Background: The present study was aimed to investigate the effects of betaine (BET) on streptozotocin (STZ)-induced diabetes mellitus (DM) in rats. Additionally, the efficiency of BET was compared with metformin (MET), a standard oral antidiabetic drug.

Methods: STZ (55 mg/kg body weight; i.p.) was injected to male Wistar rats. Rats with DM were treated with BET (1 g/kg body weight/day;) or MET (500 mg/kg body weight/day;) for 4 weeks. Blood glycated hemoglobin (HbA1c), serum glucose, lipids, hepatic and renal function tests and urinary protein levels were examined. Reactive oxygen species (ROS) formation, malondialdehyde (MDA), glutathione (GSH) levels, and ferric reducing antioxidant power (FRAP) were also determined in liver and kidney.

Results: Glucose, HbA1c, and serum lipids increased and liver and kidney function tests were impaired in diabetic rats. Hepatic and renal ROS formation and MDA levels were elevated, hepatic, but not renal GSH and FRAP levels were decreased. BET decreased blood HbA1c, serum glucose and lipid levels and urine protein levels. BET diminished hepatic and renal prooxidant status.

Conclusion: Our results indicate that BET may be effective in decreasing STZ-induced high levels of blood HbA1c, and serum glucose and lipid levels and prooxidant status in liver and kidney tissues.

Keywords: Diabetes mellitus; Betaine; Oxidative stress; Liver; Kidney; Rat.

Özet

Amaç: Bu çalışmada betainin (BET) streptozotosin (STZ) ile diabet oluşturulan sıçanlarda etkisini araştırmak amaçlandı. Ayrıca BET'in etkisi standart oral antidiyabetik ilaç olan metforminle (MET) karşılaştırıldı.

Metod: STZ (55 mg/kg vucut ağırlığı; i.p.) erkek Wistar sıçanlara uygulandı. 4 hafta süreyle diyabetik sıçanlara BET (1 g/kg vucut ağırlığı/gün; diyetle) veya MET (500 mg/kg vucut ağırlığı/gün; içme suyu) uygulaması yapıldı. Kanda glikoziem (HbA1c), serum glikoz, lipid ve karaciğer ve böbrek fonksiyon testleri ve idrarda protein düzeyleri ölçüldü. Ayrıca, karaciğer ve böbrek dokusunda reaktif oksijen türeleri oluşumu, malondialdehid (MDA), glutatyon (GSH) düzeyleri ve antioksidan aktivite (FRAP) tahmin edildi.
Betül Evran et al.: Betaine treatment decreased serum glucose and lipid levels

Bulgular: Diyabetik sıçanlarda glikoz, HbA1c ve serum lipit düzeyleri arttı, karaciğer ve böbrek fonksiyon testleri bozuldu. Karaciğer ve böbrekte ROS oluşumu ve MDA düzeyleri arttı, karaciğerde GSH ve FRAP düzeyleri azaldı. BET uygulaması kan HbA1c, serum glikoz ve lipit düzeylerini azalttı. BET ayrıca karaciğer ve böbrek prooksidan durumunu baskıladı.

Sonuç: Sonuçlarımız BET’in STZ uygulamasıyla artmış olan kan HbA1c, serum glikoz ve lipit düzeyleri ile karaciğer ve böbrek dokularında prooksidan durumu azaltmada etkili olduğunu gösterdi.

Anahtar Kelimeler: Diabetes mellitus; Betain; Oksidatif stres; Karaciğer; Böbrek; Sıçan.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease with high glucose levels caused by partial or complete insufficiency of insulin. Oxidative stress induced by hyperglycemia is accepted to play a major role in functional and structural defects seen in patients with DM and experimental models of diabetes [1–3]. Although exact mechanisms are unknown, autooxidation of glucose induced by hyperglycemia, formation of advanced glycation end products (AGEs), and activation of polyol pathway are considered to be responsible for the oxidative stress in DM [1–3].

