

Histopathological analysis of kidneys was conducted [17]. Samples were cut off (5 µm) and the sheets were stained by Hematoxylin/Eosin (H/E). The microscopic analysis was conducted. For kidney tissue; presence of glomerulosclerosis, contraction of the bowman range, thickening of the capillary basement membrane, mesangial matrix expansion, increase in reticular fibrils, hydropic changes in the tubular epithelium and vacuolization, H glycogen accumulation in the proximal tubule epithelium have been recognized as histological damage [18].

Statistical analysis

Analysis of variance (ANOVA) and Bivariate correlation were performed using SPSS 22.0 for Windows.

RESULTS and DISCUSSION

The yield of OM was 45.4 ± 2.6%. Total phenolic and flavonoid amounts of OM were 363.59 ± 2.11 mg GAE/g and 31.40 ± 0.47 mg QE/g extract, respectively. Total antioxidant capacity of OM was 252.11 ± 0,6 mg AAE/g extract.

Phenolic components in OM were determined by HPLC and results was expressed as µg/g in Table 1. Total 23 standard compounds were used. Protocatechic acid, syringic acid, caffeic acid, *p*-coumaric acid, rosmarinic acid, *p*-hydroxy benzoic acid, eriodictiol, chlorogenic acid, cinnamic acid, quercetin, rutin, ferulic acid, luteolin and apigenin were detected. Gallic acid, vanillin, sinapinic acid, catechin, epicatechin, hesperidin, *o*-coumaric acid, benzoic acid and kamferol weren't detected. Rosmarinic acid (9.6 µg/g) was the predominant compound in the OM. The least abundant component is *p*-coumaric acid (0.02 µg/g).

Table 1. The phenolic composition of *O. minutiflorum* extract.

Phenolics	Amount (µg/g)
1-Protocatechic acid	0.08
2-p-Hydroxy benzoic acid	0.10
3-Chlorogenic acid	0.3
4-Caffeic acid	1.7
5-Syringic acid	0.6
6-p-Coumaric acid	0.02
7-Ferulic acid	0.6
8-Rutin	0.6
9-Rosmarinic acid	9.6
10-Eriodictiol	1.6
11-Cinnamic acid	0.1
12-Quercetin	1.7
13-Luteolin	0.9
14-Apigenin	2.4

OM had inhibitor effect on β- carotene bleaching. The inhibition rates of OM, BHT and BHA were 53.57%, 84.26% and 94.33% at 1 mg/ml, respectively. Inhibitory effect of OM is less than that of the standards at same concentration.

DPPH scavenging property was determined as percent inhibition of the initial DPPH absorption by OM (Figure 1). The statistical differences among the different concentrations of OM were important ($P < 0.05$). OM had high DPPH scavenging activity. The scavenging capacity of OM was 16.36%, 30.44%, 57.91%, 89.96% and 90.75% at 0.1, 0.25, 0.5, 1 and 2 mg/ml, respectively. At 2 mg/ml, inhibition value of OM is near to BHT (92.15%). IC_{50} value of OM and BHT were 14.21 and 7.5 µg/ml, respectively.

Statistical differences were found among the OM and standard compounds for H_2O_2 scavenging activity ($P < 0.05$). The H_2O_2 scavenging activity of OM depend on concentration (Figure 1). The activities of OM, BHA, BHT and gallic acid were 17.30%, 64.73%, 76.97% and 137.61% at 50 µg/ml, respectively. IC_{50} rates were 129.76, 23.16, 31.09 and 17.62 µg/ml, respectively. In respect of results, OM exerted low H_2O_2 scavenging effect.

The statistical differences among the different concentrations of OM and EDTA for Fe^{2+} chelating activity were important ($P < 0.05$) (Figure 1). OM showed 79.99% Fe^{2+} chelating properties which is lesser than that of EDTA (99.21 %) at 5.0 mg/ml. OM had moderate iron binding capacity.

In reducing activity assay, the colour of the test solution change many shades of green because of reduction capacity of the antioxidants [19]. Statistical differences among the reducing activities at different concentrations of OM and BHT were important ($P < 0.05$). The increased absorbance value reflects increased reducing power. Reducing capacity of OM is higher than that of the standard at the highest concentration (Figure 1).

The iron ion-reducing antioxidant potential (FRAP) of OM was measured as 3.44 mM/l. This value was lesser than L-ascorbic acid (4.52 mM/L).

