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Antidiabetic and Antioxidant Effects of *Bryonia multiflora* Boiss. & Heldr. in a Rat Model of Streptozotocin-Induced Diabetes

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Abstract

This aim of this study is to investigate the antidiabetic, antioxidant and hypolipidemic potential of *Bryonia multiflora* BM Boiss. & Heldr. in streptozotocin (STZ) induced diabetes in rats. During 21 days, control group (NC) and diabetes control (DC) were fed only with food and water, while diabetes acarbose group (DAC) was fed with 20 mg / kg of acarbose. The DB1, DB2, DB3 groups were fed with 100 mg / kg, 200 mg/kg and 400 mg / kg BM plant extract, respectively. The body weight and biochemical parameters and antioxidant parameters were examined for all treated groups and compared against diabetic control group and normal control group. According to the results; significantly higher levels have been observed in DC group serum ALT, AST, BUN, and CRE compared to NC group ($p<0.05$), while declines have been observed in groups treated with BM extract ($p<0.05$). There has been decline in VLDL, cholesterol, and triglycerite levels in the plant extract applied groups ($p<0.05$), while HDL levels increased ($p<0.05$). On the other hand, MDA levels increased while GSH and SOD levels declined as DC group compared to the control group. MDA levels significantly declined ($p<0.05$) while SOD and GSH levels significantly increased in therapeutic groups treated with plant extracts. The results indicate that BM extract have antidiabetic effects by regulating antioxidant activities thereby improving the function of β-cells maintaining normal insulin and glucose levels. Thus the investigation results that BM has significant antidiabetic, antioxidant activity.

Keywords: Antidiabetic, antioxidant, B. multiflora, Diabetes mellitus, Oxidative stress.

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Introduction

Diabetes is a chronic endocrine disorder that is characterized by elevated levels of blood glucose, as well as disturbances in protein, carbohydrate, and lipid metabolism. The condition arises due to a decrease or deficiency in insulin secretion, or increased cellular resistance to insulin. Diabetes can lead to dysfunction or damage in multiple organs and tissues, including the heart, nerves, eyes, kidneys, and blood vessels (1,2). Efforts to complement the treatment of diabetes have recently focused on functional foods and their bioactive compounds. Elevated blood glucose levels in diabetes lead to the production of superoxide anions, which generate hydroxyl radicals, resulting in oxidative damage to cell membranes, as well as other important biomolecules such as carbohydrates, proteins, and DNA (3). The production of reactive oxygen species and oxidative stress associated with hyperglycemia contribute to the pathogenesis and progression of diabetes (4).

The prevalence of diabetes continues to increase globally, leading to a reduction in quality of life, microvascular and macrovascular complications, and even death. In response to this issue, scientists have turned to plants for their healing effects, which have been utilized in traditional medicine for centuries. Numerous studies have investigated the effects of plant extracts on various diseases. This paper aims to examine the effects of *Bryonia multiflora* (BM) extracts on diabetes and diabetes-induced complications through experimental diabetes. BM is a perennial, herbaceous plant with rhizome roots that contain active substances such as ose, steroidal sapanonin, triterpenic sapanonin, essential oil, and cucurbitacin I and cucurbitacin B (5). Literature reviews indicate that cucurbitacins and triterpenic acids are found in B types as triterpenic substances, while sterols are found as steroidal substances. Cucurbitacins possess attributes such as cytotoxic, hepatoprotective, antitumoral, anti-inflammatory, and purgative properties (6). Chemical research has revealed that BM extracts prepared with roots and herbs using specific reactions contain saponins and fixed oil. Saponins are known to exhibit various biological effects such as hypocholesterolemic, anti-carcinogenic, antioxidant, anti-inflammatory, antimicrobial, antiprotozoal, and antihypertensive effects. *Bryonia multiflora* is a member of the Bryonia L. (Cucurbitaceae) genus and is known to have anti-inflammatory, antimicrobial, cytotoxic, and antioxidant effects (7). The components of this species have been shown to possess anti-tumoral properties (8).

