Thymoquinone Therapy Improves Hyperglycemia, Erythrocyte Indices, Erythropoietin Production and Erythrocyte Osmotic Resistance in Rat Model of Streptozotocin-induced Diabetes

Tariq Helal Ashour¹*

¹Department of Laboratory Medicine, Faculty of Applied Medical Sciences, Umm Al-Qura University, Saudi Arabia.

Author’s contribution

This whole work was carried out by the author THA.

ABSTRACT

Herein, both antidiabetic and hematoprotective effect of thymoquinone (TQ) therapy was investigated in rat model of streptozotocin (STZ)-induced diabetes. After disease induction, TQ (35 mg/kg/day) was given for 28 days and fasting blood glucose level was weekly measured. At day 29, blood samples, pancreatic and kidney specimens were prepared and screened for: (1) hematological parameters: counts of red blood cells (RBCs), white blood cells (WBCs) and platelets (PLTs), erythrocyte osmotic fragility, normal hemoglobin (Hb) and glycosylated hemoglobin (HbA1c), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), (2) serum insulin and serum and renal erythropoietin (EPO) levels, (3) serum creatinine and urea levels, (4) concentrations of glutathione (GSH), superoxide dismutase (SOD) and thiobarbituric acid reactive substances (TBARS) in serum, pancreatic and renal tissues, and (5) pancreas histopathology. STZ-injected rats showed injured pancreatic islets accompanied with persistent hyperglycemia, significant decreases in serum insulin, serum and renal EPO, RBCs counts and indices, PLTs counts, and increases in HbA1c, erythrocyte osmotic, WBCs counts, and serum creatinine and urea. However, therapy with...
TQ successfully protected the pancreatic islets and significantly improved the glycemic status, insulin and EPO production, and the hematological parameters that were deteriorated in STZ-diabetic untreated rats. TQ therapy also improved renal function and reversed the decreases in GSH and SOD, and the increases in TBARs that were induced in STZ-diabetic rats. In conclusion, these findings emphasize the antihyperglycemic, antianemic and hematoprotective potential of TQ in STZ-diabetic rats. Further studies are still needed to confirm the hematoprotective efficacy of TQ in different modalities of DM.

Keywords: Thymoquinone; Diabetes; Hematological complications; Erythropoietin.

1. INTRODUCTION

Diabetes mellitus (DM) is a multifactorial and complex metabolic disorder characterized by absolute or relative deficiencies in insulin secretion and/or insulin action, chronic hyperglycemia, disturbances of carbohydrate, lipid and protein metabolism, and various complications [1]. The global incidence of DM and its associated serious complications is increasing and has become one of the major causes of mortality worldwide. The number of diabetics is expected to reach 439 million among the population worldwide in 2030 [2]. Prevention and control of DM is still a big challenge, and none of the currently used antidiabetic drugs could give a long term glycemic control without causing adverse side effects [3]. Therefore, improvement of diabetes preventative and treatment strategies has become a crucial medical demand. In this concern, a special attention is currently being focused on the use of medicinal plants and their products in prevention or correction of various metabolic disorders including DM.

Over the recent decades, Nigella sativa, commonly known as black cumin or black seed, and its main biologically active ingredient "Thymoquinone (TQ)" have gained popularity due to its pluripotent pharmacological effects [4]. In addition to its powerful antioxidant activity [5], TQ has been reported to exhibit many beneficial effects such as immunomodulatory [6], anticancer [7], anti-inflammatory and organ protective activities [5,7]. Even though the beneficial antidiabetic properties of TQ treatment on hyperglycemia and organs injury have been previously reported in different disease modalities [8,9], there remains insufficient information concerning its possible protective effect against DM-induced anemia and other hematological complications. Therefore, the present study was designed to explore this important issue and investigate the antidiabetic and hematoprotective potential of TQ supplementation therapy by using the most common experimental animal model of human diabetes induced by STZ in rats [10].

