Effects of Endothelin-A Receptor Antagonist BQ-123 on Plasma Leptin Levels Streptozotocin Induced Diabetic Rats
A Ozturk1, H Erdogan2, F Ekici3, D Aydin4, E Sogut5

ABSTRACT

Objectives: Leptin and Endothelin (ET) as important endogenous factors interact with each other which may contribute to a better understanding their role in diabetic pathogenesis.

We aimed to evaluate the relationship between Leptin and ET by investigating the influence of BQ-123, an ET-A receptor (ETaR) antagonist, on Leptin levels in rats with diabetes induced by Streptozotocin (STZ).

Methods: In this study, 24 male Wistar-albino rats were divided into three groups; Control, STZ, STZ+BQ-123 groups. Experimental diabetes was induced by delivering a single dose of 60 mg/kg intravenous STZ. The rats in the STZ+BQ-123 group received 4 mg/kg i.v. in total BQ-123 (2 mg/kg +2 mg/kg on the 39th and 40th days). The plasma specimens collected 6 hours after the last BQ-123 delivery were studied for biochemical parameters.

Results: At the end of the experiment, the weights of rats in the STZ and STZ+BQ-123 groups were significantly lower compared with the values in the Control group. The levels of blood glucose were significantly higher in the STZ and STZ+BQ-123 groups than in the Control group. While rats with STZ-induced diabetes demonstrated no changes in Leptin, PC and K+ levels, they exhibited reduced NO, Na+ and Cl- concentrations. The levels of plasma TBARS was significantly higher in the STZ group than in the Control and STZ+BQ-123 groups.

Conclusion: Although the levels of plasma Leptin was no statistically significant difference between the groups, BQ-123 groups lead to further decrease in reduced levels of Leptin than only diabetic group. Our findings have been considered that ETaR antagonists have positive impacts depending on the dosage in the diabetic rats.

Key words: Endothelin-1, experimental diabetes, Leptin, BQ-123

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INTRODUCTION

Diabetes Mellitus (DM) is the most common endocrine and metabolic disorder of our age, emerging as a result of real or functional lack of insulin, which is characterized by blood, carbohydrate, protein and fat metabolism disorder (1–3).

Today, experimental diabetes models can be formed with various materials and methods. One of the ingredients used to create an experimental diabetes model is streptozotocin (STZ). Diabetes created with STZ is a commonly used model in terms of formation of insulin dependent diabetes model of human (4).

ET's are the peptides occurring naturally in many cell types of the body and known as the most potent vasoconstrictor molecules (5, 6). There are ET isopeptides in humans and other mammals, named as ET-1, ET-2 and ET-3 which have different structural and pharmacological effects. ET-1 is the primarily ET which is synthesized by endothelial cells (7). It shows paracrine and autocrine effects via ETₐR and ET₇R on the endothelial and smooth muscle cells (8). There are many studies that have demonstrated the increase in the level of ET-1 in diabetes (9, 10). ET-1 also leads to the progression of DM (11).

Leptin is a protein hormone, that derives its name from the Greek word leptos, which means thin, produced by ob-gene in fat cells and other tissues and released to plasma (12, 13). Insulin is the most researched Leptin-associated hormone and an important regulator of the ob-gene expression (14). Leptin and other adipocytokines were associated with the type 2 DM and insulin resistance (15). It has been demonstrated that Leptin deficiency lead to severe insulin resistance in uncontrolled DM (16). Additionally, it has been informed that Leptin had an anti-diabetic effect in diabetic rats. Researches has been shown that Leptin had powerful anti-diabetic effect in the STZ- induced insulin dependent diabetic rats and transgenic mice (17).
There are some studies evaluating the relationship between Leptin and ET-1 in different diseases and systems. For example, ET-1 stimulates Leptin production with ETAR in adipocyte cultures (18). In another study conducted by Juan et al., Leptin increased the levels of ETAR in vascular smooth muscle cells (19). It has been informed that, Leptin induced ET-1 in endothelial cells (20). These data suggest that increased ET-1 levels in diabetes due to the relationship between Leptin and ET-1, may affect the plasma Leptin levels.

Although there are many studies evaluating as well as Leptin and ET-1 separately in various diseases and systems, any study that demonstrates how ET-1 and Leptin affect each other in diabetes and how ET receptor antagonists affect plasma Leptin levels in diabetes, could not find. Therefore, we aimed to investigate the effects of ETAR antagonist BQ-123 on Leptin levels in STZ-diabetic rats.