CUPRAC assay measure the reduction of the Cu^{+2}-neocuproin complex to the Cu^{+1}-neucuproin complex [20]. The absorbance of complex generated is maximum at 450 nm. Absorbance and copper reduction

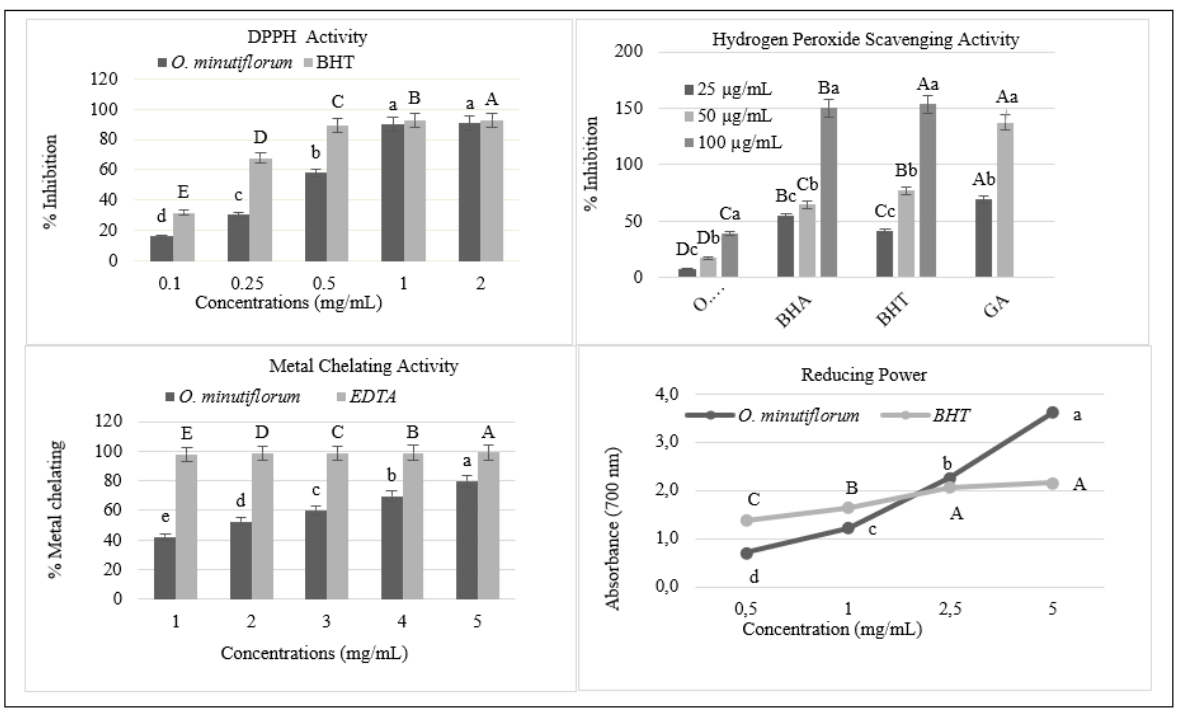

Figure 1. DPPH, H_2O_2 scavenging, metal chelating activities and reducing power of *O. minutiflorum* extract.

Lowercase and uppercase letters represent the statistical differences at $P < 0.05$.

