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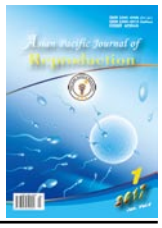

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Improvement of sexual behavior, sperm quantity and quality by Quercetin in streptozotocin-induced diabetic erectile dysfunction

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ABSTRACT

Objective: To evaluate the effect of Quercetin (QT) on erectile dysfunction and oxidative stress in penile tissue of streptozotocin-induced diabetic rats. **Methods:** Two weeks after diabetes induction, QT was treated to normal and diabetic rats for 5 wk. Sexual behavioral parameters including mount latency, intromission latency, ejaculation latency, post-ejaculatory interval, mount frequency and intromission frequency, were observed against stimulus females. Sperm count and their motility and viability were recorded. Serum glucose and testosterone levels were estimated. In penile tissue levels of cyclic guanosine monophosphate, thiobarbituric acid reactive substances and glutathione, and enzymatic activities of superoxide dismutase and catalase were measured. Histopathological changes were evaluated in a cross-section of penile tissue. **Results:** Sexual behavioral ejaculation latency, post-ejaculatory interval, mount latency and intromission latency were significantly increased while mount frequency and intromission frequency were decreased in diabetic rats. Treatment with QT corrected the male sexual behavioral levels and also enhanced the inhibited sperm count, motility and viability in diabetic rats. Serum testosterone and penile cyclic guanosine monophosphate levels were significantly increased in QT treated diabetic rats compared to untreated diabetic animals. Penile oxidative stress biomarkers were corrected by the QT treatments in diabetic rats. Histopathological evaluation revealed damaged penile tissues in diabetic rats, which was protected following QT treatment. **Conclusions:** QT eliminated the diabetic-induced sexual impairment and showed significant antioxidant effects in penile tissue. Further experimental studies are recommended for QT therapeutically usage.

1. Introduction

Diabetic-induced erectile dysfunction (ED) found a common clinical problem in male diabetic patients and that has destructive effects on their sexual life. Epidemiological studies revealed that, around 75% of diabetic patients come across with major complications in their earlier age compared to normal population[1,2]. Experimental and clinical studies have established that, diabetic-induced ED has multifactorial characteristics, involving largely of vascular and neurological insults as a result of diabetic-induced metabolic inequities[3,4]. In diabetic condition boost the expression of arginase enzyme, which decreases the availability of L-arginine as substrate to reduce the synthesis of nitric oxide (NO). Moreover, denovo diacyglycerol production promotes the protein kinase C, leading to generation of reaction oxygen species (ROS). Scarano, *et al*.[5] reported that diabetic mellitus (DM) causes infertility by following the effects on ejaculatory process that can be enlightened through a secondary complication of DM-induced neuropathy an autonomic syndrome[6]. Such pathological condition affects

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autonomic neurotransmissions which are intricate in ejaculation process and also affect vas deferens and epididymis where these two organs are important for sperm transportation via the male reproductive tract.

Chronic hyperglycemic conditions generate the ROS and nitrogen species which induces the development of advanced glycation end-product. Consecutively, this may produce variations in the bioavailability in endothelial-and neuronal-derived NO, which may damage the mechanisms of vasorelaxation in corpus cavernosum of diabetic patients[7,8]. In cellular membrane, the ROS production forms a toxic molecule called malondialdehyde which induces the peroxidation of cellular membranes as well as oxidative injury *in vivo*. The superoxide dismutase (SOD) is also a vital enzyme that eradicates the superoxide radicals. However, increased production of ROS can inhibit SOD activity, which augment the peroxynitrite and ameliorate NO levels[9].

The accumulation of ROS cause changes in penile vasorelaxation by reducing antioxidant levels, lipid peroxidation. Furthermore, DNA damage also may directly cause abnormality in cavernosal cellular structure and function[10,11]. Several preclinical studies recommended the use of antioxidants to eliminate the deleterious effects of ROS in form of ED by showing reduction in superoxide production (O_2^-) and increase endothelial NO synthase[8,10-12]. Effect of ROS generation in diabetic penile tissue revealed the O_2^- production and compared its significance related to diabetic-induced ED[8,9]. However, another important ROS molecule, namely H_2O_2, has not been assessed in diabetic cavernosum; H_2O_2 is a powerful oxidizing agent, produced after spontaneous or SOD-catalyzed mutation of O_2^-, as well as by other enzymatic reactions[12].

