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Research Article

Therapeutic effect of quercetin on the complications of nicotinamide-streptozotocin induced type-2 diabetes in male rats

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ABSTRACT

Introduction: Type-2 diabetes mellitus (T2DM) is a rapid growing epidemic disease, with a continually increasing prevalence that exhibit complications in different body organs. The nicotinamide-streptozotocin (NA-STZ) rat model induces similar conditions to T2DM in humans, therefore used for studying the drugs and natural products' effects on diabetic complications.**Aim:** This study was performed in order to investigate the therapeutic; biochemical effects of the flavonoid compound quercetin (Q) to treat the complications, with a glance at the testicular dysfunction of type-2 diabetes induced by nicotinamide-streptozotocin (NA–STZ) in male rats. **Methods:** Type-2 diabetes was induced in fasted rats by an intraperitoneal (ip) injection of STZ (100 mg/kg b. wt.; freshly prepared in citrate buffer, pH 4.5), after 15 min of nicotinamide administration (NA) (240 mg/kg b. wt.). The rats were divided into the following groups (10 animals/group): *Group 1* (Control group), *Group 2* (quercetin treated rats; Q group): Rats were given quercetin (100 mg/kg), *Group 3* (NA-STZ treated group or diabetic (D) group) and *Group 4* (NA-STZ/quercetin treated group; DQ). After 30 days, the animals were fasted overnight, sacrificed, then the blood was collected, the serum was separated,Epididymal sperm number (ESN) was collected and the testes of each animal were excised and then stored at -80oC. **Results:** Oral supplementation of quercetinsignificantly ($p<0.05$) ameliorated the glucose level in T2DM rats. Further, quercetin treatment of T2DM rats significantly ameliorated the total cholesterol, triglycerides, total proteins, albumin, urea, creatinine levels, liver transaminases (ALT, AST), alkaline phosphatase and gamma glutamyl transpeptidase activities. Furthermore, quercetin administrationsignificantly enhanced serum level of insulin, testosterone, follicle-stimulating hormone and luteinizing hormone. Additionally, quercetin administration improved the antioxidant status in the testicular tissues of T2DM rats, by enhancing the superoxide dismutase, glutathione peroxidase, catalase, acetylcholinestrase and butrylcholinestrase activities in parallel with decline the malondialdehyde nitric oxide, the pro-inflammatory markers; interleukin-6, tumor necrosis factor-alpha, nuclear factor kappa-B levels and up-regulated the relative ratio of Peroxisome Proliferator-Activated Receptor-γ (PPAR-γ) gene expression. **Conclusion:** Quercetin has potential therapeutic effects on different complications of T2DM induced nicotinamide-streptozotocin in male rats.Quercetinimproved testicular dysfunctions in T2DM, which is mediated by improving of the testicular antioxidant, anti-inflammatory status, acetylcholinestrase and butrylcholinestrase activities, also up-regulation of the PPAR-γ gene expression. Therefore, regular daily administration of quercetin is recommended to regulate the different complications of T2DM and to decrease risk of infertility in men.

Keywords: Quercetin, anti-diabetic, testicular dysfunction, nicotinamide-streptozotocin, Peroxisome Proliferator-Activated Receptor-γ gene expression**.**

INTRODUCTION:

Type-2 diabetes mellitus is a rapid growing epidemic disease, with continually increasing prevalence[1]. Type-2 diabetes mellitus (T2DM) acquire resistance and malfunctioning of insulin, which evoke hyperglycemia; increased glucose level in the bloodstream. Hyperglycemic condition