Materials and Methods

Plant Materials and Preparation of Lyophilized Extract

During June 2015, *Bryonia multiflora* (BM) was collected from Hizan town in Bitlis, Turkey, and its authenticity was confirmed by Assoc. Prof. Dr. Fevzi ÖZGÖKÇE from the Department of Botany at Yuzuncu Yil University. The herbarium number of BM was determined to be 165060 and a sample of the plant was stored at the Yuzuncu Yil University Science and Art Faculty Herbarium (VANF). To prepare the extract, dried roots of BM were first weighed as 50 g and then divided into small pieces. The aqueous extract was produced by stirring the 50 g of BM powder in 1000 mL of distilled water (dH2O) for 24 hours using a magnetic stirrer. Afterward, the mixture was centrifuged for 15 minutes at 8,000 rpm, and the filtrate was collected. The solvent was evaporated under reduced pressure at 37°C using a rotary evaporator (Rotavapor R-205; Buchi, Switzerland). The viscous extract was then transferred to a falcon tube and freeze-dried under vacuum and at a temperature of -54°C, resulting in a fine lyophilized powder. The extract was prepared using a modified version of the Dalar and Konzcak methods (9).

Experimental Animals

Female Wistar albino rats between 3-4 months old and weighing between 200-300 g were obtained from the Experimental Animal Research Center at Yuzuncu Yil University.

The rats were divided into six groups, each containing seven rats, and were housed in controlled environmental conditions with a 12-hour light/dark cycle and a humidity range of 60% to 70% and a temperature of 25°C. The rats were fed a wheat-soybean-based diet and had access to water ad libitum while living in stainless-steel cages. All rats received proper care in accordance with the "Guide for the Care and Use of Laboratory Animals" developed by the National Academy of Science and published by the National Institute of Health. The ethical guidelines and regulations for the protection of animal welfare during experiments were followed according to national and institutional guidelines.

This study was approved by the Ethic Committee of Yuzuncu Yil University under the protocol number 27552122-142.

Induction of experimental Diabetes mellitus

The animals were fasted for 12h prior to the induction of diabetes. Streptozotocin (STZ) freshly prepared in citrate buffer (0,1 M, pH 4,5) was administered intraperitoneally (i.p.)

at a single dose of 45mg/kg. After 72 h streptozotocin treated rats with blood glucose levels higher than 200 mg/dL were considered as diabetic and used in this study.

Chemicals

The substances used in this study, such as Trichloroacetic acid (TCA), thiobarbituric acid (TBA), and reduced glutathione (GSH), were obtained from Sigma Chemical Co. (St. Louis, MO, USA) in technical grade. Other substances used include butylated hydroxytoluene (BHT), ethylenediaminetetraacetic acid (EDTA), and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). β-Nicotinamide adenine dinucleotide phosphate (NADPH), trihydroxymethyl aminomethane (Tris), and 1-chloro-2,4-dinitrobenzene (CDNB) were also used. In addition, kits for antioxidant enzyme analysis were supplied by Randox Laboratories ltd. and α-Glycosidase Activity Colorimetric Assay Kit (Catalog #K690-100, BioVision, USA) was used for small intestine tissue samples. All substances used in this study were obtained from reliable sources and were of high quality.

Acute toxicity testing

The current study utilized the techniques introduced by Lorke (10) and Ibeha and EzeaJa (11). The experiment involved with twelve rats, which were divided into three groups randomly. Each group was administered with a different amount of the extract, 250 mg/kg, 500 mg/kg, and 1000 mg/kg, respectively, through oral gastric gavage. The rats were allowed to consume food and water as much as they wanted. No harmful or lethal effects were observed during the 72-hour observation period.

Experimental design

The rats were randomly divided into six groups each containing seven rats.

I. Group: Normal Control (NC) : Rats received citrate buffer (pH 4.5) (1 ml/kg, i.p.).

II. Group: Diabetes Control (DC): Rats received STZ in single dose (45 mg/kg, i.p.).

III. Group: Diabetes + Acarboz (DAC): Rats received STZ in single dose (45 mg/kg, i.p.) and Acarbose (20 mg/kg, per day) was treated to diabetic rat groups by oral gavage during 21 days experimental period..

IV. Group: Diabetes + BM (DB1): Rats received STZ in single dose (45 mg/kg, i.p.) and BM extract (100 mg/kg, per day) was treated to diabetic rat groups by oral gavage during 21 days experimental period.

V. Group: Diabetes + BM (DB2): Rats received STZ in single dose (45 mg/kg, i.p.) and BM extract (200 mg/kg, per day) was treated to diabetic rat groups by oral gavage during 21 days experimental period.

VI. Group: Diabetes + BM (DB3): Rats received STZ in single dose (45 mg/kg, i.p.) and

BM extract (400 mg/kg, per day) was treated to diabetic rat groups by oral gavage during 21 days experimental period.