Flavonoids, known to have antioxidant properties are commonly available in dietary resources. Experimental and clinical studies are reported their pharmacological importance by showing potential action against chronic diseases including DM, hypertension, inflammation and allergy[13]. Quercetin (QT) is one of such natural flavonoid mainly occurring in form of glycosidic as rutin[14]. Naturally, onions, apples, tea and red wine are rich with QT[15]. Furthermore, QT is widely used in herbal remedies and multivitamin supplements[16]. Kanter, *et al*.[17] and Al-Khamees[18] were reported that QT has attenuated testicular oxidative damage in streptozotocin (STZ)-induced hyperglycemia in rats. Thus, the existing investigation was designed to examine the potential effects of QT against diabetic-induced sexual impairments by alleviating oxidative stress in cavernosum tissues.

2. Materials and methods

2.1. Animals

This study was conducted using adult Wistar albino rats, provided by the Experimental Animal Care Center, College of Pharmacy, King Saud University. The experiment started after 7 d of acclimatization under controlled conditions. The current study protocol was in agreement with the 8th edition of the National Institute of Health (NIH) guidelines for the care and use of laboratory animals and was ethically approved by the ethical committee at the Experimental Animal Care Centre, College of Pharmacy, King Saud University.

2.2. Diabetes induction

Animals were subjected to a single IP shoot of a freshly prepared 0.1 mol/L citrate buffered STZ solution (Sigma-Aldrich, ST. Louis, MO, USA). The pH of the solution was 4.5 and dose was delivered at 65 mg/kg. Control animals received the same volume of STZ vehicle. The experimental diabetes was confirmed 48 h following the STZ injection by measuring the fasting glucose values in blood samples obtained from the tail vein. The diabetic animals considered with >250 mg/dL of fasting glucose levels.

2.3. Study design

Animals were divided into six groups with six rats in each group as follows:

1) Control animals were treated with vehicle/d (Control)
2) Normal rats were treated with QT 25 mg/kg/d (QT25)
3) Normal rats were treated with QT 50 mg/kg/d (QT50)
4) Diabetic rats were treated with vehicle/day (STZ)
5) Diabetic rats were treated with RT 25 mg/kg/d (QT25+STZ)
6) Diabetic rats were treated with RT 50 mg/kg/ (QT50+STZ)

The animals in QT groups received freshly prepared QT suspension in 0.25% carboxymethyl cellulose sodium solution once a day orally (gavage) for 5 wk starting from 2 wk after STZ injection. The equal volume of carboxymethyl cellulose sodium solution was used as vehicle. Weekly, body weights were recorded and at the end of the treatment, sexual behavior was observed by taking stimulus females. Then after overnight fasting, cardiac puncture was done for each animal to collect the blood samples under light ether anesthesia. These samples were centrifuged at 4 000 r/min for 10 min serum was suppurated in labeled sample and stored at -20 ℃ till analysis. Finally, animals were decapitated and the reproductive organs such as penile tissue, cauda epididymides and seminal vesicles were dissected, weighed and stored at -80 ℃. A cross-section of penile tissue from every group was preserved in 10% formalin solution for histopathology.

2.4. Mating behavior tests

Before diabetic induction, the sexual behavior of the animals was verified to select the sexually active ones to conduct the experiment. Twenty four hours after the treatment period sexual behavioral progress was observed under dim red illumination in a cage with glass frontal wall. Estrus levels were increased in the females

animals using *i.p.* injection of estradiol benzoate[19]. Acclimation of males to the test chamber was allowed before the test and the test was taken negative when there was no mount noticed within the next 10 min. Sexual behavioral parameter as earlier described and identified by Ahlenius, *et al*.[20] including Ejaculation latency (EL), intromission latency (IL), Post-ejaculatory (PEI), mount latency (ML), mount frequency (MF), intromission frequency (IF) were recorded.