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Streptozotocin (STZ) and thymoquinone (TQ) were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA), and were freshly prepared for immediate use. Commercial Enzyme linked immunosorbant assay (ELISA) kits for quantitative measurement of erythropoietin (EPO) and insulin hormones were purchased from Cusabio Biotech CO.(Hubei, China), and commercial ELISA kits for measurement of total reduced glutathione (GSH) level, superoxide dismutase (SOD) activity, and thiobarbituric acid reactive substances (TBARS) concentration were purchased from Cayman Chemical (Ann Arbor, MI, USA). All other used chemicals and reagents were of analytical grade and obtained from standard commercial supplies as stated under the sections of their applications.

2.2 Animals, Induction of Experimental Diabetes and Study Design

All animal procedures and experimental protocols were conducted in accordance with the standards of the National Institutes of Health (NIH) and were approved by the Animal Ethical Committee of Umm-Al-Qura University, KSA. Adult male Wistar albino rats, weighing 200–225 g, were used and housed (five rats per cage) under controlled temperature (20–25°C), 12-h light–dark cycle, and allowed free access to water and a commercial rat pellets stock diet. After acclimatize for 2 weeks, the rats were fasted overnight and then DM was induced by a single intraperitoneal injection of STZ (65 mg/kg BW) freshly dissolved in 5 mmol/L citrate buffer (pH 4.5). Three days after STZ injection, the induced diabetes was confirmed by measuring fasting blood glucose levels in blood samples.
from the tail vein with a One Touch Digital Glucometer (Roche Diagnostics GmbH, Mannheim, Germany), and rats with blood glucose level ≥200 mg/dL were considered as diabetic and used for the experiment as previously described [11].

In this study, a total of 40 rats (10 normal and 30 confirmed diabetic) were assigned into the following 3 groups: Group I (n=10): normal control rats received an equal volume of vehicle alone (control group); Group II (n=15): diabetic untreated rats (STZ group); and Group III (n=15): diabetic rats treated with oral TQ (35 mg/kg/day) for 28 consecutive days (STZ+TQ group), and this TQ's daily dose was selected based on data of a pilot study that was conducted prior to this target study. All animals were daily observed for survivability during the entire period of the study. Fasting blood glucose level of tail blood sample was measured once per week in all animal groups by the above mentioned method of One Touch Digital Glucometer. At the end of the study, rats were fasted overnight, sacrificed under diethyl ether anesthesia and their whole blood, pancreases and kidneys were collected and used for the target analyses.

2.3 Blood Sampling and Hematological Measurements

During scarification process, three whole blood samples were immediately withdrawn from the vena cava of each rat. The first sample was collected in a tube contained disodium salt of ethylene diamine tetra acetic acid (EDTA) anticoagulant and used for determination of the following hematology parameters: counts of RBCs, WBCs and PLTs, normal Hb concentration, HCT percentage, and levels of MCV, MCH, and MCHC. These hematological parameters were determined by standard hematological techniques by using Automated Analyzer, Sysmex XS 500 (Sysmex, IL, USA). In addition, glycosylated hemoglobin (HbA1c) was determined by using a full automated HbA1C analyzer (Clover A1c™, Infopia Inc., Anyang, Korea). The second sample was collected into a heparinized test tube and used for determination of erythrocyte osmotic fragility by using 1% phosphate buffered sodium chloride (NaCl) solution diluted to concentrations ranging from 0.0% to 0.9% NaCl as previously described [12]; while the last portion of the collected blood was placed in a plain centrifuge tube without any anticoagulant, and its serum was obtained and kept at -20°C until used for measurement of the serum levels of kidney function biomarkers (creatinine and urea), EPO and insulin hormones and the biomarkers of antioxidation and oxidative stress as described below.