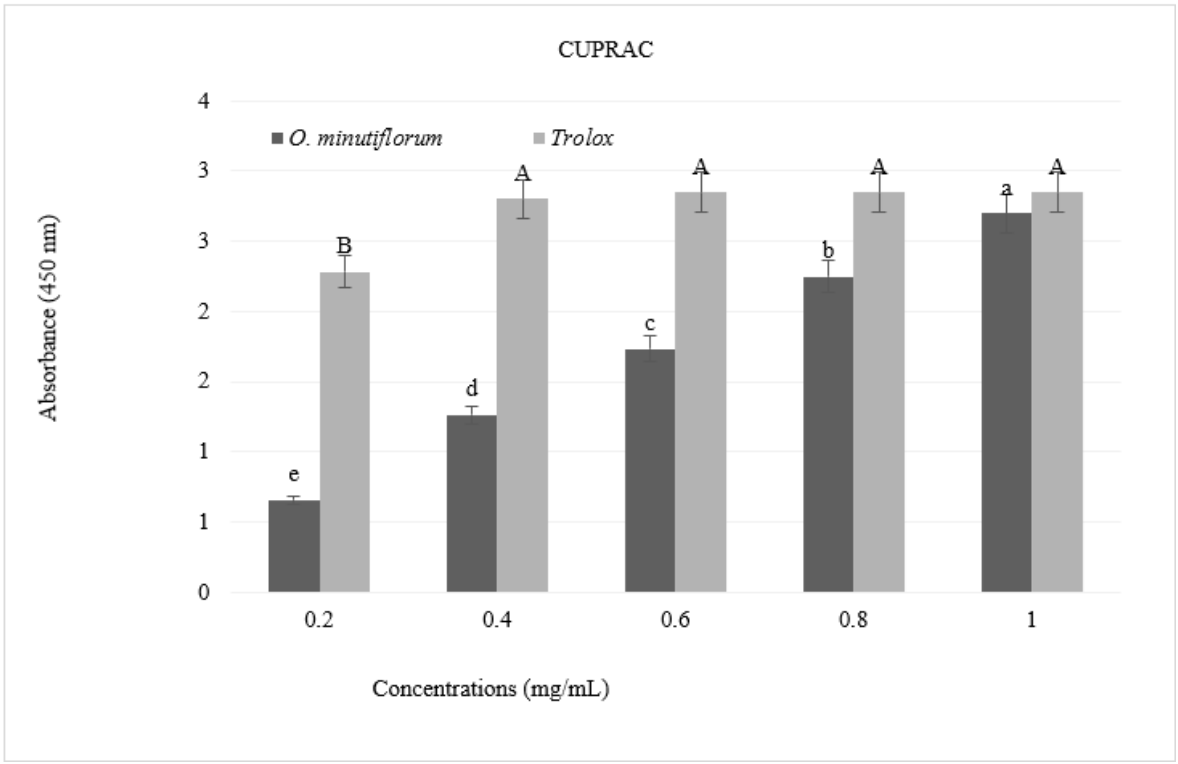

Figure 2. Cu^{+2} reducing activity of OM and Trolox.

activity is increased depending on the concentration of the extract ($P < 0.05$). Absorbance value of OM is lower than the value of Trolox. However, the value obtained at a concentration of 1 mg / ml (2.70) is quite close to the value of Trolox (2.85) at the same concentration (Figure 2). Antioxidant properties of the different extracts of *O. minutiflorum* were previously determined [21]. Also, total phenol contents of the extracts were measured. In our previous study, the main compound in *O. minutiflorum* essential oil was determined as carvacrol (90.87%). Also, the methanol extract of *O. minutiflorum* had found to be potential antioxidant, radical scavenger and reducing agent [11].

The antidiabetic property of OM was calculated as % inhibition of the α-amylase enzyme and compared with the standard acarbose (Figure 3). Also, the extract and acarbose concentration (IC_{50}) value, which inhibited the activity of the enzyme by 50%, was determined. α-Amylase enzyme inhibitory activity of OM and acarbose was statistically different ($P < 0.05$). IC_{50} values of OM and the acorbose were calculated as 7.85 and 6.43 mg/ml, respectively. The antidiabetic property of OM was calculated as % inhibition of the α-glucosidase enzyme and compared with the standard acarbose (Figure 3). Also, IC_{50} values of the extract and acarobose was determined. α- Glucosidase enzyme inhibitory activity of OM and acarbose was statistically different ($P < 0.05$). IC_{50} values of OM and the acorbose were calculated as 1.51 and 1.94 mg/ml, respectively. It was determined that α-glucosidase enzyme inhibitory capacity of OM was higher than acarbose. The essential oil compositions of two subspecies of *O. vulgare* (subsp. *vulgare* and subsp. *hirtum*), and their antimicrobial, antioxidant and enzyme inhibitor capacities were studied [22].

There was a notable reduction in body weight of diabetic control group (Group 3) compared to non-diabetic control group (24.19%) during experimental period. No weight decrease was recorded in non-diabetic rats treated with extract (Group 1 and 2). In diabetic groups (Group 4 and 5), weight loss was observed in both doses of the extract (14.92% and 20.06%, respectively) until the 21^{th} day, but the weight decrease was lower compared to diabetic control group (Group 3). Weight loss was recorded in rats given carvacrol and glibenclamide (Group 6 and Group 7) (Table 2).