The studies demonstrate that a decrease in Leptin levels may occur in STZ-induced diabetic rats. How this decrease affects in diabetic rats given ETAR antagonist BQ-123 became our scientific curiosity. Because, there are some studies demonstrating increase in ET-1 and endothelium caused up-regulation of Leptin secretion levels in diabetes.

Hence, Leptin levels which were already decreased in diabetes may be reduced more with the application of ET receptor antagonist. It is known that, decrease in Leptin levels in diabetes caused adverse effects. So, it should be determined that how ETAR antagonist affected the increased levels of Leptin in diabetes.

SUBJECTS AND METHODS

Before the experiment, the approval was taken from Gaziosmanpasa University Local Ethics Committee for Animal Experiments and animal rights were protected (2010-HADYEK-029). In the experiment, 24 male Wistar albino rats weighting 180-250 g were used. Rats were kept
in cages at room temperature 21±2 °C with a 12 h light/dark cycle, fed with standard rat pellet food and tap water.

**Chemicals**

STZ and BQ-123 were obtained from Sigma Chemical Co., (St. Louis, MO). STZ was dissolved in cold phosphate-citrate buffer solution (0.1 M, pH=4.5). Buffer solution was prepared freshly and protected from light. BQ-123 dissolved in0.9 % NaCl.

**Procedure for diabetes**

Rats were randomly divided into 3 groups and each group included 8 rats (Control group, n=8, STZ group n=8, STZ+BQ-123 group n=8). Blood-glucose levels of the each rat, fasted for 12 h, were measured with a sugar measuring instrument (glucometer) (PlusMED ACCURO pM1300) and recorded. After that, by measuring the weight of each rats, STZ 60 mg/kg, which was prepared freshly by dissolving in phosphate-citrate buffer (pH=4.5), was administered *i.p.* to the rats in STZ and STZ+BQ-123 groups. For the control group, phosphate-citrate buffer with the same volume was administered *i.p.* Then, the feeding of rats was released. Fasting blood glucose levels were measured again 72 h after the STZ administration, the rats with the level of 200 mg/dl or higher were considered as diabetic. In order to complete the formation of diabetic physiopathology, rats were kept under the appropriate conditions for 40 days after the STZ implementation. The diabetes procedure was prepared with the reference of few studies that have been done before (21, 22).

**Treatment procedure**

The rats in STZ+BQ-123, STZ group, 39th and 40th days after the application, (2 mg/kg+2mg/kg), a total of 4mg/kgBQ-123 was administered *i.v.* from tail vein. For the rats in control and STZ group, instead of BQ-123, same volume of saline was given. Rats were
sacrificed by taking 4-5 ml cardiac blood samples under anesthesia with, Ketamine (30 mg/kg) and xylazine (5 mg/kg) 6 hours after the last BQ-123 implementation.

The Measurement of plasma leptin

Leptin levels were measured in the plasma obtained after centrifugation of blood by using the method of Enzyme-Linked Immunosorbent Assay (ELISA). The Mouse / Rat ELISA Leptin kit (Biovendor, Cat no: RD291001200R, North Carolina, USA) was used for determination of rats’ leptin levels by ELISA device (Organ tecnicareade 230 S). The results were read at 450 nm with the help of spectrophotometer (GBC Cintra 10e) and calculated as ng/ml. Leptin ELISA kit is based on the sandwich principle. Microtiter layer is covered with monoclonal antibody which is sensitive against a single antigenic portion of Leptin molecule. Patient samples containing large molecules of Leptin were incubated in 'rabbit anti-Leptin' antibody-coated layer and the sandwich complex was formed. After incubation, unbound material is washed and in order to determine the bound Leptin, 'anti rabbit' peroxidase is added. Substrate solution is added, forming color intensity is directly proportional to the amount of Leptin in patient serum (23).