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is predicted as the causative connection between diabetes and its complications. The chronic hyperglycemia creates oxidative stress and consequent oxidative damage in different tissues due to overproduction of reactive oxygen species (ROS), as a result of glucose auto-oxidation and protein glycosylation[2]. ROS have been shown to inactivate key enzymes of glucose metabolism in both the glycolytic pathway and the electron transport chain coupled to oxidative phosphorylation[3]. Further, oxidative stress can cause depolarization and calcium uptake in insulin producing pancreatic isletcells[4], a phenomenon that stimulates insulin secretion, resulting in hyperinsulinemia. Furthermore, oxidative stress can damage several biomolecules like proteins, lipids and DNA, leading to the inactivation of enzymes affecting DNA integrity and cellular membrane composition[2]. The possible complications of T2DM include fatty liver disease, cardiovascular disease, Kidney damage (*nephropathy*), Eye damage (*retinopathy*), foot damage, skin conditions, hearing impairment, nerve damage (*neuropathy*), and may increase the risk of Alzheimer's disease[5]. Diabetes has also been associated with reproductive disorders in both men and women[6].The nicotinamide-streptozotocin(NA-STZ) rat model induces similar conditions to T2DM in humans, therefore used for studying the drugs and natural products' effects on diabetic complications[7].

Quercetin (3,3',4',5,7-pentahydroxyflavone) is the most abundant of all flavonoids and is found in significant amounts in green vegetables, onions, fruits, and legumes[8]. Quercetin (Q) exhibits anti-diabetic[5]and cell protection activity that owing to its antioxidant, quenching of ROS and anti-inflammatory actions[9]. Q can protect pancreatic β cells from inflammatory damage, and has the ability to reduce aldose reductase, an enzyme involved in the conversion of glucose to sorbitol via polyol pathway. The accumulation of sorbitol in different organs of the body evokes various diabetic complications[10].

The present study was aimed to investigate and clarify the therapeutic effect of the quercetin (Q) on the complications; with a glance at the testicular dysfunctioninT2DM induced by nicotinamide- streptozotocin (NA-STZ) in male rats.

Materials and Methods:

Chemicals

Nicotinamide (NA), Streptozotocin (STZ), and quercetin dihydrate were obtained from Sigma Chemicals. All other chemicals used in this study were of high analytical grade.

Animals

Male albinorats, weighing 220–250 g were obtained from the Nile pharmaceutical Co., Cairo, Egypt. Animals were housed at the animal facility, at the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt. Rats were kept under standard laboratory conditions of light/dark cycle (12/12 h), a temperature of 25±2°C and a humidity of 60±5% with free access to food (standard laboratory-pellet-diet) and drinking water *ad libitum*. The animals were allowed to acclimatize for 1 week before starting the experiment. The study was conducted in accordance with international guidelines for animal experiments and approved by the Ethical Committee at the NCRRT.

Animal Experiments

The rats were divided into the following groups (10animals/group)

***Group 1*(Control group; C):** only distilled water was administered orally to the rats by gastric intubation.

***Group 2* (NA-STZ treated group;D):**Type-2 diabetes was induced in fasted rats (for 16 h, with free access to drinking water) by an intraperitoneal (ip) injection of STZ (100 mg/kg b. wt., freshly prepared in citrate buffer, pH 4.5), after 15 min of a single-dose administration of nicotinamide (NA) (240 mg/kg, dissolved in normal saline)[11]. Progression of diabetes was confirmed by blood glucose level assaying at 72 hours after the NA-STZ injection. Ultimately, rats with fasting blood glucose levels of more than 250 mg/dl were considered diabetic and used as D group[12].

***Group 3*(quercetin treated rats; Q)**: normal rats were administeredquercetin by gastric- intubation (100 mg /kg b. wt.)[13].

***Group 4* (NA-STZ/quercetin treated group; DQ):**Diabetic rats were received quercetin orally (100 mg/kg b. wt.).

After 30 days, the animals were fasted overnight, anesthetized by light ether, and then they were

sacrificed by cervical dislocation. Part of the blood was collected in fluoride-containing tubes for determination of fasting blood glucose. The rest of blood was collected in glass tubes free of anti-coagulants, allowed to clot for 30 min at 25°C, centrifuged at 1200 g at 4°C using universal centrifuge (16R, Germany, at NCRRT) and sera were separated for the different biochemical assessments. The body weights of all animals, epididymal sperm number,thepercentage viability and total sperm abnormalities were assessed[14,15].The testes of each animal were excised immediately, washed with ice cooled saline, the wet weight was taken and then stored at -80°C.