2.5. Evaluation of count, motility and viability

The spermatozoa samples were acquired from the cauda epididymis and vas deferens and preserved in 1 mL of modified Krebs Ringer-bicarbonate buffer (pH 7.4) for 10 min in 37 ℃. The samples were assessed for sperm content, % motility and sperm viability by using Sperm Class Analyzer (Microptic Diagnostic System, Barcelona, Spain).

2.6. Penile tissue biochemistry

In the homogenates of penile tissues, cyclic GMP was determined by the immunoassay kit (R&D Systems, USA), while the TBARS concentrations were assayed as malondialdehyde equivalent using Zepto Metrix kits. The technique equipped to quantify penile GSH levels was provided by Sedlak and Lindsay[21]. Penile antioxidant enzymes activities including SOD and CAT were respectively estimated by Kono[22] and Aebi[23] methods. A spectrophotometer (LKB-Pharmacia, Mark II, Ireland) was to read the colorimetric biochemical results.

2.7. Histopathological procedures

Across sectional portion of a penile tissue from each group of treatment were preserved in 10% buffered formalin. After embedding the samples in paraffin blocks, they were sectioned into a thickness 5 μm using an optical rotary microtome. The produced sections were then stained with H & E stain. The histological examination of the stained samples was conducted in a blind fashion.

2.8. Statistical analysis

Achieved data expressed as means±SE. All the statistical calculations were made by using Graph Pad Prism (version 5) software. Statistical significances were carried out by Tukey-post hoc test considering $P<0.05$ is significant.

3. Results

In diabetic rats, EL, PEI, ML and IL latencies increased significantly ($P<0.001$) while MF and IF frequencies were inhibited ($P<0.001$) compared to control animals. QT treatment with the higher doses to the diabetic rats markedly inhibited the latency time as compared to untreated diabetic animals. The mounting and intromission frequencies were markedly ($P<0.001$) lowered in diabetic animals compared to normal animals. Administration of the higher dose of QT to diabetic rats significantly enhanced the MF ($P<0.01$) and IF ($P<0.05$) frequency time (seconds) compared to untreated diabetic group (Figure 1).

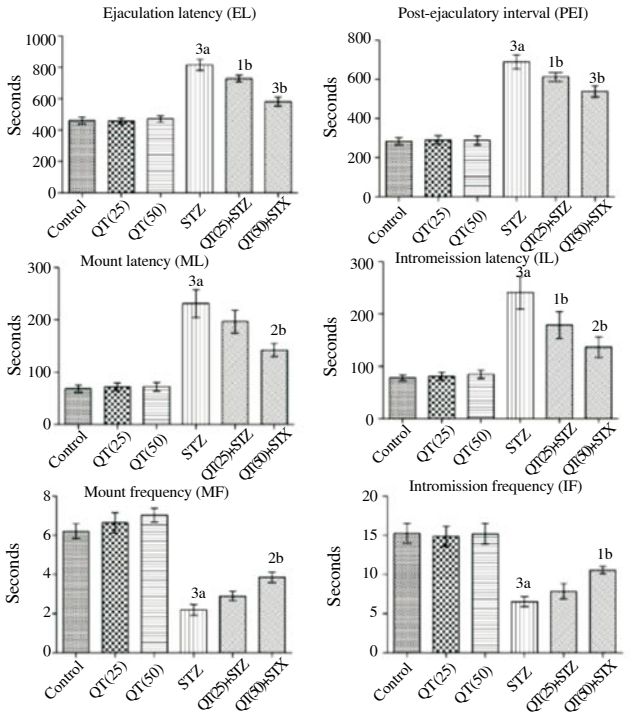

Figure 1. Effect of QT on sexual behavioral parameters including ML, IL, EL, PEI, MF and IF of normal and diabetic rats.

Results were expressed as mean±SE (n=6) and statistically analyzed by one-way ANOVA followed by Tukey-post hoc test. [1]$P<0.05$, [2]$P<0.01$ and [3]$P<0.001$. 'a' indicates control group *vs* STZ, QT(25) and QT(50) groups, while 'b' indicates STZ group *vs*. QT(25)+STZ and QT(50)+STZ groups.