Liver dysfunction and nephropathy are common complications of DM. Diabetic nephropathy is described with proteinuria, hypertension, and loss in renal function. It has been shown that DM diminishes prooxidant-antioxidant balance and causes inflammatory, apoptotic, and degenerative changes in liver [4, 5]. Similarly, DM was observed to increase oxidative stress and induce functional and structural defects in renal tissue [4, 6–8]. Based on these findings, several scientists have proposed antioxidant compounds to support treatment of DM. In this regard, effects of various antioxidant compounds (sulphydryl compounds, vitamin C and E, taurine, polyphenols) have been investigated [9].

Betaine (tri-methyl glycine; BET) is a choline metabolite formed in liver by the activities of mitochondrial choline dehydrogenase and betaine aldehyde dehydrogenase. Additionally, vegetables are alimentary sources of BET [10]. BET functions as an osmolite and shows antioxidant and antiinflammatory effects [10, 11]. Accordingly, BET treatment was reported to be useful against some oxidative stress-induced conditions such as atherosclerosis [12], liver [13–16], and kidney [17–20] disorders. In addition, it has been suggested that there is a relationship between the alteration of BET levels and development of DM complications [21, 22]. However, the effect of BET treatment on DM is poorly investigated [23, 24].

Metformin (MET) is an antidiabetic drug widely used in the treatment of DM [25]. MET was reported to ameliorate insulin resistance and have antioxidant, antiinflammatory and antiapoptotic effects [25–27]. These properties of MET contribute to its effect in preventing or ameliorating diabetic complications including hepatosteatosis [28, 29] and diabetic nephropathy [6, 28].

The present study aimed to investigate the ameliorative effects of BET on blood glycated hemoglobin (HbA1c), serum glucose and lipid levels, oxidative stress, and hepatic and renal dysfunction in STZ-induced diabetic rats and the efficiency of BET was compared with MET, an oral antihyperglycemic drug with antioxidant potential.

Methods

Chemicals

STZ, BET-hydrochloride and other chemicals were purchased from Sigma-Aldrich (USA). MET-HCl was obtained from Bilim İlaç Sanayii (Istanbul, Turkey).

Animals

Male Wistar rats (3–4 months; 220–240 g body weight) were used for all experiments. Animals were obtained from Experimental Medical Research Institute of Istanbul University. They were kept in wire-bottomed stainless cages. All the animals were housed (three to four per cage) under a daily cycle of 12 h light and 12 h darkness, supplied with food and water ad libitum. Experimental settings such as feeding of animals and maintenance conditions were standardized for all the animals to minimize the variations. The experimental procedure used in this study met the guidelines of the Animal Care and Use Committee of the University of Istanbul.

STZ-induced diabetes model and treatments

Rats were divided into control and experimental groups. Six rats were randomly selected as control group and fed commercial food. Rats in experimental group were injected intraperitoneally with a single dose of STZ dissolved in citrate buffer (0.1 M; pH 4.5) at a dose of 55 mg/kg body
weight (BW). Administration of STZ was performed after an overnight fasting. Three days later, blood glucose levels were determined in whole blood samples collected from the tip of the tail. Only rats with fasting blood glucose levels higher than 250 mg/dL [5, 7] were included in DM group.

Rats with DM were divided into three subgroups as follows: untreated (n = 7), BET-treated (n = 7), and MET-treated rats (n = 7). Total food and water intake was recorded daily and diabetic rats were fed with BET containing diet or MET in drinking water for 4 weeks. The daily consumptions of BET and MET were equivalent to 1 g/kg BW and 500 mg/kg BW, respectively.

At the end of the treatments, rats were placed in individual metabolic cages for 24 h and urine samples were collected. They were centrifuged at 1750×g for 10 min at 4°C and frozen at −20°C. The day after, rats were sacrificed by collecting their blood into dry and heparin-containing tubes by cardiac puncture under sodium thiopental (50 mg/kg BW; i.p.) anesthesia. Serum samples were obtained by centrifugation at 1500×g for 10 min. Liver and kidney tissues were rapidly removed, washed in ice-cold saline and kept in ice. The materials were stored at −80°C until they were needed for analysis. In our study, all samples were analyzed at the same time in a single run for each assay to avoid inter-assay variations.