Assessment of the blood biochemical parameters

The different blood biochemical parameters were determined in the serum using thebiochemical blood analyzer; ALFA WASSERMANN DIGNOSTIC TECHNOLOGYIES, LLC, ACE, Alera, USA at RCFF.Serumsamples were also used for the determination of the lactate dehydrogenase (LDH)[16] and acid phosphatase (ACP)[17]activities.

The enzyme-linked immunosorbent assay (ELISA)

Insulin, testosterone (T), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) concentrations in serum, interleukin -6 (IL-6) and tumor necrosis factor–alpha (TNF-α), nuclear factor-kappa-B (NF-κB), acetylcholinestrase and butrylcholinestrase in the testes' homogenates were determined using an ELISA kits for rat (Glory Science Co., Ltd, USA). The measurements were done according to the catalogue-instruction guidelines.

Preparation of testis homogenates

Parts of the testis with known weight were homogenized in phosphate buffered saline (0.05 M, pH 7.4) in 1:10 w/v ratio; using Teflon homogenizer (Universal laboratory aid, Type MPW-309, Poland), then centrifuged at 1200 g for 15 min at 4°C and stored at -80°C.

Assessment of the antioxidant status** in the **testis

The Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) activities, glutathione (GSH) content, malondialdehyde (MDA) and nitric oxide (NO) were assessed using Bio-diagnostic kits[18-23].

Relative ratio of Peroxisome Proliferator-Activated Receptor-γgeneexpressionin the testisby quantitative Real Time–Polymerase Chain Reaction

RNA was extracted from testes' frozen tissues, and reverse transcription-polymerase chain reaction (RT-PCR) was performed as described previously[24,25].

RNA Isolation and Reverse Transcription

Briefly, testes' tissues (100 mg) was homogenized in 1 ml icecoldTRIzol reagent (Invitrogen, U.S.A.) using a Polytron Homogenizer andsubsequently incubated for 10 min at room temperature. Sampleswere mixed with chloroform (0.2 ml) and incubated for 3 min atroom-temperature, followed by centrifugation (12,000 g, 15 min). Thetop aqueous phasewas isolated and 0.5 ml isopropanolwas added, sampleswerere-centrifuged (12,000 g, 10 min) and the resulting RNApelletwas washed with 75% ethanol and centrifuged again (7500 g, 5 min).The supernatants were discarded and the RNA pellets were air-dried(10 min), then dissolved in diethyl pyrocarbonate (DEPC), then incubatedat55–60 °C for 10 min. The concentration of isolated nucleic acidswas determined spectrophotometrically by measuring the absorbanceat 260 nm. All samples were stored at −80 °C until the analysis ofcDNA synthesis reaction that was performed using the ReverseTranscription System (Promega, Leiden; Netherlands). Total RNAwas incubated at 70 °C for 10 min to prevent secondary structures.TheRNA was supplemented with $MgCl_2$ (25 mM), RTease buffer(10×), dNTP mixture (10 mM), oligo d(t) primers, RNase inhibitor(20 U) and AMV reverse transcriptase (20 U/µl). This mixture was incubatedat 42 °C for 1 h.

Quantitative Real Time-PCR

QRT-PCR was performed using an optical 96-well plate with an ABIPRISM7500 fast sequence detection system(Applied Biosystems, Carlsbad,California) and universal thermal cycling conditions (95 °C for10 min, 40 cycles of 95 °C for 15 s and 60 °C for 60 s). Each 10 µl reactioncontained 5 µlSYBR Green Master Mix (Applied Biosystems), 0.3 µlgenespecific primers (10 µM), 2.5 µl cDNA and1.9 µl nuclease-free water. The sequences of PCR primer pairs used foreach gene are as follows[26], PPAR-γ: sense: 5'-

AACCGGAACAA-ATGCCAGTA-3', antisense:5'-TGGCAGCAGTG-GAAGAATCG-3', GAPDH (Glyceraldehyde-3-PhosphateDehydrogenase): sense: 5'-TATGA TGACA TCAAG AAGGTGG-3', antisense: 5'-CACCACCC TGTTGCTGTA-3'.