Preparation of tissues supernatant and erythrocyte pellets

At the end of the 21-day experiment, the rats were given anesthesia via intraperitoneal injection of ketamine at a dose of 5 mg per 100 g of body weight. Blood samples were collected through cardiac puncture using a syringe for biochemical analysis. The serum samples were obtained by centrifuging the blood samples at 4 kg for 15 minutes at 4°C, and enzyme levels were measured in the resulting serum samples. The blood samples were immediately placed in two silicon disposable glass tubes with EDTA as an anticoagulant for biochemical analysis. The first tubes were used to measure glycosylated hemoglobin (HbA1c) levels, while the second tubes were centrifuged at 4 kg for 15 minutes at 4°C to obtain erythrocyte pellets. The pellets were then washed three times with physiological saline (0.9% NaCl).

Small intestine, brain, kidney, and liver tissues were dissected and placed in petri dishes. The tissues were washed with physiological saline (0.9% NaCl) and stored at -78°C for analysis. The tissues were homogenized for 5 minutes in 50 mM ice-cold KH_2PO_4 solution (1:5 w/v) using a stainless steel probe homogenizer (20 KHz frequency ultrasonic, Jencons Scientific Co.) and then centrifuged at 7000g for 15 minutes. All procedures were carried out at 4°C. The resulting supernatants and erythrocyte pellets were used to determine the constituents of ADS and MDA contents (12,13). Additionally, α-glycosidase activities in small intestine tissue supernatant samples were investigated.

Biochemical analysis

The concentration of MDA in erythrocytes and tissues was determined using the TBA reactivity method described by Jain et al. (14) while the concentration of GSH in erythrocytes and tissues was measured using the method described by Beutler et al. (15). GST activity was assayed by measuring the conjugation of glutathione with CDNB at 340 nm as described by Mannervik and Guthenberg (16). The decrease in absorbance of NADPH at 340 nm was used to assay GR activity, according to Carlberg and Mannervik (17). GPx activity was measured by catalyzing the oxidation of glutathione by cumene hydroperoxide using a method based on that of Paglia and Valentine (18). SOD activity was calculated by measuring the inhibition percentage of formazan dye formation at 505 nm (19). CAT activity was determined by measuring the rate of H_2O_2 consumption and the decrease in absorbance at 240 nm using the method described by Aebi (20). The α-glycosidase activity in small intestine tissue samples was measured colorimetrically at

410 nm by hydrolyzing the Substrate Mix to release p-nitrophenol (BioVision kits, USA).

Measurement of biochemical parameters

Several parameters were measured using an automated analyzer (COBAS 8000/ROCHE/Germany/Serial No 1296-08) with Roche kits. These parameters include ALT, AST, LDH, glucose, lipid profile (total triglyceride, total cholesterol, LDL-cholesterol, and HDL-cholesterol), creatinine, blood urea nitrogen, and urea. Insulin and c-peptide levels were measured using an ELISA Enzyme-linked Immunosorbent Assay Kit based on the 450 absorbance.

Measurement of Blood Glucose Levels

Fresh blood samples were collected from the tail vein of the rats. The blood glucose levels were determined with a blood glucose meter (ACCU-CHEK Active, Roche). These measurements were performed on days 0, 7, 14, and 21.

Analysis of data

The data obtained from the experiments were presented as mean values and standard deviations. The statistical analysis of the data was conducted using Minitab 13 for Windows software. The means and standard deviations were calculated for all the parameters using standard methods. One-way analysis of variance (ANOVA) was used as a statistical test to determine any significant differences between the means of the experimental groups. A significance level of $p < 0.05$ was accepted.

Results

Acute toxicity studies

Animals showed tolerance to testing three (250, 500 and 1000 mg/kg) doses of BM lyophilized extract. Extract in doses as high as 1 g/kg that were found to be non-lethal. Highest dose of extract did not show any noticeable signs of toxicity and mortality after 3 days of administration onceper day. Therefore, the extract is safe for long term administration.

BM extract effect on body weight and blood glucose levels

Table1. Effect of BM aqueous extract supplements on body weight and glucose level of experimental groups during 21 days