**Biochemical analyses in blood and serum**

Blood hemoglobin A1c (HbA1c) levels were measured in heparinized blood using a turbidimetric inhibition immunoassay (Roche Diagnostics, Mannheim, Germany). The levels of serum glucose, total cholesterol, triglyceride, total protein, albumin, urea nitrogen (BUN), creatinine, and activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) were determined on an automated clinical chemistry analyzer (Roche Diagnostics, Mannheim, Germany).

**Urinary protein determination**

Urinary samples were centrifuged at 1750×g for 10 min, supernatants were diluted and protein levels were measured using Bradford reagent [30].

**Reactive oxygen species (ROS) determination in liver and kidney tissues**

Liver and kidney tissues were homogenized in ice-cold 0.15 M KCl (10%; w/v). ROS production in liver and kidney homogenates were measured fluorometrically [31]. Tissue homogenates were incubated with 100 μM 2′,7′-dichlorodihydrofluorescein diacetate at 37°C for 30 min. The fluorescence of 2′,7′-dichlorofluorescein was determined using a microplate fluorometer (Fluoroskan Ascent FL, Thermo scientific Inc, USA) with an excitation of 485 nm and emission of 538 nm. Results were expressed as relative fluorescence units (RFU).

**Determination of malondialdehyde (MDA) levels in tissues**

MDA levels in tissue homogenates were assessed using thiobarbituric acid in accordance with the method of Ohkawa et al. [32]. The breakdown product of 1,1,3,3-tetraethoxypropane was used as a standard.

**Glutathione (GSH) determination in tissues**

GSH levels were measured in tissue homogenates with 5,5-dithiobis-(2-nitrobenzoate) at 412 nm [33].

**Ferric reducing antioxidant power (FRAP) determination in tissues**

Total antioxidant capacity was analyzed using a ferric reducing antioxidant power (FRAP)-assay in postmitochondrial fraction [34]. Tissue homogenates were centrifuged at 600×g for 10 min and supernatants were recentrifuged at 10,000×g for 20 min at 4°C. FRAP assay was performed in the final supernatant fraction. This assay uses antioxidants as reductants in a redox-linked colorimetric method. At low pH, the ferric-tripryridyltriazine complex is reduced to the ferrous form, which is monitored by measuring the change in absorbance at 593 nm. Protein levels in postmitochondrial fractions were determined using bicinchoninic acid [35].

In our laboratory settings, intra-assay coefficients of variations for FRAP, GSH, ROS and MDA assays were 3.2%, 6.1%, 4.6% and 10.4%, respectively.

**Histopathologic analyses**

Liver and kidney tissue samples were fixed in 10% formalin solution. After routine processing, tissue samples were embedded in paraffin blocks and 3 μm thick sections were...
obtained. Sections were stained with haematoxylen and eosin (H and E) and evaluated under light microscope.

Statistical analyses

The results were expressed as mean ± SE. One-way analysis of variance (ANOVA) followed by Tukey’s honestly significant difference post-hoc test was used for equal variances. Kruskal-Wallis test was performed for unequal variances. In all cases, a difference was considered significant when p < 0.05.

Results

Body weights, food and water consumption

Daily food and water intakes increased in STZ rats. MET decreased both food and water intakes in STZ rats, but BET treatment did not have any effect on these intakes. Final body weights were reduced in STZ rats as compared to control group. However, there were no changes in final body weights after BET or MET treatments in STZ rats (Table 1).

Blood HbA1c, serum glucose and lipid levels

STZ treatment caused significant increases in blood HbA1c, serum glucose, triglyceride, and cholesterol levels as compared to controls. BET treatment caused significant decreases in blood HbA1c, serum glucose, triglyceride, and cholesterol levels in STZ rats. MET treatment also decreased these parameters except cholesterol (Table 2).