2.4 ELISA Assays

At the end of the experiment, concentrations of serum insulin, serum and renal EPO, serum, pancreatic and kidney GSH, SOD and TBARS were measured in all experimental groups by ELISA using a fully automated ELISA system (Human 7 Diagnostics, Germany). Before the assays, the pancreatic and renal tissue homogenates were prepared by homogenizing a portion of each isolated pancreas and kidneys in RIPA lysis buffer (1:6 w:v), centrifuged at 10,000 rpm for 10 min at 4°C, and the supernatant was obtained. The concentrations of the total proteins in the extracted supernatants were measured using the BioSpec-nano (Shimadzu Corporation, Japan). During the assays, all samples were processed in duplicate and according to the manufacturers' instructions.

2.5 Histopathological Examination

A small portion of the pancreas from each rat was fixed in 10% formalin solution for sectioning (5 μm-thickness slices) and staining with hematoxylin and eosin (H&E) to enable histological examination. For histopathological investigations, the stained sections were examined with a light microscopy via a pathologist in a blinded fashion for the presence of the hallmarks of pancreatic islets injury.

2.6 Statistical Analysis

The results were expressed as mean ± standard deviation (SD) and statistical analysis was carried out using SPSS program for Windows (version 16.0; SPSS Inc., Chicago, IL, USA). Differences among the groups were investigated using one-way analysis of variance (ANOVA) followed by a Student's t-test. Differences between percentages of RBC hemolysis, HbA1C and mortality rate for the groups were analyzed by χ² test. The statistical probabilities, P<0.05 and P<0.01, were considered statistically significant and highly significant, respectively.
3. RESULTS

3.1 Antidiabetic Activity of TQ Therapy on STZ-rat Model of DM

After induction of DM with STZ, fasting blood glucose level of tail blood sample was measured once per week in all animal groups until the end of the study. Compared with normal control non-diabetic group, this fasting blood glucose increased significantly and persistently in STZ-diabetic untreated rats (STZ group) until day 28 (Table 1). On the contrary, treatment of these STZ-diabetic rats with TQ had resulted in a remarkable and persistent anti-hyperglycemic effect (Table 1). Serum insulin level was also measured by the end of the experimental period, and it was significantly lower in diabetic untreated rats than that of normal controls or diabetic rats treated with TQ (Table 1). Moreover, glycosylated hemoglobin (HbA1c), a form of hemoglobin used primarily to identify the average plasma glucose concentration over prolonged periods of time, was measured in all experimental groups at the end of the study, and the results showed that its percentage was significantly higher in STZ-diabetic rats than that of STZ-diabetic rats treated with TQ (Table 1). This in turn reflects the potential antihyperglycemic efficacy of TQ therapy during the entire period of the experiment. Furthermore, all animal groups were daily monitored for deaths and to determine the accumulative survival rate during the entire period of the experiment. Furthermore, all animal groups were daily monitored for deaths and to determine the accumulative survival rate during the entire period of the study, and the results showed that its percentage was significantly higher in STZ-diabetic rats than that of STZ-diabetic rats treated with TQ (Table 2 and Fig. 2C). This in turn reflects the potential antihyperglycemic efficacy of TQ therapy during the entire period of the experiment.

3.2 Hematoptotective Effect of TQ Therapy on STZ-rat Model of DM

The observed hematological parameters of all tested experimental groups are shown in Table 2 and Fig. 2. Rats injected with STZ and left without treatment showed remarkable anemia reflected by significant decreases in RBCs count, Hb concentration, HCT% and values of MCH and MCHC (Table 2 and Fig. 2A&B). In contrary, therapy of these STZ-diabetic rats with TQ significantly ameliorated the developed anemia and rescued the deteriorated erythrocyte indices (Table 2 and Fig. 2A&B). In a like manner, the platelet count was significantly lower in STZ-diabetic untreated group than that of STZ-diabetic rats and treated with TQ (Table 2). In contrary, the total WBC count was highest in the diabetic rats untreated than that of TQ-treated diabetic or normal control rats (Table 2). Collectively, this part of data indicate that after 4 weeks of STZ-induced diabetes in rats there was significant anemia and hematological disturbances that were collectively alleviated and improved by TQ therapy.