Measurement of serum TBARS levels

Serum TBARS levels were measured by Mihara and Uchiyama method. In this, in TBA test reaction, malondialdehyde (MDA) or MDA-like substances produce a pink color by entering reaction with TBA and give the maximum absorbance at 532 nm. The reaction was carried out at 90 °C and at pH = 2-3 for 15 min. In order to bring down the proteins, the samples were stirred with two-fold volume of 10% cold trichloroacetic acid (w/v). The particles were brought down by centrifugation and the reaction of liquid part of the supernatant and equal volume of 0.67% of (w/v) TBA was performed in boiling water bath for 10 minutes. After
cooling, it was read with the spectrophotometer at 532 nm. The results were calculated as nmol/ml (24).

**Measurement of the level of serum PC**
Detection of protein carbonyl groups; was performed on the basis of the spectrophotometrically measurement of stable hydrazone compounds formed as a results of reaction of protein carbonyl groups with 2,4-dinitrophenyl hydrazine at 370 nm. During calculations, $\varepsilon = 22000$ M$^{-1}$ cm$^{-1}$ was accepted as molar absorption coefficient at 370 nm for 2.4 dinitrophenyl hydrazine. Protein carbonyl levels were calculated as nmol/ml (25).

**Measurement of serum NO Level**
Amount of nitrite in the serum was determined by the Griess reaction after deproteinization. Total nitrite (nitrite + nitrate) was evaluated with the modified cadmium reduction method. Nitrate reduction was provided at the end of the 90-minute incubation with copper (Cu)-coated cadmium granules deproteinized sample supernatant in pH 9.7 glycine buffer. Produced nitrites; were determined with sulfanilamide and related N-naphtylethylenediamine (NNDA) diazotization. A pink color occurred as a result of the reaction was read with the help of spectrophotometer at a wavelength of 545 nm. Nitrate concentration was determined by substracting the resulting concentration from the first concentration (26).

**Other biochemical analyses**
Plasma Na, K, Cl values were measured with the help of an auto analyzer (Cobas C 501, Tokyo, JAPAN) by using indirect Na$^+$, K$^+$, Cl kits.

**Statistical analysis**
Statistical analysis was performed with "SPSS 19.0 for Windows". For the comparison of the differences between the groups, "One-way ANOVA" test was used. In which case the data did
not comply with the normal distribution \((p \leq 0.05\) according to the Levene test), instead of this test, "Kruskal-Wallis Analysis of Variance" was applied. When the differences between groups were found significant in one-way analysis of variance \((p \leq 0.05)\), the groups were compared in pairs with "Tukey HSD" which is one of the Post Hoc tests. When the differences between groups were found significant in Kruskal-Wallis Analysis of Variance \((p \leq 0.05)\), the groups were compared in pairs with "Mann-Whitney U test (Bonferroni correction)". Results were expressed as mean ± standard deviation.

RESULTS

Live Body Weights of Rats during the Test

The weights of the rats were measured at the beginning, in the middle and at the end of the experiment, they were recorded as; for the control group; 184.37±12.37, 193.13±11.63, 239.38±15.68 g and for STZ group; 208.13 ± 7.53, 200.00 ± 10.35, 191.25 ± 11.57 g; for STZ + BQ-123 group, 231.25 ± 25.03, 218.75 ± 18.47, 203.50 ± 29.90 g. The weights were compared at the end of the experiment between the groups, the weights of rats in STZ group \((p=0.0001)\) and STZ + BQ-123 group \((p=0.015)\) were significantly lower than the control group. Diabetic rats lost weight.

Fasting Blood Glucose Levels in Rats during the Test

The fasting blood-glucose levels of the rats compared on the basis of the groups, no significant difference was found between the groups at the beginning of the experiment \((p=0.384)\). In the measurements performed 72 hours after the application of STZ, the blood glucose levels of rats in control, STZ and STZ+BQ-123 groups were recorded as 127.00±15.32, 491.75 ± 72.65, 501.13 ± 75.75 mg/dl, respectively. The blood glucose levels of the rats in STZ and STZ + BQ-123 groups were found significantly higher than the levels
of the control group (for both \( p=0.0001 \)). An experimental diabetes model was achieved in rats with STZ.

**Plasma Levels of Leptin, Serum Levels of PC, TBARS, NO**

The plasma Leptin levels of the rats in the experimental groups were, in the control 0.92 ± 0.58 ng/ml, in STZ group; 0.58 ± 0.34 ng/ml, STZ+BQ-123 group; 0.38±0.29 ng/ml. Leptin levels were analyzed according to the groups, but no significant difference was found \( (p=0.055) \) (Table 1; Figure 1).