Gel Electrophoresis

10 µl of PCR product was analyzed on 2% agarose gel with ethidiumbromide staining and the productwas visualized on an ultraviolet transilluminatorthengel documentation was performed. PCR products weresemiquantified by using a gel documentation system(Bio Doc Analyzesuppliedby Biometra, Germany. The data were analyzed with the ABIPrism sequence detection system software and quantified using thev1·7 Sequence Detection Software from PE Biosystems (Foster City,CA). The relative expression of the studied genes was calculated usingthe comparative threshold cycle method. All values were normalized to the GAPDH genes[27].

Statistical analyses

All statistical analyses were conducted by using the statistical package for Windows Version 15.0 (SPSS Software, Chicago, IL). The results for continuous variables were expressed as mean±standard error. Values were compared by one-way analysis of variance (ANOVA) and significance of p values < 0.05 was considered statistically significant.

Results

Effect of quercetin on rats' body, testis weights and sperm count

The data in Table (1) showed that significant decreases in the final body weight, testicular weight, the total count and viability of sperms in T2DM animals, while, sperms-abnormalities were increased, compared to control animals. However, the diabetic group treated with quercetin showed amelioration of these parameters.

Effect of quercetin on Fasting blood glucose level

Table 2 showed that the fasting blood glucose (FBG) level was significantly increased (p< 0.001) by 3.5 folds in T2DM group, as compared to the control group, approving the establishment of diabetes. The diabetic group supplemented with quercetin showed a significant decrease in the FBG levels to 1.2 folds of the normal control value.

Table 1: The total body weight, testis weight, sperm count, viability % and Total sperm abnormality % of different experimental groups:

Parameter / Group	The total body weight (g)	Testis weight (g)	Sperm count (number of sperms/rat x10^6)	Viability %	Total sperm abnormality %
C	311.67 ± 10.77	2.55 ± 0.08	161.33 ± 6.70	91.17 ± 1.60	3.03 ± 0.22
D	185.00 ± 7.64[a]	1.83 ± 0.06[a]	115.00 ± 4.18[a]	60.33 ± 2.90[a]	8.70 ± 0.3[a]
Q	300.00 ± 5.32[b]	2.27 ± 0.07[b]	160.50 ± 6.7[b]	89.67 ± 1.36[b]	3.08 ± 0.22[b]
DQ	245.00 ± 7.64[a,c]	2.16 ± 0.07[a,c]	12933 ± 2.93[a,c]	79.50 ± 2.23[a,c]	4.33 ± 0.24[a,c]

C: control group, D: diabetic group; induced by NA and STZ, Q: quercetin treated group, DQ: diabetic animals treated with quercetin. [a]: Statistical significant difference as compared to the control group, [b]: non-significant difference as compared to the control group, [c]: Statistical significant difference as compared to the diabetic group.

Table 2: The blood biochemical parameters of different experimental groups:

Group / Parameter	C	D	Q	DQ
Glucose (mg/dl)	99.50 ± 2.57	350 ± 10.90[a]	100.17 ±2.11[b]	118.50 ±5.08[a,c]
ALT (U/L)	20.00 ± 2.60	45.17 ± 2.94[a]	22.17 ± 0.91[b]	23.33 ± 1.45[a,c]
AST (U/L)	52.50 ± 2.88	85.50 ± 3.25[a]	51.17 ± 1.42[b]	60.50 ± 2.83 [a,c]
ALP (U/L)	136.17 ± 6.44	206.67 ± 5.68[a]	139.00 ± 8.45[b]	156.50 ± 13.24[a,c]
GGT (U/L)	5.00 ± 0.21	10.0 ± 0.33[a]	5.33 ± 0.84[b]	5.83 ± 0.83[a,c]
Urea (mg/dl)	24.83 ± 1.17	60.00 ± 1.77[a]	25.17 ± 1.45[b]	39.17 ± 1.58[a,c]
Creatinine (mg/dl)	0.38 ± 0.03	0.72 ± 0.05[a]	0.39 ± 0.02[b]	0.55 ± 0.021[a,c]
TC (mg/dl)	45.33 ± 1.36	59.67 ± 1.33[a]	42.17 ± 1.42[b]	49.33 ± 2.43[a,c]
TG (mg/dl)	75.00 ± 3.11	91.50 ± 2.88[a]	72.33 ± 2.47[b]	80.83 ± 1.52[a,c]
TP (mg/dl)	7.71 ± 0.05	5.80 ± 0.16[a]	7.27 ± 0.13[b]	6.07 ± 0.09[a,c]
A (mg/dl)	4.45 ± 0.07	3.30 ± 0.08[a]	4.08 ± 0.12[b]	3.94 ± 0.07 [a,c]

The legend is as Table 1.

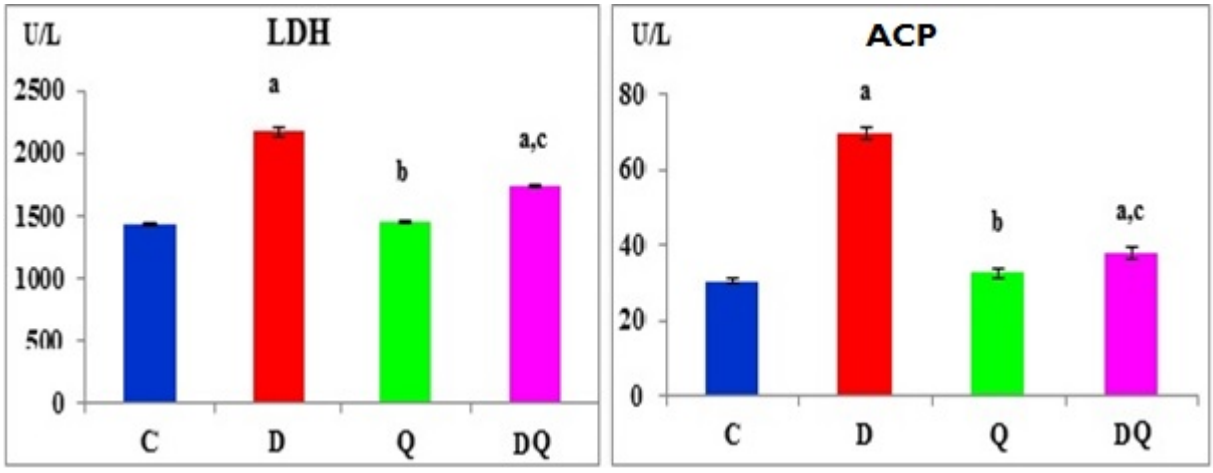

Figure 1: The activity of serum lactate dehydrogenase (LDH) and acid phosphatase (ACP) of different experimental groups. The legend is as Table 1.