	GROUPS					
	NC	DC	DAC	DB1	DB2	DB3
	Mean 7 SD	Mean 7 SD	Mean 7 SD	Mean 7 SD	Mean 7 SD	Mean 7 SD
Body weight (g)						
Beginning	213,85±5.55	213.71±7.97	205.71±11.81	272.85±22.31	251.01±7.02	249.14±44.63
Finally	219.57±10.21	203.57±5.74*	196.14±8.31	250.57±26.20	245.28±22.04	228.14±11.53*
Three hour period Blood glucose (mg/dL)						
0. Hour (Fasting blood glucose)	84.23±9.28	330.47±159.32	388.81±150.71	158.47±36.25	130.38±38.56	236.91±91.25
1.Hour (After consumption)	85.95±8.39	363.61±142.04	390.09±145.41	158.52±32.59	138.66±39.49	235.91±93.49
3.Hour (After consumption)	90.23±6.61*	388.14±131.79	409.33±128.70	152.09±55.97	130.23±51.92	191.81±62.83
21 day period Blood glucose (mg/dL)						
Day 0	84.42±12.76	261.42±68.73	225.85±21.16	212.01±13.01	291.14±41.58	207.42±4.19
Day 7	82.91±9.85	363.71±146.21	472.14±105.51*	158.85±50.57*	151.81±45.62*	310.57±64.19*
Day 14	88.66±8.41*	377.04±145.34	393.01±134.72*	164.08±28.11*	137.71±30.16*	188.95±39.04
Day 21	88.85±5.33	341.47±146.66	323.09±140.51	146.14.±44.86*	109.76±42.87*	165.09±63,09

Changes in beginning and final body weight in control and experimental groups are shown in Table 1. Significant weight loss was observed in final diabetic control group compared to initial animals. During the study period, the normal control rats gained weight, while STZ-induced diabetic rats exhibited a lower body weight. Table 1 summarizes the levels of glucose in normal and diabetic animals. The NC group blood glucose level increased 3.h compared with 0 hour whereas the levels of blood glucose significantly decreased in diabetic rats treated with BM extract after 2 and 3 weeks.

Effect of the BM extract on liver and renal serum biomarkers of experimental groups

The serum levels of ALT, AST, URE, CRE and BUN were significantly increased in the diabetic control rats compared to the normal control rats. The serum level of LDH decreased in the diabetic control rats compared to the normal control rats. The serum levels of ALT, were significantly decreased in the treated groups with BM extract compared to the diabetic control group. The treatment groups renal serum biomarkers such as URE, CRE, BUN were decreased compared to the diabetic control group (Table2)

Table2. Effect of BM aqueous extract supplements on liver and renal serum biomarkers of experimental groups

GROUPS						
Parameters	NC Mean 7 SD	DC Mean 7 SD	DAC Mean 7 SD	DB1 Mean 7 SD	DB2 Mean 7 SD	DB3 Mean 7 SD
ALT U/L	70.94±18.57	176.55±19.91[a]	139.88±19.91[ab]	143.75±36.53[ab]	98.72±19.83[b]	210.91±49.87[a]
AST U/L	146.81±30.33	239.61±71.33[a]	248.57±17.84[a]	239.05±63.28[a]	289.57±54.08[a]	372.24±66.05[ab]
Üre mg/dL	49.21±4.62	58.52±6.09[a]	87.24±1.85[ab]	48.31±11.51	48.37±16.86	78.61±18.95[ab]
CRE mg/dL	36.10±4.02	40.01±5.71	52.01±5.40[ab]	38.8±3.50	37.8±3.80	61.01±14.70[ab]
BUN mg/Dl	23.14±2.03	27.42±2.82[a]	40.57±1.13[ab]	22.57±5.51	22.42±7.82	36.85±8.74[ab]
LDH U/L	1623.5±274.6	1610.2±247.9	1630.1±211.7	2008.8±462.9	1847.2±470.6	1658.1±449.9

Effect of the B. multiflora extract on lipid profile

Table 3. Effect of BM aqueous extract suppleents on lipid profile of experimental groups

GROUPS						
Parameters	NC Mean 7SD	DC Mean 7SD	DAC Mean 7SD	DB1 Mean 7SD	DB2 Mean 7SD	DB3 Mean 7SD
TG (mg/dL)	134.07±31.06	110.62±33.02	78.71±16.05[ab]	130.51±32.29	92.97±22.58[a]	56.71±9.57[ab]
TC (mg/dL)	68.05±5.81	43.88±9.33[a]	69.52±2.31[b]	58.31±6.06[ab]	57.18±8.51[ab]	69.81±7.91[b]
HDL (mg/dL)	51.52±5.06	38.35±5.83[a]	57.64±6.69[b]	47.81±8.43[b]	44.12±8.02	54.28±7.74[b]
LDL (mg/dL)	5.58±0.99	5.94±1.41	11.42±2.23[ab]	8.31±1.73[ab]	6.24±2.05	11.07±2.07[ab]
VLDL (mg/dL)	28.28±4.61	20.71±4.28	15.82±3.23[ab]	24.14±5.61	18.57±4.35[a]	11.71±2.62[ab]

The serum levels of TG, TC, HDL and VLDL were markedly decreased in the diabetic group compared with the normal group (Table 3). TG levels dropped significantly in plant extract applied rats. VLDL, TC, TG levels notably dropped in all of the groups applied with plant extract, while HDL levels went up close to the healthy control group.