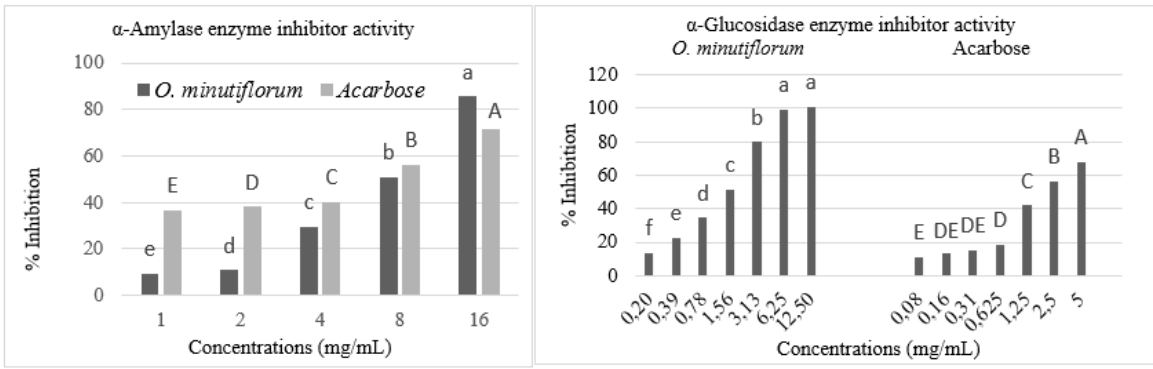

Figure 3. α-Amylase and α-glucosidase inhibitor activities of OM.

The blood glucose amounts are given in Table 3. The end of 21 days, the blood glucose amounts of groups given OM and carvacrol were highly decreased in contrast to Group 3 rats ($P < 0.05$). But, the high blood glucose amount in diabetic control group rats continued.

Serum triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL), creatinine, BUN (urea nitrogen), Ckd-Epi, ALT and AST amounts in all groups were given in Table 4. The serum TG, cholesterol, BUN, creatinine, Ckd-Epi, AST and ALT amounts were considerably increased in Group 3 compared to non-diabetic control group. Administration of OM (20 and 40 mg/kg extract) to non-diabetic group rats notably decreased TG amounts compared to the non-diabetic control group. Also, administration of OM (both concentrations), carvacrol and glibenclamide to diabetic group rats significantly decreased TG amounts compared to diabetic control group. In contrast, serum HDL amounts were lesser in diabetic control group rats than control group rats. It had been determined that HDL amounts of OM (40 mg/kg) treated non-diabetic group rats were same with non-diabetic control group rats. Also, administration of OM (both concentrations), carvacrol and glibenclamide to diabetic group rats significantly increased HDL amounts in comparison with Group 3 rats ($P < 0.05$).

Table 2. Body weight values of rats.

Groups	1.	6.	12.	21.	Difference (%)
Cntrl	263.00^{Cd*}	300.20^{Bb}	307.80^{Bb}	346.00^{Aa}	31.56
Group 1	310.50^{Da}	331.33^{Ca}	336.67^{Ba}	347.33^{Aa}	11.86
Group 2	302.50^{Cab}	320.33^{Ba}	327.33^{Bab}	332.83^{Aa}	10.03
Group 3	315.50^{Aa}	254.17^{Bcd}	242.50^{Bc}	239.17^{Bbc}	-24.19
Group 4	278.17^{Ac}	254.00^{Bcd}	245.17^{BCc}	235.33^{Cbc}	-14.92
Group 5	301.67^{Aab}	249.33^{Bd}	244.50^{Bc}	241.17^{Bbc}	-20.06
Group 6	312.67^{Aa}	255.67^{Bcd}	248.33^{Bc}	224.83^{Cc}	-28.09
Group 7	296.33^{Ab}	266.50^{Bc}	240.00^{Cc}	240.00^{BCb}	-19.01

*Uppercase letters in the same row represent the statistical differences between values obtained at four different days at $P < 0.05$. Lowercase letters in the same column represent the statistical differences between groups at $P < 0.05$. Control: Water + pellet diet, Group 1: 20 mg/kg extract, Group 2: 40 mg/kg extract, Group 3: Diabetic control group, Group 4: STZ + 20 mg/kg extract, Group 5: STZ + 40 mg/kg extract, Group 6: STZ + 10 mg/kg carvacrol, Group 7: STZ + 10 mg/kg glibenclamide.

Table 3. Serum glucose levels in groups (mg/dL).

Groups	1. day	3. day	21. day
Group 3	$69.5 \pm 10.45^{Ba*}$	398.25 ± 80.88^{Aa}	402.17 ± 50.75^{Aa}
Group 4	75.67 ± 12.42^{Ca}	481.25 ± 15.35^{Aa}	224.17 ± 45.53^{Bb}
Group 5	73.83 ± 9.58^{Ca}	456.6 ± 76.88^{Aa}	269.67 ± 64.39^{Bb}
Group 6	74.00 ± 8.92^{Ca}	435.0 ± 54.56^{Aa}	249.50 ± 37.89^{Bb}
Group 7	74.17 ± 9.72^{Ca}	412.5 ± 100.93^{Aa}	254.33 ± 27.72^{Bb}

Group 3: Diabetic control group, Group 4: STZ + 20 mg/kg extract, Group 5: STZ + 40 mg/kg extract, Group 6: STZ + 10 mg/kg carvacrol, Group 7: STZ + 10 mg/kg glibenclamide.