3.3 Effect of TQ Therapy on Serum and Renal Erythropoietin in STZ-diabetic Rats

As gathered clinical data have proven that anemia is common in diabetic patients and is mainly due to EPO deficiency secondary to diabetic nephropathy [13,14], the concentration of this hormone was therefore measured in the sera and kidney tissues of all experimental groups. As illustrated in Fig. 3, after 4 weeks both systemic and renal levels of EPO were significantly reduced in the STZ-diabetic untreated rats and these levels were significantly restored in STZ-diabetic rats treated with TQ (Fig. 3). Further confirmation, the serum levels of creatinine and urea were measured in all animal groups and their values were higher in untreated diabetic rats than that of diabetic rats treated with TQ (Fig. 4), suggesting the renoprotective effect of TQ therapy against diabetic induced renal injury.

3.4 Effect of TQ Therapy on Erythrocyte Osmotic Fragility in STZ-diabetic Rats

Changes in RBCs hemolytic pattern after their exposed to different concentrations of NaCl solution were measured in all animal groups at the end of the experiment to determine the erythrocyte osmotic fragility and resistance among the tested animals. As shown in Table 3, erythrocyte osmotic fragility of STZ group was significantly higher than that of diabetic rats treated with TQ or normal non-diabetic control group at each tested NaCl solution. This observation can therefore indicate the stabilizing effect of TQ supplement on erythrocyte osmotic resistance.
Fig. 1. Histopathological findings. (A) Pancreatic tissues of normal rats show normal islets of Langerhans (Yellow arrow). (B) Pancreatic tissues of streptozotocin (STZ)-induced diabetic rats show diffuse necrosis with massive inflammatory cell infiltration. (C) Pancreatic tissues of STZ-diabetic rats treated with thymoquinone (TQ; 35 mg/kg/day; for 28 days) show the alleviating effect of TQ therapy on injured pancreatic islets.

Fig. 2. Effect of thymoquinone therapy (TQ; 35 mg/kg/day, orally for 28 days) on red blood cell (RBC), normal hemoglobin (Hb) and glycosylated hemoglobin (HbA1c) in streptozotocin (STZ)-induced diabetes in rats. $^*$P < 0.05 versus control group, $^#$P < 0.05 versus STZ group.
Table 1. Effect of thymoquinone therapy (TQ; 35 mg/kg/day, orally for 28 days) on survivability, blood glucose and serum insulin levels in streptozotocin (STZ)-induced diabetes in rats

| Group            | Fasting blood glucose (mg/dL) | Serum insulin (µU/mL) | Total mortality (%) |
|------------------|-------------------------------|------------------------|---------------------|
|                  | Week 1                        | Week 2                 | Week 3              | Week 4                 | Day 28            |                      |
| Control (n = 10) | 81.1±3.17                    | 79.3±5.14              | 80.5±6.16           | 80.3±4.22              | 4.2±0.09         | 0                    |
| STZ (n = 15)     | 231.2±13.2**                 | 353.8±17.5**           | 377.5±15.8**        | 316.2±11.7**           | 1.15±0.08**      | 13.3*                |
| STZ + TQ (n = 15)| 160.2±10.6*                  | 142.5±8.3**            | 123.5±13.5**        | 112.2±13.1**           | 3.7±0.22         | 0*                   |

Values of blood glucose and serum levels are represented as mean ± SD. *P < 0.05 and **P < 0.01 versus control group, #P < 0.05 versus STZ group.

3.5 Systemic and Tissue Levels of Antioxidant and Oxidative Stress Indices

Finally, the levels of GSH (a non-enzymatic antioxidant defense element), activities of SOD (an example of enzymatic antioxidant defense mechanism), and concentrations of TBARS (an index of lipid peroxidation and oxidative stress) were measured in the sera samples and the pancreatic and kidney tissue homogenates of all animal groups by the end of the experimental period. As demonstrated in Table 4, significant reductions in GSH contents and SOD activities, as well as marked elevations in TBARS concentrations were detected in the tested biological samples of STZ-diabetic untreated rats compared with that in normal controls. On the other hand, treatment of these STZ-diabetic rats with TQ significantly restored both GSH and SOD to almost their normal values, and also significantly decreased the elevations of serum and tissue levels of TBARS (Table 4).