TBARS levels of rats in experimental groups in the control, STZ and STZ+BQ-123 groups were found as 1.55±0.16 nmol/ml, 1.97±0.24 nmol/ml, 1.65±0.10 nmol/ml, respectively. TBARS values of the STZ group were significantly higher than the control \( (p=0.0001) \) and STZ+BQ-123 groups \( (p= 0.0004) \), (Table 1; Figure 2).

PC levels of rats in experimental groups in the control, STZ and STZ+BQ-123 groups were found as 903.88±280.45 nmol/ml, 930.50±252.09 nmol/ml, 1040.50±400.41 nmol/ml, respectively. PC levels were analyzed according to the groups, there was no statistically significant difference between the groups \( (p=0.665) \), (Table 1; Figure 3).

NO levels of rats in experimental groups in the control, STZ and STZ+BQ-123 groups were measured as 46.32±3.85 nmol/ml, 38.52±2.26 nmol/ml, 37.48±4.88 nmol/ml, respectively. NO levels of STZ \( (p=0.0001) \) and STZ+BQ-123 \( (p=0.0001) \) groups were statistically significant lower than the values of the control group (Table 1; Figure 4).

**Other biochemical parameters**

The serum Na\(^+\) concentration levels were measured in control, STZ and STZ+BQ-123 were 137.75±3.01 mmol/L, 126.75±7.57 mmol/L, 128.50±12.34 mmol/L, respectively Serum Na\(^+\) concentration values of STZ group, were significantly lower than the values of the control group \( (p=0.001) \). There was no significant difference between the other groups.
Serum K\(^+\) concentrations values were measured in the control, STZ and STZ + BQ-123 groups as 5.45 ± 0.64 mmol/L, 6.03 ± 1.99 mmol/L and 5.08 ± 0.97 mmol/L, respectively. There was no a statistically significant difference between the groups in terms of values of serum K\(^+\) concentrations \((p = 0.375)\). Serum levels of Cl\(^-\) concentration were measured in the control, STZ and STZ+BQ-123 groups as 97.69 ± 0.93 mmol/L, 84.61 ± 5.38 mmol/L, 88.12 ± 9.89 mmol/L, respectively. In terms of Cl\(^-\) concentration in serum levels of the groups, the values of STZ group \((p = 0.0001)\) and STZ+BQ-123 group \((p = 0.0001)\) were significantly lower than the values of the control group.

**DISCUSSION**

In our study, the blood glucose levels of STZ and STZ + BQ-123 groups measured 72 hour after STZ application were significantly higher than the values of the control group. This finding supports that, model of diabetes was successful, and 60 mg/kg dose of STZ was sufficient. In the rats that diabetes was formed after administration of STZ, during the test, for a period of 40 days, polyphagia, polydipsia, polyuria, and hyperglycemia was observed. These findings observed in diabetic rats were consistent with the clinical signs of diabetes.

Hypoleptinemia accompanies to hyperglycemia observing due to reduced insulin secretion which occurs due to the destruction of the pancreas with the effect of STZ in this model \((27, 28)\). Previous studies demonstrate that, serum Leptin levels decreased in diabetes \((27, 29)\). Studies in STZ-diabetic rats have indicated that the levels of serum Leptin and Leptin mRNA noticeably diminished \((28)\). The levels of plasma Leptin also decreased in Type II DM patients \((30)\) and in Type I DM patients \((31)\). In our study, although Leptin levels in STZ group were lower than the control group, this was not statistically significant \((p = \)
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0.055). Despite the decrease in Leptin levels in diabetic groups, the reason that we did not find significantly difference may be related with STZ dose and duration.

ET-1 biosynthesis increases in diabetes, and therefore this peptide causes vascular complications. There are some studies that have shown an increase in ET-1 levels in diabetes. For example, in a study conducted by Makino and Kamatojin STZ-diabetic rats, as well as the formation of hyperglycemia, ET-1 levels were found increased significantly in STZ-diabetic rats (9).