Total sperm count was considerably ($P<0.001$) decreased in diabetic rats and that found significantly ($P<0.05$) increased in QT (50 mg/kg/d) treated animals compared to untreated diabetic animals. Motility and Viability percentages were also inhibited ($P<0.001$) in diabetic rats compared to control group. Higher dose of QT treatment to diabetic rats enhanced the percentage inhibition ($P<0.05$) of motility and viability while compared to untreated diabetic animals (Figure 2).

STZ-induced hyperglycemia was markedly ($P<0.05$) lowered by the QT (50 mg/kg/d) for 5 wk compared to the STZ group. Serum testosterone levels were significantly ($P<0.001$) inhibited in diabetic animals as compared to normal values. Treatments with lower and higher doses of QT to diabetic rats markedly $P<0.05$ and $P<0.01$

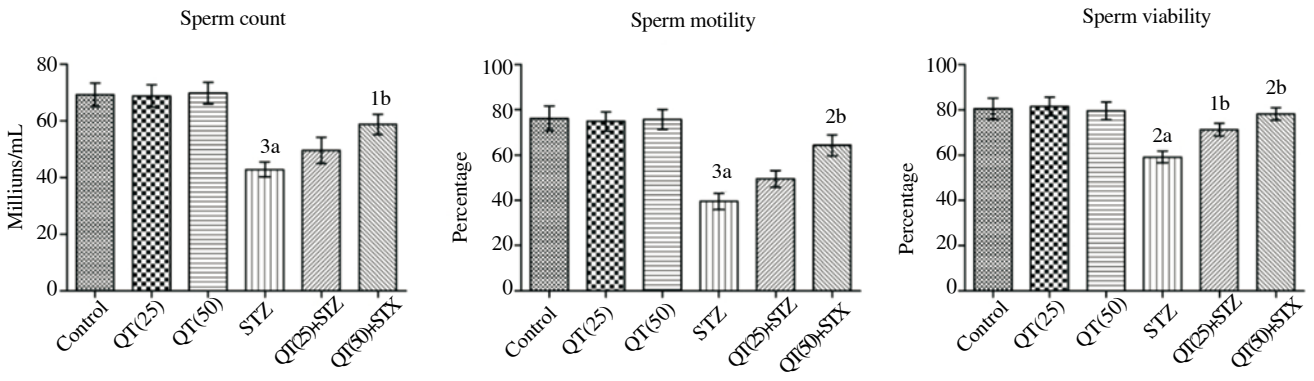

Figure 2. Effect of QT on sperm count, sperm motility and sperm viability of normal and diabetic rats.

Results were expressed as mean±SE (n=6) and statistically analyzed by one-way ANOVA followed by Tukey-post hoc test. [1]P<0.05, [2]P<0.01 and [3]P<0.001. [a] indicates control group *vs.* STZ, QT(25) and QT(50) groups, while [b] indicates STZ group *vs.* QT(25)+STZ and QT(50)+STZ groups.

enhanced the serum testosterone levels compared to STZ group (Figure 3).