Effect of the B. multiflora extract on HbA1c, serum insulin, c-peptide and α-glycosidase activity in small intestine tissue

Table 4. Effect of BM aqueous extract supplements on HbA1c serum insulin, C-peptide and α-Glucosidase activity in small intestine levels of experimental groups

GROUPS						
Parameters	NC	DC	DAC	DB1	DB2	DB3
	Mean 7SD	Mean 7SD	Mean 7SD	Mean 7SD	Mean 7SD	Mean 7SD
İnsulin (pg/mL)	594.17±44.74	415.28±30.82[a]	516.76±103.42	480.11±32.27[ab]	561.78±45.87[b]	521.59±29.96[ab]
HbA1c (%)	4.45±0.64	7.29±1.42[a]	6.31±1.46	4.01±0.91[b]	4.64±1.36[b]	4.41±0.29[b]
α-Glukozidaz	32.55±5.88	56.35±9.66[a]	52.74±3.21[a]	65.32±8.64[a]	46.89±3.53[a]	67.87±10.33[a]
C-Peptide	577.76±67.77	465.51±33.58[a]	582.16±86.48[b]	473.28±44.16[a]	588.89±109.74	568.52±114,11

As shown in Table 4, at the end of the experiment, the serum insulin, C-peptide levels of diabetic control rats were significantly decreased ($p < 0.05$) compared to those of normal control rats. In contrast, the administration of BM extract to diabetic rats significantly increased all of these parameters compared with the diabetic control rats. However, HbAlc significantly increased in diabetic control group, which were found near to normal in the treatment group.

Effect of the BM extract on lipid peroxidation and antioxidant defense systems

Table 5. Effect of B.multiflora aqueous extract supplements on lipid peroxidation and antioxidant defense systems of experimental groups.

	Parameters	GROUPS NC Mean 7SD	DC Mean 7SD	DAC Mean 7SD	DB1 Mean 7SD	DB2 Mean 7SD	DB3 Mean 7SD
Erythrocyte (U/ml)	MDA	9.49±2.15	12.5±1.79^{a}	10.10±2.47^{b}	10.35±1.03^{b}	5.39±1.56^{ab}	8.41±3.37^{b}
	GSH	4.56±0.50	3.77±0.47^{a}	4.92±0.69^{ab}	4.52±0.57^{ab}	4.53±0.24^{ab}	3.58±0.86^{a}
	GST	9.10±2.40	10.10±1.07	10.01±2.01	9.10±1.80	9.80±1.20	7.50±0.90
	GR	0.17±0.07	0.11±0.05	0.09±0.02^{a}	0.10±0.02	0.06±0.03^{a}	0.08±0.02^{a}
	CAT	371.16±41.70	384.80±53.23	460.65±53.93^{ab}	443.90±57.68^{ab}	412.32±50.44	321.38±62.46
	GPX	2298.83±527.79	2485.72±307.60	2261.19±256.81	1931.97±282.75^{b}	2142.65±272.38	1921.59±272.98^{b}
	SOD	2284.78±25.51	2270.41±28.29	2262.58±21.40	2287.06±9.69	2286.37±14.31	2278.71±9.77
Brain (U/g)	MDA	32.19±6.53	72.62±3.13^{a}	38.11±8.49^{b}	41.54±3.92^{b}	44.59±10.57^{ab}	53.29±7.67^{ab}
	GSH	24.59±5.99	28.39±9.79	25.65±6.64	23.43±2.66	24.17±5.39	26.33±3.09
	GST	12.5±1.79	13.78±0.76	13.21±2.58	14.66±2.65	13.52±2.58	13.21±5.33
	GR	0.98±0.21	0.92±0.12	0.88±0.18	0.64±0.06^{ab}	0.45±0.13^{ab}	0.87±0.18
	CAT	29.43±1.57	25.84±1.52	35.65±7.15	25.36±1.72	25.84±1.30	23.45±1.41
	GPX	2604.26±75.97	2607.28±38.34	2642.33±46.03	2670.88±74.05	2703.32±45.65^{ab}	2495.67±75.29^{ab}
	SOD	2175.07±14.92	2196.48±67.47	2166.92±46.73	2174.95±39.91	2114.42±50.61^{ab}	2097.06±54.78^{ab}
Kidney (U/g)	MDA	169.21±38.81	231.02±21.54^{a}	212.42±53.09	186.51±41.59^{b}	181.33±33.66^{b}	199.56±18.76^{b}
	GSH	51.74±2.67	45.71±12.33^{a}	43.63±12.48	57.77±6.68^{b}	46.83±4.23	47.06±5.43
	GST	8.23±0.85	8.72±1.07	14.06±8.77	10.10±3.19	10.10±2.47	14.24±1.58^{b}
	GR	0.80±0.22	0.47±0.10^{a}	0.62±0.10^{b}	0.67±0.10^{b}	0.43±0.08^{a}	0.50±0.17^{a}
	CAT	318.75±22.59	154.59±30.84^{a}	205.80±64.58^{ab}	160.33±28.31^{a}	145.73±7.81^{a}	172.05±32.39^{a}
	GPX	1587.17±292.42	1879.62±170.63^{a}	1864.48±240.02	1520.98±175.92^{b}	1957.49±72.91^{a}	1812.13±137.75
	SOD	2166.04±49.06	2189.9±26.28	2235.34±43.27^{ab}	2215.07±77.85	2223.78±32.89^{ab}	· 2183.84±32.43
Liver (U/g)	MDA	53.10±9.92	70.12±13.88^{a}	44.13±4.00^{ab}	46.35±7.00^{b}	55.69±5.40^{b}	56.71±13.59
	GSH	50.29±4.43	51.64±5.15	56.34±2.31^{ab}	57.46±1.23^{ab}	53.57±2.79	54.38±2.26
	GST	61.76±10.52	69.75±7.48	74.03±4.82	72.72±8.49	72.24±10.12	58.85±9.86^{b}
	GR	0.47±0.11	0.48±0.09	0.37±0.08	0.31±0.08	0.31±0.06^{a}	0.20±0.04^{ab}
	CAT	446.06±62.27	319.70±62.47^{a}	319.70±58.84^{a}	325.69±41.64^{a}	414.47±64.64^{b}	260.84±36.05^{a}
	GPX	1394.66±238.55	1233.72±204.32	829.23±130.39^{ab}	868.6±158.97^{ab}	1586.74±281.17	921.81±164.89^{a}
	SOD	2102.89±47.71	1985.05±67.79^{a}	1945.73±67.84^{a}	2000.87±43.55^{a}	2029.74±60.21	2072.76±85.63^{b}