There are some studies evaluating the relationship between Leptin and ET-1, on different systems and diseases. For example, in a study conducted by Xiong et al., it has been found that, ET-1 stimulated the production of Leptin adipocytes cultures with ET_A R (18). Also in another study; Quehenberger et al. found that Leptin induced ET-1 in endothelial cells (23). This data also suggest that, due to the relationship between Leptin and ET-1 levels, increased levels of ET-1 levels in diabetes may affect plasma Leptin levels. In the light of these data, even the decrease in Leptin levels in STZ group was not statistically significant; the levels of Leptin in BQ-123 decreased more. If so, it may be said that, ET_A R antagonist BQ-123 reduced the levels of ET which increased in diabetes. However, a longer duration of diabetes, administration time of BQ-123 can provide a meaningful detection of reduction in the levels of Leptin. The topic may be evaluated better with new studies with different doses and times.

DM is a chronic metabolic disorder and at the same time as an increased oxidative stress situation (32). Oxidative stress acts as a leading factor in DM and its complications (33, 34). Several studies inform that free radicals and reactive oxygen species increased in DM rats and patients (34-38). Lipid peroxidation occurring due to free oxygen radicals is one of the most important causes of cell damage. Lipid peroxidation is defined as a chemical event initiated by free radicals and contains the oxidation of unsaturated fatty acids of membrane structure.
(Turk et al., 2002). TBARS, produced endogenously in the body, is an important indicator of lipid peroxidation (39) and the studies indicate that tissue and plasma TBARS levels increased in diabetes. In our study, in the serum of STZ-diabetic rats, TBARS levels which are an indicator of lipid peroxidation, were increased significantly compared with control group. This increase in lipid peroxidation in diabetic rats is similar to other studies on diabetic rats and humans. In a study conducted by Turk et al., a significant increase of TBARS levels in patients with Type 2 DM has been reported (40). In another study conducted by Ruberz et al., it has been detected that, plasma TBARS levels in STZ-diabetic rats were significantly higher than the control group (41). In another study conducted by Jeyashanthi et al., an increase in serum, liver and kidney tissues levels of TBARS of STZ-diabetic rats was detected (42). Our study is in line with these data.

Researchers have used various agents against DM such as melatonin (43–46). We used BQ-123 that is a selective ET_{A}R antagonist and decreases ET-1 levels in serum (47). Although the effect of BQ-123 on TBARS levels was investigated, we did not find any study about how BQ-123 affects the increased levels of TBARS in diabetes. However, in renal ischemia and reperfusion injury, BQ-123 has as the effects of antioxidant, decreased the increased levels of TBARS (48). In another study conducted by Lund et al., it has been determined that, BQ-123 reduced cardiac TBARS levels (49). BQ-123 as an ET_{A}R antagonist inhibited the formation of lipid and protein oxidation products (50). Our results are in line with these studies. In the group treated with BQ-123, levels of TBARS showed a significant decrease compared with STZ group. In our study, it can be said that, BQ-123 has a positive effect by reducing the increased levels of TBARS in diabetes.

It has been reported that there is a significant protein oxidation caused by oxidative damage in diabetic patients (22) and diabetic rats (51). In our study, there was no significant difference between the groups in terms of serum PC levels. To keep longer duration of
diabetes and examination on tissue samples may help to show the change in the levels of PC. In our study, due to checking only serum PC levels, in 40-day period, protein damage may not be reflected in the serum levels.

NO, was produced enzymatically by nitric oxide synthetase (NOS) from L-arginine (52). It has been reported that, in diabetes, bioactivity of NO, basal NO production and L-arginine which is a NOS substrate decreased, and some researchers indicate that, in diabetes, NO production has been damaged due to decrease in the plasma levels of L-arginine (53). Decreased levels of NO, contributes the vascular damage, by facilitating interactions of platelet-vascular wall and causing adhesion of circulating monocytes to endothelial surface (54). Therefore, it is important to keep the NO levels in the physiological limits. In our study, in terms of NO levels, there is significant difference between the values of control and STZ groups. A significant reduction in NO levels occurred in diabetic rats. The studies indicate that, NO levels decrease in diabetes. In another study conducted by Zhao et al., it has been determined that, expression of eNOS decreased in the aortas of STZ-diabetic rats (55). In the study conducted by Tessari et al. on diabetic patients, it has been reported that, concentrations of NO significantly decreased in the blood of diabetic patients (56). Our study is in line with these data. In our study, NO levels of rats in STZ and STZ + BQ-123 group decreased significantly compared to control rats, and the effect of BQ-123 which increases the level of NO was not detected.