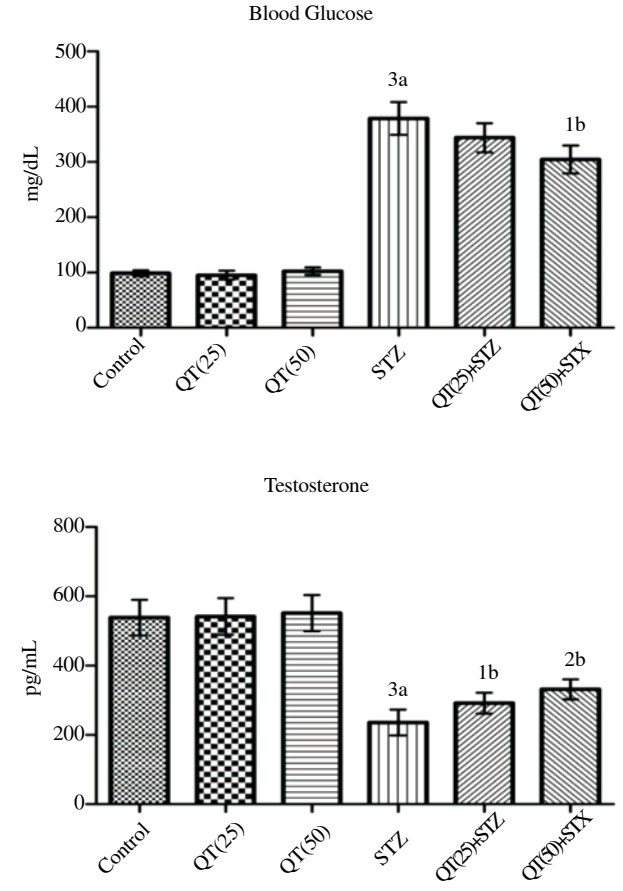

Figure 3. Effect of QT on serum blood glucose and testosterone levels of normal and diabetic rats.

Results were expressed as mean±SE (n=6) and statistically analyzed by one-way ANOVA followed by Tukey-post hoc test. [1]P<0.05, [2]P<0.01 and [3]P<0.001. [a] indicates control group *vs.* STZ, QT(25) and QT(50) groups, while [b] indicates STZ group *vs.* QT(25)+STZ and QT(50)+STZ groups.

Penile cGMP levels in diabetic rats were significantly (P<0.01) inhibited compared to controls. Treatments with higher the dose (50 mg/kg/d) of QT to diabetic rats for 5 wk significantly (P<0.05) enhanced the penile cGMP levels compared to untreated diabetic rats (Figure 4).

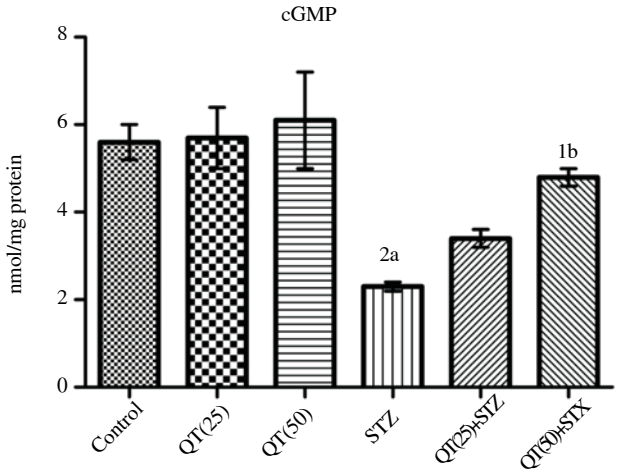

Figure 4. Effect of quercetin (QT) on penile levels of cGMP in normal and diabetic rats.

Results were expressed as mean±SE (n=6) and statistically analyzed by one-way ANOVA followed by Tukey-post hoc test. [1]P<0.05, [2]P<0.01 and [3]P<0.001. [a] indicates control group *vs.* STZ, QT(25) and QT(50) groups, while [b] indicates STZ group *vs.* QT(25)+STZ and QT(50)+STZ groups.

Oxidative biomarkers showed significant changes in penile tissue of diabetic rats. The TBARS levels increased from (2.78±0.31) nmol/mg protein to (6.34±0.78) nmol/mg protein while GSH levels decreased from (7.84±0.94) nmol/mg protein to (3.87±0.27) nmol/mg protein in penile tissue of diabetic rats. Similarly, enzymatic activities of SOD and catalase were markedly (P<0.001) attenuated in the penile tissue of the diabetic untreated animals compared to their respective untreated control ones. Treatment with higher dose (50 mg/kg/d) or QT to diabetic rats for 5 wk potentially (P<0.05) eliminated the diabetic-induced alterations in oxidative stress biomarkers (Figure 5).