4. DISCUSSION

In the present study, both antidiabetic and hematoprotective activity of thymoquinone (TQ), the main biological active component of Nigella Sativa with pluripotent pharmacological actions [7,9], were observed in rat model of STZ-induced DM. TQ therapy had resulted in significant improvement effects on STZ-induced pancreatic islets injury, persistent hyperglycemia, anemia and deteriorations on erythrocyte indices and osmotic stability, erythropoietin production and antioxidant status.

Due to its high selective cytotoxicity to the insulin-producing β cells of the pancreatic islets of Langerhans, STZ has clinically been approved for treating insulinomas and metastatic cancer of pancreatic islets β cells [15], and has been long used for inducing diabetes model on various experimental animals in medical research [10]. Its peculiar cytotoxicity and diabetogenic effect is based upon a fact that STZ is selectively transported into the pancreatic β cells by the glucose transport protein (GLUT2) causing damage to the DNA and irreversible cell destruction, though other mechanisms may also contribute such as generation of reactive oxygen species and oxidative stress [10,16]. In constancy...
with these facts, the histopathological findings of the present study showed that the pancreatic islets of STZ-injected rats and left without treatment exhibited diffuse necrosis with excessive inflammatory cells infiltration (Fig. 1B), and these histopathological changes were accompanied with intrapancreatic depletion of antioxidant defense elements and enhancement of lipid peroxidation (Table 4).

The antidiabetic beneficial effect of TQ therapy was achieved in the present study and conformed at the biochemical and histological levels that were evidenced by protection of the pancreatic islets and glycemic status, as well as by restoring serum insulin levels, antioxidant and lipid peroxidation circumstances in their pancreatic tissues. These findings are in full agreement with those previously reported by other researchers [8,9]. Based on these observations, it seems that TQ produced its antihyperglycemic effect and improved insulin production due to protection of the intact functional pancreatic β-cells and/or stimulation of insulin secretion. In support, Rchid and co-workers [17] have demonstrated that extracts of Nigella sativa induced insulin release in isolated rat pancreatic islets in concentration-dependent manner. In addition, TQ might also be mediated its antihyperglycemic effect by extrapancreatic actions. This latter suggestion based on the previous reports revealed that TQ protected mice offspring from diabetes by inhibiting the production of free radicals and the diabetogenic and pro-inflammatory mediators [18]. Moreover, Nigella sativa extracts have also found to regenerate pancreatic β-cells, decrease the intestinal absorption of glucose and have insulin-like stimulation of tissue glucose uptake [19].

Glycosylated hemoglobin (HbA1c) is a form of hemoglobin formed in a non-enzymatic pathway by the exposure of normal hemoglobin to high blood glucose levels, and its higher percentage is a strong predictor of uncontrolled hyperglycemia and diabetes complications [20]. To confirm the antihyperglycemic stability of TQ therapy during the entire period of the induced diabetes, HbA1c was therefore measured in all experimental groups at the end of the study. Interestingly, therapy with TQ significantly normalized the elevated HbA1c that was observed in STZ-diabetic untreated rats (Table 2 and Fig. 2).

Among the interest findings in the present work is the improvement effect of TQ therapy on the impaired erythrocyte indices induced in STZ-diabetic rats. Anemia is a common feature in diabetic patients, and its development aggravates the risks of diabetic complications [14,21]. Consistent with these clinical facts, a clear anemic status was developed in the STZ-diabetic untreated rats but not in STZ-diabetic rats were treated with TQ (Table 2 and Fig. 2). TQ improved Hb concentration in diabetic animals has also been reported earlier by Sankaranarayanan and Pari [8]. Ayinla et al. [22] have also reported similar findings with Nigella Sativa extract therapy in alloxan-diabetic rats.