Hepatic and renal function tests in serum and urinary protein levels

Significant increases in serum AST, ALT, and LDH activities were observed in STZ rats. BET treatment did not alter these enzyme activities, but they decreased due to MET treatment. Increased BUN and decreased total protein and albumin levels in serum were detected in STZ rats, but creatinine levels remained unchanged. These parameters did not alter due to BET or MET treatments in STZ rats. Only, increases in albumin levels were detected following MET treatment. Additionally, both BET and MET treatments diminished high levels of urine protein in STZ rats (Table 3).

ROS, MDA, FRAP, and GSH levels in the liver and kidneys

Significant increases in ROS and MDA levels and decreases in FRAP and GSH levels were observed in the liver of STZ rats. Both BET and MET treatments decreased liver ROS and MDA levels, but GSH and FRAP levels remained unchanged (Figure 1). In kidneys of STZ rats, ROS and MDA levels also increased, but there were no changes in FRAP levels.

Table 1: The effects of betaine (BET) and metformin (MET) treatments on final body weight, food and water intakes in diabetic rats induced by streptozotocin (STZ) (Means ± SE).

| Parameters          | Control (n = 6) | STZ (n = 7) | STZ + BET (n = 7) | STZ + MET (n = 7) |
|---------------------|----------------|-------------|-------------------|------------------|
| Food intake (g/day/rat) | 17.2 ± 0.70a  | 32.4 ± 0.95  | 30.2 ± 1.52       | 23.1 ± 1.33      |
| Water intake (mL/day/rat) | 34.4 ± 3.00a  | 108 ± 4.35   | 132 ± 12.6        | 50.0 ± 3.46a     |
| Final body weight (g) | 256 ± 11.3a   | 182 ± 11.6   | 182 ± 6.67        | 177 ± 4.99       |

*p < 0.05 as compared to STZ group.

Table 2: The effects of betaine (BET) and metformin (MET) treatments on blood hemoglobin A1c (HbA1c), serum glucose, triglyceride and cholesterol levels in diabetic rats induced by streptozotocin (STZ) (Means ± SE).

| Parameters      | Control (n = 6) | STZ (n = 7) | STZ + BET (n = 7) | STZ + MET (n = 7) |
|-----------------|----------------|-------------|-------------------|------------------|
| HbA1c (%)       | 3.53 ± 0.02a   | 8.89 ± 0.35 | 7.21 ± 0.31*      | 6.14 ± 0.20*     |
| Glucose (mg/dL) | 115 ± 5.11a    | 374 ± 17.9  | 308 ± 13.9a       | 293 ± 12.2a      |
| Triglyceride (mg/dL) | 36.7 ± 3.38a | 57.3 ± 4.31 | 31.9 ± 6.18a      | 36.0 ± 3.22a     |
| Cholesterol (mg/dL) | 43.7 ± 2.51a  | 76.9 ± 3.62 | 55.9 ± 6.90a      | 75.9 ± 7.75      |

*p < 0.05 as compared to STZ group.
and GSH levels. Both BET and MET treatments decreased kidney ROS and MDA levels. Additionally, kidney GSH levels increased after MET treatment in STZ rats (Figure 2).

Liver and kidney histology

In livers of STZ-diabetic rats, mild mononuclear inflammatory cell infiltration was observed in portal areas. Mild proliferation in biliary duct epithelium and mild inflammatory cell infiltration in portal areas were also seen. BET or MET treatment did not significantly affect these findings (Figure 3). Normal kidney histology was observed in STZ-diabetic rats. BET or MET treatments did not alter histologic findings in kidney (Figure 4).