*Values expressed are mean of three independent experiments ± standard deviation (± SD). Uppercase letters in the same row represent the statistical differences between values obtained at three different days at $P < 0.05$. Lowercase letters in the same column represent the statistical differences between groups at $P < 0.05$.

Table 4. The serum triglycerides, cholesterol, HDL-C, BUN, Ckd-Epi levels, and AST and ALT activities in groups.

	Groups							
	Control	1	2	3	4	5	6	7
TG (mg/d)L	$165.30 \pm 4.62^{b*}$	143.50 ± 13.13^{bc}	96.83 ± 8.98^{d}	306.33 ± 60.07^{a}	123.00 ± 23.86^{bcd}	118.50 ± 55.49^{cd}	60.33 ± 20.57^{e}	112.40 ± 14.93^{cd}
TC (mg/dL)	81.00 ± 6.56^{bc}	67.83 ± 0.98^{d}	66.25 ± 4.27^{d}	94.40 ± 5.68^{a}	80.60 ± 5.55^{bc}	82.30 ± 3.21^{b}	70.33 ± 6.12^{cd}	87.17 ± 15.82^{ab}
HDL (mg/dL)	53.50 ± 3.00^{bc}	51.00 ± 1.00^{c}	56.57 ± 3.44^{bc}	50.50 ± 4.51^{c}	58.83 ± 5.71^{b}	74.17 ± 4.54^{a}	71.50 ± 4.20^{a}	70.75 ± 6.75^{a}
BUN (mg/dL)	23.00 ± 1.41^{cd}	19.67 ± 0.52^{d}	19.17 ± 0.75^{d}	39.50 ± 3.27^{a}	27.17 ± 3.19^{bc}	30.00 ± 4.32^{b}	32.00 ± 4.00^{b}	30.17 ± 8.52^{b}
CRE (mg/dL)	0.35 ± 0.05^{ab}	0.30 ± 0.0^{b}	0.30 ± 0.0^{b}	0.40 ± 0.1^{a}	0.20 ± 0.0^{c}	0.33 ± 0.0^{b}	0.32 ± 0.04^{b}	0.40 ± 0.0^{a}
CKD-EPI (mg/dL)	171.20 ± 8.35^{b}	174.00 ± 3.61^{b}	170.50 ± 5.86^{b}	186.75 ± 6.13^{a}	172.25 ± 7.76^{b}	160.33 ± 4.18^{c}	160.17 ± 5.42^{c}	157.17 ± 3.87^{c}
AST (U/L)	74.67 ± 11.15^{bcd}	69.50 ± 6.77^{cd}	64.00 ± 6.29^{d}	140.83 ± 14.11^{a}	75.25 ± 12.42^{bcd}	86.67 ± 17.10^{b}	78.75 ± 9.54^{bc}	75.60 ± 7.23^{bcd}
ALT (U/L)	58.17 ± 1.94^{e}	50.33 ± 3.72^{f}	53.80 ± 4.97^{ef}	163.50 ± 9.98^{a}	126.00 ± 7.94^{b}	115.67 ± 10.26^{c}	88.00 ± 6.83^{d}	81.00 ± 4.58^{d}

Control: Water + pellet diet, Group 1: 20 mg/kg extract, Group 2: 40 mg/kg extract, Group 3: Diabetic control group, Group 4: STZ + 20 mg/kg extract, Group 5: STZ + 40 mg/kg extract, Group 6: STZ + 10 mg/kg carvacrol, Group 7: STZ + 10 mg/kg glibenclamide, TG: Total triglycerides, TC: Total Cholesterol, HDL-C: High Density Lipoprotein-cholesterol, BUN: Urea nitrogen, CRE: Creatinine, AST: Aspartate amino transferase, ALT: Alanine amino transferase.

*Values expressed are mean of three independent experiments ± SD. Lowercase letters in the same row represent the statistical differences between groups at $P < 0.05$.