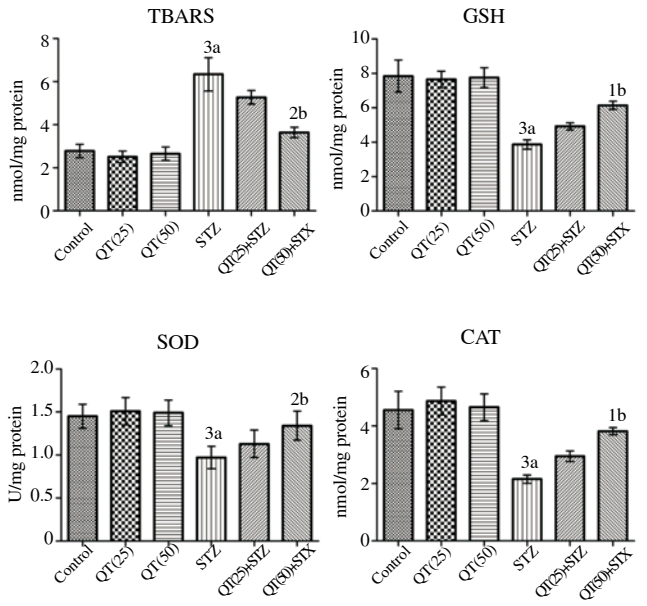

Figure 5. Effect of QT on levels of thiobarbituric acid reaction substance, glutathione (GSH), SOD and catalase in penile tissue of normal and diabetic rats. Results were expressed as mean±SE (n=6) and statistically analyzed by one-way ANOVA followed by Tukey-post hoc test. [1]P<0.05, [2]P<0.01 and [3]P<0.001. 'a' indicates control group *vs* STZ, QT(25) and QT(50) groups, while 'b' indicates STZ group *vs* QT(25)+STZ and QT(50)+STZ groups.

Histopathological investigation of the penis tissue showed (A) cross sections covered by skin underneath which normal erectile tissue formed of numerous blood vessels lined by flat epithelium. Corpora cavernosa and spongiosa are normal with no significant inflammatory reaction in the control group; (B) Corpora cavernosa covered by cuboidal epithelial cells and numerous blood vessels lined by flat epithelium with scattered inflammatory cells in the diabetic group; (C) vascular subcutaneous tissue with numerous small blood vessels lined by flat epithelial cells. The corpora cavernosa showed some destruction in the tissue, while corpora spongiousum is normal in higher dose (50 mg/kg/d) QT treated group (Figure 6).

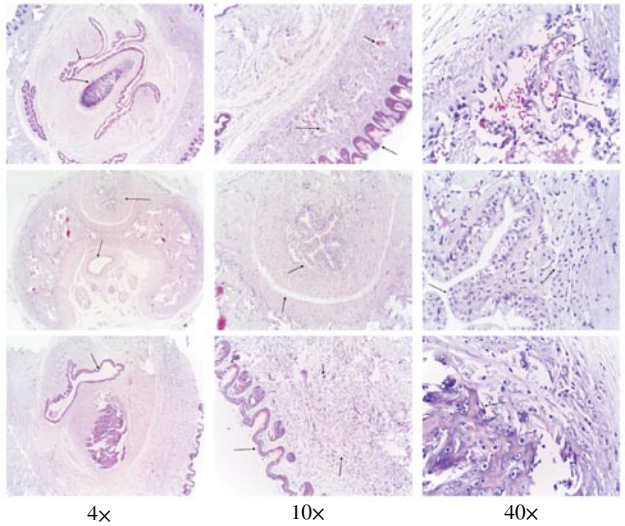

Figure 6. Histopathological features of penis tissues of (A) control, (B) diabetic untreated and (C) diabetic QT treated rats.
Figure with different magnification showing almost normal features with few scattered inflammatory cells in diabetic untreated group and some corpora cavernosa destruction in diabetic QT treated group.

4. Discussion

Present data revealed the beneficial properties of QT on STZ-induced erectile dysfunction in Wistar rats. We found marked reduction in sexual performance of diabetic rats compared to controls. Five weeks of QT treatment to diabetic animals considerably enhanced the sexual activity by reducing the EL, PEI, ML and IL latencies while enhancing the MF and IF frequencies. Diabetes induced significant oxidative impairments in the penile cells. Administration of QT to diabetic rats markedly reduced the penile cellular damage and its oxidative stress induced by DM. Our histopathological screening further confirms the potential properties of QT against hyperglycemia-induced damage in penile tissue.