According to the results, the levels of MDA content significantly increased ($p < 0.05$) in all tissues of DM group as compared to normal control (NC). In our study, liver,

kidney, and brain and erythrocyte MDA levels increase in diabetic control group compared with control group, while BM extract applied therapeutic groups MDA decreased, while SOD, GSH, and CAT levels increased. The tissues as brain, kidney, erythrocyte and liver levels of GST increased DM groups as compared to NC. In general, fluctuations in the ASS elements level was recorded as close to control values. Summery while STZ induced diabetic rats caused a decrease in antioxidant defense system constituent's level as a result of oxidative stress condition in the rats; BM extract restored the STZ-induced diabetic rats ADS constituents towards to NC group. After treatment, most of these parameters had returned to normal (Table 5).

Discussion

Phenolic compounds such as alcaloids, flavonoids, tannins, glycosides, terpenoids, sterols, considered to have antihyperglisemic effect which controls hyperglysemia (21).

Our literature review aimed to reach a limited number of research on effects of BM on various diseases, while no research has been abouton the effect of this plants effect on diabetes.

Chemical research has reveal that BM extracts with water and alcohol prepared with root and herbs using specific reactions hold saponosides and fixed oil. Chemical research has concluded that BM's extracts with water and alcohol prepared with root and herbs using specific reactions hold saponosides and fixed oil. Saponins are 0-glycisides with stereoidal or triterpenic structure forming a complex with cholesterol. They can hemolize erytrons and usually have triterpenic or stereoidal aglycone, stable foaming when its aqueous solutions are shaken. It has been asserted that saponin including plants have various biological effects such as antioksidant, hypocholesterolemic, anti-carcinogenic, antioxidant, anti-inflammatory, antimicrobic, antiprotozoal, and antihypertensive (7,21). Declines have been reported on 0th, 7th, 14th, and 21st days in blood glucose values of extract applied groups starting from the first week. Significant declines have been reported in blood glucose levels of all of the extract applied groups after the second week, while similar values with the healthy group have been reported last week in blood glucose levels of groups treated with BM extract. The reason for this decline might be the active substances in the plant to induce insüline secretion in pancreas, or the plant's built-in phytochemical compounds refunction as islet cells in time to originate a protective effect against hyperglycemia.