Changes in the malondialdehyde (MDA) concentration and SOD, CAT and GSH-Px activities in kidney tissues and serums of all groups are showed in Table 5. SOD activities in kidney tissues ($P= 0.97$) and serums ($P= 0.973$) weren't statistically important between the control and treated groups. CAT activity was decreased in kidney tissues and serums of Group 3 compared to control group rats. The highest CAT activity was determined in kidney tissues of diabetic group rats treated with glibenclamide. The administration of OM (20 and 40 mg/kg extract), and carvacrol to diabetic group rats significantly increased CAT activities compared to diabetic control group rats in kidney tissues. Also, CAT activity of Group 1 was higher than that of control group in both kidney tissues and serum samples ($P= 0.002$). CAT activities of diabetic group rats given OM (20 and 40 mg/kg extract) and carvacrol were statistically the same in the serum samples. The administration of OM (40 mg/kg extract) and carvacrol to diabetic group rats significantly increased GSH-Px activitiy compared to Group 3 in kidney tissues. GSH-Px activity of rats treated with glibenclamide were found the statistically same with that of diabetic control groups rats. GSH-Px activity of Group 1 were found the statistically same with that of

control groups rats (P= 0.102). GSH-Px activities in serum samples were not statistically important among diabetic control group and treated groups rats with OM extracts, carvacrol and glibenclamide (P= 0.475). Also, there was a significant increasing in MDA amount in serum and kidney tissue of Group 3 compared to control group. Treatment of OM (20 and 40 mg/kg extract), carvacrol to diabetic group rats notably decreased MDA concentrations compared to Group 3 in serum (P= 0.04) and kidney tissues (P= 0.001). These reductions were higher at diabetic groups which treated with OM (20 and 40 mg/kg extract) than diabetic groups which treated with carvacrol and glibenclamide. Also, it had been determined that the administration of OM (20 and 40 mg/kg extract) to non-diabetic groups had positive effects on MDA reduction compared to control group rats.

Table 5. Changes in the concentration malondialdehyde and the antioxidant enzyme activities in kidney tissue and serum.

		Groups							
		Control	1	2	3	4	5	6	7
Kidney Tissue	SOD (ng/mL)	1.76 ± 0.37*^{a}	1.68 ± 0.71^{a}	1.62 ± 0.43^{a}	1.53 ± 0.42^{a}	1.63 ± 0.41^{a}	1.82 ± 0.85^{a}	2.02 ± 1.80^{a}	1.80 ± 0.46^{a}
	GSH-Px (U/mL)	32.33 ± 4.08^{b}	36.11 ± 8.76^{ab}	33.75 ± 1.51^{b}	23.69 ± 8.30^{c}	37.35 ± 12.84^{ab}	44.47 ± 8.32^{ab}	43.22 ± 6.18^{ab}	51.39 ± 9.31^{a}
	MDA (nmol/mL)	196.24 ± 11.47^{ab}	186.17 ± 18.74^{ab}	164.42 ± 12.80^{b}	176.29 ± 35.43^{ab}	192.97 ± 23.17^{ab}	202.27 ± 24.70^{a}	211.07 ± 19.96^{a}	178.01 ± 13.60^{ab}
	SOD (ng/mL)	1.02 ± 0.08^{bcd}	0.91 ± 0.24^{cd}	0.86 ± 0.17^{d}	1.27 ± 0.20^{b}	1.08 ± 0.28^{bcd}	0.93 ± 0.24^{cd}	1.18 ± 0.11^{bc}	1.66 ± 0.12^{a}
Serum	CAT (ng/mL)	1.64 ± 1.41^{a}	1.29 ± 0.63^{a}	1.43 ± 0.63^{a}	0.99 ± 0.48^{a}	1.51 ± 1.02^{a}	1.62 ± 0.61^{a}	1.56 ± 0.58^{a}	1.51 ± 0.87^{a}
	GSH-Px (U/mL)	27.89 ± 3.20^{ab}	29.44 ± 3.23^{a}	15.91 ± 4.51^{c}	15.13 ± 3.35^{c}	16.05 ± 2.07^{c}	17.41 ± 2.56^{c}	18.12 ± 5.88^{c}	20.95 ± 6.09^{bc}
	MDA (nmol/mL)	107.44 ± 11.11^{a}	73.90 ± 3.45^{b}	101.20 ± 16.15^{ab}	88.92 ± 19.69^{ab}	92.21 ± 26.72^{ab}	93.16 ± 32.61^{ab}	90.75 ± 5.62^{ab}	91.86 ± 10.92^{ab}
	ALT (U/L)	0.85 ± 0.05^{ab}	0.70 ± 0.30^{abc}	0.64 ± 0.15^{bc}	0.97 ± 0.13^{a}	0.85 ± 0.18^{ab}	0.53 ± 0.04^{c}	0.61 ± 0.10^{bc}	0.86 ± 0.31^{ab}

Control: Water + pellet diet, Group 1: 20 mg/kg extract, Group 2: 40 mg/kg extract, Group 3: Diabetic control group, Group 4: STZ + 20 mg/kg extract, Group 5: STZ + 40 mg/kg extract, Group 6: STZ + 10 mg/kg carvacrol, Group 7: STZ + 10 mg/kg glibenclamide,

* Values expressed are mean of three independent experiments ± SD. Lowercase letters in the same row represent the statistical differences between groups at P < 0.05.