Epidemiological studies showed the higher prevalence of severe ED in diabetic men compared to normal healthy males[1,2]. Experimentally-induced diabetic models are well-established to elaborate the mechanism of diabetic-induced ED and also a recommended model for establishing the beneficial effect of any new compound against diabetes ED[9]. In present study, sever sexual impairment was found in STZ-induced diabetic rat by inhibiting the EL, PEI, ML and IL latencies and increasing the MF and IF frequencies. Our observations are in harmony with other reports, which showed fewer in sexual behaviors of diabetic animals compared controls[5]. In QT supplemented diabetic rats, we found the characteristics of all sexual behavioral tests enhanced, suggesting that QT produces protective action in one of the diabetic-induced metabolic syndrome.

It is well established that the testosterone levels decreases in diabetic conditions. However, testosterone depletion is not only a factor for reduction in the mating behavior because it has seen its replacement could not improve the diabetic-induced adverse effects on sexual behavior. However, hyperglycemia-provoked sexual imbalance may be a result of the inhibition of testosterone, by the direct or indirect actions of insulin and/or glucose on the adrenergic complex[24]. Our findings also demonstrated a significant decrease in testosterone levels compared to control group in serum. The QT treatment markedly increased the inhibited testosterone levels in our diabetic rats which showed potentials of QT against diabetic-induced ED. Zhang and his colleagues[10] studied the QT effect on intracavernous pressure of STZ-induced diabetic rats and clearly documented the potential effect of QT against ED. Earlier reports documented that diabetes causes inhibition in sperm count, motility and viability[5], similar changes have noted in present study. The decreased values of sperm numbers, motility and viability were significantly enhanced by the QT treatment. It supports the beneficial effect of QT against diabetic-induced ED.

Since the evaluation of the conducted mating behavior tests in the current study relayed upon physical observation, further

biochemical analysis including oxidative stress and inflammatory process were used to explore QT mode of action against diabetic-induced erectile dysfunction. cGMP concentrations were markedly lowered in the current investigation in penile tissues. Similar inhibition was demonstrated experimentally in earlier studies[25,26]. In several preclinical studies it is clearly documented the oxidative impairment in the male reproductive system[27–29]. It is established that hyperglycemia provokes the production of glycation end-products, reactive oxygen and nitrogen species and impairs no bioavailability and negatively affects penile tissues, resulting in alterations in vasorelaxation mechanisms of the endothelium[7,30]. In one study, the transfer of adenoviral gene bearing EC-SOD lowered the corporal superoxide anion, enhanced the cavernosal cGMP, improved NO bioavailability, and restored the erectile functions in diabetic animals[8]. In the present study, we explored oxidative stress biomarkers including TBARS and GSH levels along with SOD and CAT activities in penile tissues and found decreased GSH levels, suppressed antioxidant enzymes activities and triggered TBARS. These abnormalities were markedly corrected by QT, which suggests it therapeutic value in improving ED in diabetic rats through repairing of oxidative damage in penile tissue. The antioxidant properties of QT are mainly attributed to its chemical structure, which contains multiple hydroxyl groups with anti-radical and scavenger activities. Moreover, the chemical structure of QT possesses double bond and carbonyl groups, which stabilize the flavonoid via conjugation and electron delocalization. QT also may undergo one or two electron oxidation to produced metal ions chelating derivatives including semiquinone and quinine type compounds[31].

In summary, we found that diabetic rats exhibited decreased sexual performance sperm count inhibition with low motility, viability, reduced antioxidant enzymes activities, along with elevated lipid peroxidation and inhibited GSH levels. QT treatment might attenuate ED in diabetic animals partly by improvement in sexual performance and suppressing oxidative injury. We recommend further preclinical researches to estimate the utility and usefulness of QT as a new approach in ED.

Conflict of interest statement

The author declares that he has no conflict of interest.

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