Na⁺/K⁺-ATPase ionic pump has an important role for providing low Na⁺ and high K⁺ in the cell and maintaining the cell hemostasis (57). Deterioration of Na⁺/K⁺-ATPase enzyme activity in diabetes, leads disrupting in the ion balance and decrease of K⁺ levels in the cell. In diabetes, due to K⁺ ions shift towards the extracellular fluid from inside of the cell, increase of K⁺ ions in extracellular fluid is expected. The reason for shifting of K⁺ ions towards the extracellular fluid is insulin deficiency or resistance seen in diabetes (58). In our study,
although it was not statistically significant, serum $K^+$ concentration levels in diabetic rats were significantly higher than control. Serum $Na^+$ concentration levels were significantly lower in the diabetic rats than control. $Cl^-$ concentration levels in serum were lower in diabetic rats than in the control group. Especially, $Cl^-$ is involved in the provision of osmotic pressure in the plasma and intracellular liquid. Therefore, it is important to balance the levels of $Cl^-$ in diabetes. Some studies supported that the defect of $Na^+/K^+$-ATPase activity in experimental diabetes lead to the active transport of cations play an important role in chronic diabetic complications such as neuropathy, nephropathy, and retinopathy. Reduced $Na^+/K^+$-ATPase activity of red blood cell membrane is considered especially as a strong marker for diabetic neuropathy (59). In the study conducted by Raccah et al., on a case of type 1 diabetes, decrease in $Na^+/K^+$-ATPase activity of red blood cell membrane was associated with the formation of peripheral neuropathy (60). Therefore, to control and balance $Na^+$ and $K^+$ ions is important in diabetes.

The results of this study demonstrate that, Leptin levels in STZ-diabetic rats, decrease but it is not statistically significant. BQ-123 treatment reduced more Leptin levels in diabetes, although it is not statistically significant. This result may be related to the administration time and dose of BQ-123. In the STZ-induced diabetes model, there was no statistically significant difference in levels of PC, $K^+$, but $Na^+$ and $Cl^-$ levels decreased significantly. BQ-123 treatment did not have any effect on this decrease. It is known that, in diabetes, oxidative damage increases and a marked increase in the level of TBARS occurred which an important indicator of oxidative damage is. In the group treated with BQ-123, BQ-123 had a BQ-123 by causing significantly decrease in TBARS levels of the rats. In experimental diabetes, the effects of an ET$_A$R antagonist, BQ-123 and its possible relationship with Leptin are needed to put forward a more precise manner with new studies with different doses and application times. Different ET$_A$R antagonists can be applied in this context. It is obvious that, very
complex relationships between autocrine and paracrine factors and their interactions in changing pathophysiological conditions is a topic which needs more investigations. Also, contributions of these changing to homeostasis in pathophysiological conditions are a topic worth exploring and investigating but it is quite complicated.

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AUTHORS’ NOTE

The authors had no conflicts of interest to declare in relation to this article.
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Table 1: Plasma levels of Leptin (ng/ml) and serum levels of TBARS (nmol/mL), PC (nmol/mL), NO (nmol/mL).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Leptin (ng/mL)</th>
<th>TBARS (nmol/mL)</th>
<th>PC (nmol/mL)</th>
<th>NO (nmol/mL)</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.93 ± 0.58</td>
<td>1.55 ± 0.16</td>
<td>903.88 ± 280.45</td>
<td>46.32 ± 3.85</td>
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<tr>
<td>STZ</td>
<td>0.58 ± 0.35</td>
<td>1.97 ± 0.24</td>
<td>930.50 ± 252.09</td>
<td>38.52 ± 2.26</td>
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<tr>
<td>STZ+BQ123</td>
<td>0.38 ± 0.29</td>
<td>1.65 ± 0.10</td>
<td>1040.50 ± 400.41</td>
<td>37.48 ± 4.88</td>
</tr>
</tbody>
</table>
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Fig 1: Leptin levels in the experimental groups (ng/ml): There is no significant difference between the groups.

Fig 2: TBARS levels in the experimental groups. TBARS levels of STZ group were significantly higher than the control (*p=0.0001) and STZ+BQ-123 (*p=0.0004) groups.
Fig 3: PC levels in the experimental groups. There is no significant difference between the groups.

Fig 4: NO levels in the experimental groups. The other groups were found to be significantly lower than the control group (*p=0.0001).