H & E stained preparations were used for histological evaluation of kidney tissues taken from all experimental groups. In light microscopic examination, renal sections of the non-diabetic control group, renal bodies (renal corpuscules), proximal and distal curved tubules, peritubular capillaries and medullar rays were properly located in the kidney tissue parenchyma. Glomeruli and tubules showed normal histologic features in non-diabetic control group (Figure 4A). Diabetic control group (Group 3) showed important and significant degenerative changes in renal tissues in comparison with non- diabetic control group. In renal tissues of diabetic control group, histopathological findings such as tubular dilatation, hemorrhage, necrosis and tubular vacuolization were recorded (Figure 8D). Although the renal tissues of Group 6 (STZ + carvacrol) and Group 7 (STZ + glibenclamide) showed histological features similar to the control group, histopathological findings such as tubular dilatation and vacuolization were observed (Figure 4G, H). When the renal tissues of Group 4 (STZ + 20 mg/kg extract) were examined by light microscopy, there was a considerable decrease in histopathological damage compared to diabetes group. In addition to normal renal histology, only some distal tubule dilatations were observed

in the sections of this group (Figure 4E). In Group 5 (STZ + 40 mg/kg extract), kidney damage decreased compared to the diabetic group but histopathological findings such as tubular dilatation and vacuolization continued (Figure 4F). Histopathological findings, which are detected in diabetic control group kidney, were not found in Group 1 (20 mg/kg extract). The renal histology of this group showed a similar structure to the non-diabetic control group (Figure 4B). However, although damaged structures such as tubular dilatation, which were thought to be related to the dose of carvacrol increased in Group 2 (40 mg/kg extract) were recorded, no significant histopathological findings were observed in the kidney sections of this group (Figure 4C).