Serum ALT, AST, and LDH levels of all of the groups have been measured through on the last day of our study, after sacrificing all the rats. ALT and AST levels of diabetes control group have been higher compared to normal control group. Hepatocyte damage changes the

transport functions and membrane permeability, hence causes enzyme leak (23). Due to the damage, enzyme leak might have caused increase in our diabetic groups. However, ALT level has been significantly lower in DB1 and DB2 groups treated with BM extract, compared to diabetes control group (DC) ($p<0.05$). Based on this, we might consider that active substances in the BM extract regulate liver enzyme activities. Diabetes mellitus is related to abnormal lipid profile (24). Due to the Streptozotosin dose, it damages insulin generating β-cells, causing insulin insufficiency in the organism. In cases of insulin insufficiency, lipid metabolism collapse, and mobilization of fatty acids from fatty tissue increase, hence fatty acid level in the blood goes up as well (25). In our study, HDL and LDL levels have been higher in all of the groups treated with BM extract, compared with diabetes control group, collaterally with the general dose increase. Triglyceride levels dropped significantly in plant extract applied rats. VLDL, cholesterol, triglyceride levels notably dropped in all of the groups applied with plant extract, while HDL levels increased close to the healthy control group. Phytochemical compounds found in the extracts used in our study activate insulin release in order to provide regeneration of beta cells, and as a result of this, it is possible to say that HDL level increased due to the increase in lipoprotein lipase activity, and hepatic triglyceride lipase activity (26). It is considered that saponins that are found in BM plant structure might regulated lipid metabolism which is effected negatively by diabetes. Many studies have been published asserts that saponins decrease liver lipid, plasma triglyceride concentration and cholesterole, while increasing HDL level (27).

In our study, there have been an increase of insulin levels in all of the groups as a result of the comparison between diabetes control group (DC) and DB1, DB2, DB3 groups with different doses of BM extract applied. On the other hand, increase has been reported in C-peptide levels of all the groups proportional to the increase of extract dose. This might be the result of normalization insulin release, regulating pancreas functions by extracts. HbA1c, known as glycosylated hemoglobin is a compound that its amount where is due to glucose concentration levels, formed by glucose and hemoglobin (28). In case of diabetes, HbA1c level increases notably (29).

In a study, increase occurred in HbA1c levels of diabetes control groups, while there has been decrease in therapeutic groups. This result is stated to occur due to the regulation of insulin release. In our study, glycosylated hemoglobin (HbA1c) level of diabetes control group has been examined higher than control group, as expected. There has been a significant decrease ($p<0.05$) in all of the extract applied groups regarding HbA1c levels compared with diabetes control (DC) group Decreasing of HbA1c levels of all of the plant extract applied groups to

the level of normal control group can be considered as the fact that active substances such as cucurbitacins found in the plants used in this study induce functional cells of pancreas, causing glucose generating in the liver to drop, or effecting the absorbing of glucose in gastro-intestinal channel, and decrease HbA1c levels accordingly. Glucose levels reported throughout this study confirm these results. Glucose levels reported throughout this study confirm these results.

In a study on the antidiabetic effects of dioscorea batatas, BUN and CRE levels of diabetes groups has been found higher than control group. However, significant decreases have been reported in therapeutic groups as to these parameters, the mentioned extract is suggested to strengthen kidney functioning by inhibiting extra-cell water raise (30).

In our study, serum urea levels of DB1, DB2, DB3 groups with different doses of DC, DAC, and BM extract have been found significantly higher ($p<0.05$) in DC, DAC, and DB3 groups, while urea levels of groups DB1 and DB2 have been found insignificantly less ($p>0.05$) than control group. Urea levels of control group (NC), diabetes acarbose group (DAC), DB1, DB2, DB3 groups compared to diabetes control group (DC) decrease have been found in NC, DB1, and DB2, while only the decrease in control (NC) group has been found significant ($p<0.05$). There has been a significant increase in the groups DAC and DB3 ($p<0.05$). Besides, in our study, levels of creatinin (CRE) and BUN decrease was examined in diabetes control group, as expected. This might be the result of nephropathy, one of the complications of diabetes. Applied different doses of BM extract, DB1 and DB2 groups was shown similar decrease results with the control group. BM extract might have regulatory effects for renal functional disorders by prohibiting diabetic complications with its built in phytochemicals.

Diabetes mellitus is related to abnormal lipid profile (24). Streptozotosin creates insulin insufficiency by damaging insulin generating β cells depending on the dose. Lipid metabolism deterioration of in case of insulin insufficiencies, causing mobilization of fatty acids from fatty tissue, hence increasing the fatty acid level in blood (25).