There are multi-factors and multiple etiologies responsible for development of anemia secondary to DM; however, it is mostly due to EPO deficiency [13,14]. EPO is a glycoprotein hormone produced mainly by the renal peritubular cells. Chronic hyperglycemia in diabetic patients may lead to hypoxia in the renal interstitium, resulting in tubulointerstitial damage and impaired production of EPO [23]. In the current study, reduction of both renal and systemic levels of EPO was also detected in STZ-diabetic untreated rats and therapy with TQ had significantly restored it almost to their levels of normal controls (Fig. 2). Additionally, kidney function was deteriorated in STZ-group but not in STZ-TQ group (Fig. 4). In other words, the observed anti-anemic effect of TQ could be in part due to its well-known renoprotective property [5] against the induced renal injury with subsequent improvement of EPO production and enhancement of RBC synthesis as supported by the restored levels of MCH and MCHC (Table 2).

Furthermore, it is well established in the literature that erythrocyte in diabetic patients have increased osmotic fragility and positively correlated with increasing of HbA1c percentage [24]. To confirm this hypothesis, changes in RBCs hemolytic pattern after their exposed to different concentrations of NaCl solution were measured in all tested animal groups and their data supported the above hypothesis and were aligned with the reported values of HbA1c (Table 2), and also showed the protective effect of TQ therapy on erythrocyte osmotic resistance (Table 3). This favorable effect of TQ on erythrocyte osmotic stability had also been reported by Harzallah et al. [25] in rat model of colon cancer. Also, the present findings were aligned with the serum levels of measured antioxidant and oxidative stress biomarkers (Table 4), and therefore could support a further hypothesis that enhanced oxidative stress and decreased antioxidant status have important
impact on increased erythrocyte deformability and fragility, and any means that can reduce oxidative stress may be beneficial for stabilizing erythrocytes [26,27].

In the present study, the observed hematoprotective property of TQ therapy was not only restricted to erythrocyte indices but also involved WBCs and PLTs. WBCs count was higher, and conversely the PLTs count was lower, in the STZ-diabetic untreated rats than STZ-diabetic-TQ-treated- or normal control rats (Table 1). Epidemiological studies have evidenced the direct impact of hyperglycemia on the increased count of WBCs in diabetic patients [28]. On the other hand, platelet aggregation and adhesion ability has been shown in diabetic patient with long term poor glycemic control [29], and low platelet count has been considered as an important risk factor for development of ischemic stroke in diabetic patients [30].

Table 2. Hematological findings. Values are represented as mean ± SD. *P < 0.05 versus control group, #P < 0.05 versus STZ group

| Group parameters | Control (n = 10) | STZ (n = 12) | STZ + TQ (n = 15) |
|------------------|-----------------|-------------|-------------------|
| RBC (x10^6/µL)   | 8.68±0.21       | 5.05±0.69   | 8.03±0.31         |
| Hb(g/dl)         | 13.68±0.55      | 8.54±0.37   | 13.00±0.21        |
| HbA1c(%)         | 6.31±0.27       | 11.03±1.22  | 7.03±0.18         |
| HCT(%)           | 42.61±2.88      | 28.8±0.21   | 41.80±3.35        |
| MCV (fl)         | 61.33±3.85      | 56.12±4.27  | 60.40±4.34        |
| MCH (pg)         | 20.25±0.77      | 16.77±1.04  | 19.10±1.12        |
| MCHC (g/dl)      | 32.42±1.23      | 19.00±1.25  | 30.07±1.71        |
| Platelets (x 10^3/µL) | 1025.27±91.83 | 875.32±59.61 | 1000.36±70.76 |
| WBC (x 10^3/µL)  | 11.12±0.82      | 15.66±0.71  | 12.51±1.80        |

STZ group: Streptozotocin-diabetic rats, STZ+TQ group: Streptozotocin-diabetic rats treated with oral thymoquinone (35 mg/kg/day; for 28 consecutive days), RBC: Red blood cell, Hb: Hemoglobin, HbA1c: Glycosylated hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelet, and WBC: White blood cell