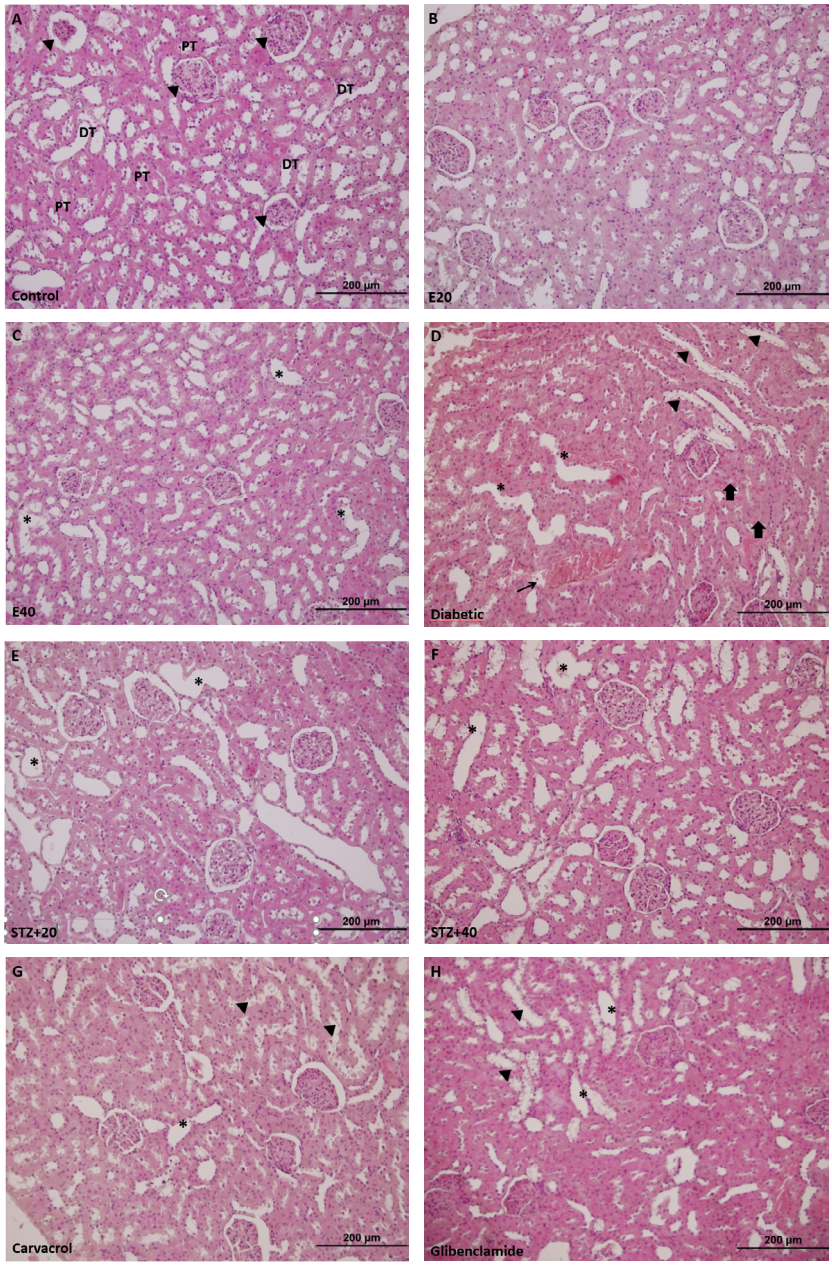

Figure 4. Photomicrographs of the histopathology of rat kidney tissue in all groups. X200.

E20: Group 1, E40: Group 2, STZ+20: Group 4, STZ+40: Group 5,

A- PT: Proximal tubule, DT: Distal tubule; Arrowhead: corpusculum renale malpighi. C-*: tubular dilatation D- Arrow: hemoraji, *: tubular dilatation, Thick arrow: necrosis, Arrowhead: tubular vacuolization. E-F-*: tubular dilatation. G-H- *: tubular dilatation; Arrowhead: tubular vacuolization

Lemhadri et al. [12] concluded that the water extract of *Origanum vulgare* had anti-hyperglycemic property in STZ treated rats. Eidi et al. [23] reported the efficacy of oil and methanolic extract of *Salvia officinalis* on serum glucose. They pointed out that the extract of *S. officinalis* had hypoglycemic effect in diabetic rats. Administration of carvacrol was significantly decreased the blood glucose amount and increased the antioxidant enzyme levels [24]. Kadan et al. [25] observed that the antidiabetic properties of the *Ocimum basilicum* extract were due to the its phenolic components. Elberry et al. [26] examined to healing efficacy of methanol extract of *Marrubium vulgare* in diabetic rats on hyperglycemia and dyslipidemia. They recorded that *M. vulgare* significantly decreased blood glucose level and significantly raised tissue glycogen levels. Esmaeili and Yazdanparast [27] clearly demonstrated that *Teucrium polium* extract could decrease high blood sugar amount by increasing insulin secretion from the pancreas without possible metabolic changes.

Kumar et al. [28] studied the antidiabetic property of *Albizia odoratissima*. Methanolic extract of *A. odoratissima* showed the protective effect on preservation of living tissues (kidney, pancreas, spleen and liver) in diabetic albino mice.

In the paper reported by Sendoğdu et al. [29], the ethanolic extract of *Vitis vinifera* leaf exerted antioxidant and antidiabetic activity in diabetic rats. Also, it had been reported that these effect due to their condensed tannins and flavonoids. The administration of carvacrol in diabtic rats caused a little decrease in serum glucose amount and notably decrease in serum TC, AST, ALT and partially protective effect on liver enzymes [30]. The treatment of *Copaifera duckei* oleoresin demonstrated alterations on the urea, creatinine, and transaminase and reduction on total cholesterol, triacylglycerides, and glucose. It had a healing effect on pancreas damage caused by diabetes [17]. *Swertia kouitchensis* extract displayed improved antioxidant capacity and antihyperglycemic activity in diabetic mice [16]. Treatment with *Achillea santolina* extract decreased blood glucose amount, pancreatic MDA while raised SOD and CAT activity in diabetic rats [15].

Conclusion

In conclusion, OM and carvacrol administration showed important decrease in glucose, TC, TG, creatinine, ALT, AST levels and important increase in the HDL level as well as SOD, CAT and GSH-Px activities. OM and carvacrol have ability to protect renal tissues against complications resulting from diabetes mellitus. These results revealed that OM and carvacrol possess antidiabetic property in diabetic rats due to its high antioxidant activity. This is the first work on antidiabetic activity of OM. Further researches are required to clarify the molecular mechanisms of these effects.

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