A study found HDL level in diabetic group significantly low compared to control group (31). In our study, we reported coherent results with previous studies, with the finding of low HDL levels in diabetic control group compared to control group. The reason for decrease fall is that fatty acid flow from adipose tissue occurs because of the decrease of inhibition of hormone sensitive lipase. Besides in our study, VLDL level of diabetes control group has been found dropping compared to normal control group. As a result of low lipoprotein lipase enzyme activity induced by diabetic insulin insufficiency, the decrease occurred in VLDL carrying endogene triglycerides and chylomicron catabolism carrying exogen triglycerides

taken with food might be considered the reason for VLDL level drop in diabetic rats. Scientific research has stated cholesterol and triglyceride levels increase in experimentally established diebetic rats. (30,31,32). In our study, groups treated with BM extract have reported higher HDL and LDL levels compared with diabetic control group, collaterally with dose increase. Plant extract has found to decrease triglyceride levels significantly in diabetic rats. All of the groups treated with plant extract have shown notable decrease in VLDL, cholesterol, triglyceride levels to the contrary of what was expected in diabetic groups, while HDL levels increased based on dose, yielding similar results with healthy control group. The reason for changes in cholesterol level might be considered as the activity of enzymes joining cholesterol which is effected by the circumstances of diabetes. Saponins which BM plant indicates might be considered as regulating the lipid metabolism effected negatively by diabetes. There are studys on saponins decreasing liver lipid and plasma triglyceride concentration as well as cholesterol, while increasing HDL level (27). Saponins cause cholesterol precipitation by forming compounds with cholesterol within intestine lumen, while reducing the access of including which cholesterol micelles into mucosa cells through effecting their size and/or stability, and ruin the membrane transport functionality because of its effect on mucosa cell membrane's cholesterol. Saponins so reduce the absorbation of cholesterol and increase the excretion of cholesterol and bile acid, together with neutral sterols such as coprostorol and plant sterols (33,34,35). Decline of hepatic cholesterol level which is closely related to the inhibition of cholesterol absorbation causes liver HMG-CoA reductase activity and low density lipoprotein receptor level increase (33,36,37). Built in plant saponins might reduce blood cholesterol through this mechanism by inhibiting exogen and endogen hypercholesterolemia.

Diabetic hyperglycemia enhances oxidative stress by causing free radical formation, leaving the antioxidant system insufficient (38). A study states that MDA concentrations increase in rats with STZ-formed diabetes, while superoxide dismutase (SOD) and catalase activity decrease (39). Plasma MDA high levels of diabetic patients was examined , while SOD, GSH, and catalase levels have been examined low (40). Another study asserted that GSH levels were low while MDA levels are high in diabetic subjects, concluding that oxidative stres increases, while antioxidant capacity decreases (41). In our study, liver, kidney and brain and erythrocyte MDA levels increase in diabetic control group compared to control group, while BM extract applied therapeutic groups MDA drops, while SOD, GSH, and CAT levels go up. It is possible to draw the conclusion that the last product of lipid peroxidation, MDA activity's drop in therapeutic groups and SOD, GSH, CAT levels increasing, occurred because

antioxidant phytochemicals in the extract works as a sweeper on free radicals that increase in diabetes, or because they inactivate oxidants, hence inhibiting the diabetes originated oxidative stress. Glutathione S Transferase (GST) enzyme in liver, kidney, lung, erythrocyte, testicles, placenta, carcase, and heart muscle, makes detoxification as a intracell carrier and connector (42,43,44). A study where erythrocyte GST activity of rats with STZ-formed diabetes, show that GST activity in the diabetic group increased compared to control group (45). In our study, GST activity levels of diabetes control group (DC) have been examined high in all of the tissues, compared to the control group. This increase might be considered as a result of resistance mechanism against the oxidative stress situation. All of the groups treated with BM displayed lower GST activity levels in brain, erythrocyte and in liver, as the dose increases. Extract might be considered to reduce diabetic oxidative stress by directly effecting free radicals or by inhibiting hyperglycemia in rats with STZ formed diabetes. Some studies have shown that plant extract supplementation may have an antioxidative role in diabetic rats (46).

According to the results of our study amount almost all the parameters applied in therapeutic groups treated with plant extracts the levels examined in diabetic individuals as same levels as could be observed in healthy individuals, indicate that BM has an effect of stimulating or renewing on functional cells of pancreas tissue, also effecting insulin secretion, hence this plant might have healing effect over diabetes related complications, and diabetes as a whole.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest. None of the authors has a commercial interest, financial interest, and/or other relationship with manufacturers of pharmaceuticals, laboratory supplies, and/or medical devices or with commercial providers of medically related services.

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