Discussion

STZ is a wide spectrum antibiotic frequently used to induce experimental DM. By damaging pancreatic β cells, STZ-induces a type 1-like DM. Thus, rat models of STZ-induced DM are convenient for studies on complications of DM [36]. A single STZ dose of 55–65 mg/kg is capable to induce functional and structural changes in different tissues of rats. In our study, rats were given 55 mg/kg STZ intraperitoneally. This treatment caused an increase in blood HbA1c, and serum glucose and lipid levels, and a disturbance in examined liver and kidney function tests. Increases in ALT, AST, and LDH activities together with hepatic histopathologic changes indicated the presence of hepatic dysfunction in STZ rats. On the other hand, elevated serum BUN levels, diminished total protein and albumin levels, and increased urinary protein levels were observed despite normal histologic appearance of kidney sections and unchanged creatinine levels. As is known, elevated BUN and creatinine levels are markers for renal injury. Elevated creatinine levels are seen only in advanced stage kidney damage whereas BUN levels increase earlier. Therefore, elevated BUN levels together with normal kidney histopathology and unchanged creatinine levels, as seen in our study, has been suggested to indicate a mild renal injury [37]. Under these conditions, significant increases in ROS production and lipid peroxide levels were detected in the liver and kidney of DM rats, indicating a prooxidant status in these tissues. These findings are in accordance with previous studies [4–8].

In the current study, the effect of BET treatment on blood glycemia, lipids, hepatic and renal function tests as well as oxidative stress in liver and kidney tissues were evaluated in STZ-induced diabetic rats. Additionally, the efficiency of BET was compared to that of MET. It has been reported that MET ameliorated insulin resistance and had antioxidant, antiinflammatory and antiapoptotic effects [25, 38]. These properties of MET may contribute to its preventive or ameliorative effect on complications of DM including hepatic [5, 28] and renal complications [6, 39]. In the current study, MET ameliorated hepatic function tests and decreased high levels of serum glucose and triglyceride, blood HbA1c, and urinary protein in STZ rats. Additionally, ROS and lipid peroxide levels diminished in the liver and kidney of MET-treated STZ rats. However, this treatment did not alter histopathologic appearance in these tissues.

On the other hand, BET has antioxidant and antiinflammatory effects. Although it does not interact directly with oxidants, the effect of BET on the metabolism of sulfur-containing substances in the liver was suggested to be responsible for its antioxidant activity [40]. BET administration decreases homocysteine levels, and increases S-adenosyl methionine (SAM) and GSH levels which have strong antioxidant effects in organism [10, 11]. Among them, SAM has a direct antioxidant activity, scavenges...

Table 3: The effects of betaine (BET) and metformin (MET) treatments on alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activities and urea nitrogen (BUN), creatinine, total protein and albumin levels as well as urinary protein levels in diabetic rats induced by streptozotocin (STZ) (Means ± SE).

| Parameters                      | Control (n=7) | STZ (n=7) | STZ+BET (n=7) | STZ+MET (n=7) |
|---------------------------------|---------------|-----------|---------------|---------------|
| ALT (U/L)                       | 37.8±1.89a    | 81.6±6.80 | 67.0±7.67     | 55.7±3.48a    |
| AST (U/L)                       | 83.2±4.03a    | 143±21.4  | 128±8.61      | 95.1±8.01a    |
| LDH (U/L)                       | 313±30.1a     | 699±38.8  | 568±69.2      | 396±21.9a     |
| BUN (mg/dL)                     | 17.2±1.49a    | 79.7±6.81 | 76.4±11.4     | 68.0±3.82     |
| Creatinine (mg/dL)              | 0.42±0.02     | 0.50±0.05 | 0.40±0.04     | 0.42±0.03     |
| Total protein (g/dL)            | 6.32±0.12a    | 5.46±0.20 | 5.60±0.17     | 5.82±0.12     |
| Albumin (g/dL)                  | 3.48±0.07a    | 2.97±0.09 | 3.21±0.14     | 3.41±0.06a    |
| Urinary protein (mg/mg creatinine) | 1.46±0.25a | 2.13±0.11 | 1.53±0.14a    | 1.01±0.11a    |

*p < 0.05 as compared to STZ group.
Figure 1: The effects of metformin (MET) and betaine (BET) treatments on liver reactive oxygen species (ROS), malondialdehyde (MDA), glutathione (GSH), and ferric reducing antioxidant power (FRAP) levels in diabetic rats induced by streptozotocin (STZ) (Means ± SE). *p < 0.05 as compared to STZ group.