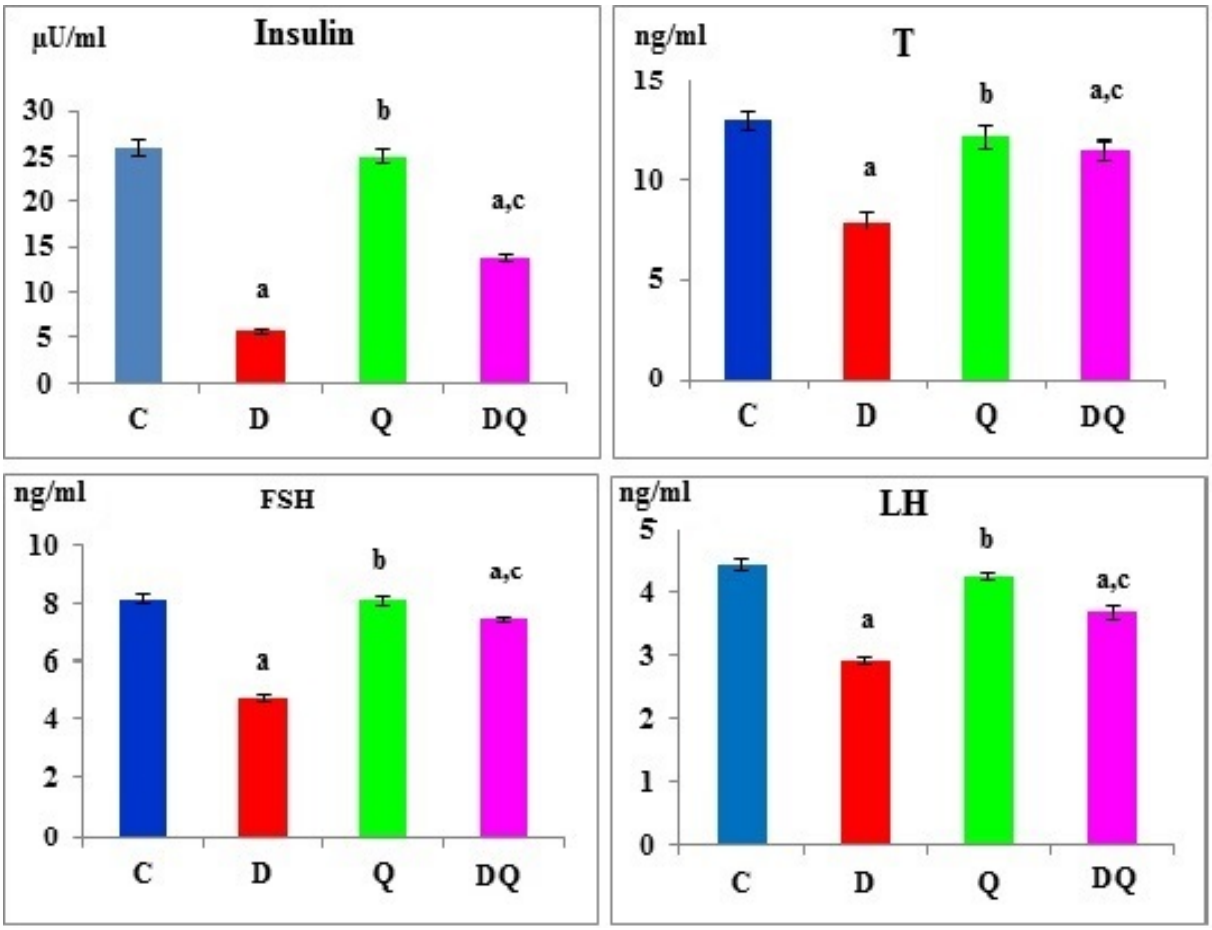

Figure 2: The hormonal changes in the serum of different experimental groups. T: testosterone, FSH: follicle-stimulating hormone and LH: luteinizing hormone. The legend is as Table 1.

Effect of quercetin on different blood biochemical parameters

Table 2 showed the level ofdifferent blood biochemical parameters. The activities of the hepatic markers enzymes; Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) , Alkaline phosphatase (ALP), and Gamma-glutamyl transpeptidase (GGT)were significantly increased in T2DM group, but showed significant amelioration in quercetin treated diabetic rats.