Table 3. Effect of thymoquinone therapy (TQ; 35 mg/kg/day, orally for 28 days) on erythrocyte osmotic fragility in streptozotocin (STZ)-induced diabetes in rats. P < 0.05 and #P < 0.01 versus normal control group, *P < 0.05 versus STZ-diabetic group

| Group      | 0.0% NaCl | 0.1% NaCl | 0.3% NaCl | 0.5% NaCl | 0.7% NaCl | 0.9% NaCl |
|------------|-----------|-----------|-----------|-----------|-----------|-----------|
| Control (n = 10) | 89.7±3.4  | 81.3±3.7  | 67.4±4.3  | 31.8±2.1  | 12.1±1.6  | 2.4±0.2   |
| STZ (n = 12)   | 94.6±3.3* | 91.3±4.5* | 84.3±2.2* | 57.2±3.1* | 38.6±3.5* | 27.8±1.7* |
| STZ+TQ (n = 15)| 90.2±2.4* | 83.7±3.2* | 70.6±3.7* | 38.1±3.2* | 17.5±2.7* | 9.7±0.6*  |
Table 4. Effect of thymoquinone therapy (TQ; 35 mg/kg/day, orally for 28 days) on pancreatic, serum and renal antioxidant and lipid peroxidation status in streptozotocin (STZ)-induced diabetes in rats. Values are represented as mean ± SD. ‘P < 0.05 and **P < 0.01 versus control group, 
#P < 0.05 versus STZ group

| Group         | GSH (µmol/mg protein) | SOD (U/mg protein) | TBARS (nmol/mg protein) |
|---------------|------------------------|--------------------|-------------------------|
|               | Pancreas | Serum | Kidney | Pancreas | Serum | Kidney | Pancreas | Serum | Kidney |
| Control (n = 10) | 35.6±2.7 | 11.5±1.1 | 60.4±6.7 | 23.5±4.1 | 4.2±0.3 | 253.2±17.3 | 43.3±5.2 | 25.4±2.8 | 37.4±5.9 |
| STZ (n = 12)    | 3.7±0.4** | 2.4±0.3** | 11.4±1.7*** | 4.6±0.9** | 0.9±0.1** | 71.5±9.7** | 1100.5±113.6** | 450.2±31.3** | 926.7±46.6** |
| STZ+TQ (n = 15) | 27.9±2.2# | 9.4±1.2# | 56.7±8.7# | 19.5±1.8# | 3.6±0.4# | 217.3±26.2# | 112.2±14.6# | 64.4±10.8# | 88.4±13.2# |

GSH: Total glutathione, SOD: Superoxide dismutase, and TBARS: Thiobarbituric acid reactive substances
Finally, there is a strong body of evidence that induced oxidative stress and impaired endogenous antioxidant defense system is crucially implicated in the pathophysiology of DM and its associated chronic systemic complications [31,32]. In an inverse line, the potential free radical scavenging, antioxidant activity and lipid peroxidation suppression property of TQ have been documented in various disease modalities [4,5]. These two facts were also confirmed in the current study, whereby administration of TQ significantly alleviated the depletion effect of induced diabetes on GSH levels and SOD activities (typical examples of antioxidant defense elements), as well as significantly decreased the elevated concentrations of TBARS (an index of lipid peroxidation and oxidative stress) in the serum, pancreatic and renal tissues of STZ-diabetic rats.

5. CONCLUSION

The collective data of the current study indicate the therapeutic value of TQ in alleviating pancreatic damage, hyperglycemia, hypoinsulinemia, anemia and other deteriorated hematological parameters, erythropoietin production and erythrocyte osmotic resistance in rat model of DM induced by STZ. Moreover, it also significantly improved antioxidant and suppressed lipid peroxidation status at the systemic and organ levels. According to the obtained results in this study it could be deduced that therapy with TQ has a favorable antihyperglycemic and hematoprotective effect on DM and its hematological complications. However, further studies are still needed to confirm this potential benefit of TQ therapy in different modalities of DM.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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