Figure 2: The effects of metformin (MET) and betaine (BET) treatments on kidney reactive oxygen species (ROS), malondialdehyde (MDA), glutathione (GSH), and ferric reducing antioxidant power (FRAP) levels in diabetic rats induced by streptozotocin (STZ) (Means ± SE). *p < 0.05 as compared to STZ group.
Figure 3: The effect of betaine (BET) and metformin treatments on liver histology in diabetic rats induced by streptozotocin (STZ) (H&E; ×100/×200).

Figure 4: The effect of betaine (BET) and metformin treatments on kidney histology in diabetic rats induced by streptozotocin (STZ) (H&E; ×100/×200).
Betül Evran et al.: Betaine treatment decreased serum glucose and lipid levels

Ethical Considerations: This study was approved by the Animal Care and Use Committee of the University of Istanbul (Project No: 2013/77).

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In conclusion, all of the above mentioned findings indicate that BET may be a candidate supplement in the treatment of DM and prevention of its complications.

In the current study, BET treatment decreased blood HbA1c, serum glucose and lipid levels, and urine protein excretion and reduced the prooxidant status in liver and kidney tissues of DM rats. However, it did not ameliorate hepatic and renal function tests in serum and histopathologic findings in the liver or kidney. As a well known anti-diabetic drug, MET was used in our study to compare the effects of BET. Our results are showing that BET exerts effects similar to that of MET in STZ-induced diabetic rats, but they do not imply any superiority. Therefore, investigating the effects of the co-treatment of BET and MET in STZ-treated rats can be interesting.

Increased hepatic and renal function tests in serum and histopathologic findings in the liver of DM rats have not been clearly elucidated yet. Jeong et al. [22] have found mildly decreased serum glucose and fructosamine levels, AST, ALT, and LDH activities together with amelioration in histopathology of pancreatic β cells in DM rats fed on 1% BET containing diet for 3 weeks. In another study, Jung et al. [24] have observed that 1% BET supplemented diet for 5 weeks resulted in decreases in insulin and triglyceride levels and amelioration of insulin resistance in db/db mice. They also found decreased lipid peroxide and increased GSH levels and catalase activities in the liver. However, BET treatment (500 mg/kg for 2 weeks with oral route) did not decrease blood glucose levels in STZ rats, but it prevented and/or delayed complications of diabetic retinopathy by inhibiting retinal neovascularization [45].

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ROS and chelates iron ions and thereby inhibits hydroxyl radical generation [41]. Accordingly, BET was reported to protect tissues including liver and kidney against oxidative stress mediated injury. BET treatment was reported to be useful in the alleviation of alcoholic-[13] and non-alcoholic fatty liver [14], and necrotic [15] and fibrotic [16] lesions in the liver. Previous studies have also demonstrated protective effects of BET against carbon tetrachloride-[17], high fructose diet-[19] and cisplatin-[20]-induced nephrotoxicity.

Decreased liver [21] and kidney [42] levels of BET were reported in animal models of DM. In human studies, increased urinary excretion and decreased serum levels of BET were shown to be correlated with DM or its complications [22, 43, 44]. However, effects of BET supplementation in rats with DM have not been clearly elucidated yet. Jeong et al. [23] have found mildly decreased serum glucose and fructosamine levels, AST, ALT, and LDH activities together with amelioration in histopathology of pancreatic β cells in DM rats fed on 1% BET containing diet for 3 weeks. In another study, Jung et al. [24] have observed that 1% BET supplemented diet for 5 weeks resulted in decreases in insulin and triglyceride levels and amelioration of insulin resistance in db/db mice. They also found decreased lipid peroxide and increased GSH levels and catalase activities in the liver. However, BET treatment (500 mg/kg for 2 weeks with oral route) did not decrease blood glucose levels in STZ rats, but it prevented and/or delayed complications of diabetic retinopathy by inhibiting retinal neovascularization [45].

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