Further, the levels of the urea,creatinine, total cholesterol (TC) and triglycerides (TG)weresignificantly increased, but the total protein (TP) and albumin levels showed significant decrease in the T2DM group, compared to the control groups.However, T2DM animals treated by quercetin showed amelioration of these biochemical parameters

Fig. 1 showed that the activity of LDHand ACPweresignificantly increased ($p<0.001$) in the T2DMgroup,to152.6% and 228%of the corresponding normal values. While,T2DM group supplemented with quercetinimproved the LDH and ACPactivitiesto 123%and121.7%respectively, compared to the control group.

Effect of quercetin on the serum hormonal changes

Fig. 2 showed that the T2DM rats displayed a significant ($p<0.001$) decline in the level of serum insulin T, FSH and LH to 20%, 61%, 57.9% and 65.9% of their corresponding normal values. Conversely, the diabetic rats supplemented with quercetin showed significant ($p < 0.001$) improvement in the level of these hormones towards normal values.

Table 3: the antioxidant status in the testes of different experimental groups:

Parameter / Group	MDA (nmol/g tissue)	NO (µmol/g tissue)	GSH (mg/g tissue)	SOD (U/g tissue)	CAT (U/g tissue)	GSH-Px (U/g tissue)
C	95.63 ± 1.08	6.07 ± 0.14	9.24 ± 0.19	30.00 ± 0.78	9.42± 0.16	18.50 ± 1.12
D	157.81 ± 1.20[a]	15.08 ± 0.24[a]	6.62 ± 0.22[a]	18.13 ± 0.63[a]	7.77± 0.16[a]	8.33 ± 0.42[a]
Q	96.23 ± 0.68[b]	5.833 ± 0.14[b]	9.46 ± 0.08[b]	28.81 ± 0.53[b]	9.35 ± 0.04[b]	17.50 ± 0.99[b]
DQ	105.37 ± 1.11[a,c]	7.03 ± 0.26[a,c]	8.34 ± 0.32[a,c]	27.16 ± 0.30[a,c]	9.20 ± 0.22[a,c]	16.83 ± 1.17[a,c]

MDA: malondialdehyde, NO: nitric oxide, GSH: reduced glutathione, SOD: superoxide dismutase, CAT: catalase, GSH-Px: glutathione peroxidase. The legend is as Table 1.

Effect of quercetin on the oxidative stress and antioxidant status in the testes

The data in Table 3 showed that MDA and NO levels were significantly increased to 165% and 248%, but the GSH content and the antioxidant enzymes, SOD, CAT, GSH-Px activities were significantly decreased to 71.6%, 60.3%, 82.5% and 45% in the testis tissue of the T2DM group, as compared to the corresponding normal values. However, the T2DM group supplemented with quercetin showed amelioration of these parameters towards normal values.

Effect of quercetin on the pro-inflammatory markers in the testes

Table 4 showed that IL-6, TNF-α and NF-κB levels were significantly increased in the T2DM group to 289%, 331% and 357% as compared to the control group. But, the T2DM group supplemented with quercetin showed a significant decline in their levels to 166%, 123% and 176% of their normal values, respectively.

Table 4: The proinflammatory cytokines of different experimental groups

Parameter / Group	IL-6 (Pg/ml)	TNF-α (Pg/ml)	NF-κB (Pg/ml)
C	31.07 ± 1.69	30.70 ± 1.26	39.33 ± 1.15
D	89.87 ± 3.27[a]	101.62 ± 3.77[a]	140.67 ± 5.48[a]
Q	35.39 ± 2.54[b]	31.53 ± 1.10[b]	39.83 ± 1.42[b]
DQ	51.58 ± 4.41[a,c]	37.97 ± 1.59[a,c]	69.33 ± 1.98[a,c]

The legend is as Table 1.

Effect of quercetin on the activity of acetylcholinestrase and butrylcholinestrase in the testes

The AchE and BchE activities were significantly decreased to 39% and 68.5% in the T2DM group, however, the T2DM group supplemented with quercetin showed a significant enhancement to 78.7% and 90.3%, respectively as compared to the corresponding normal values.

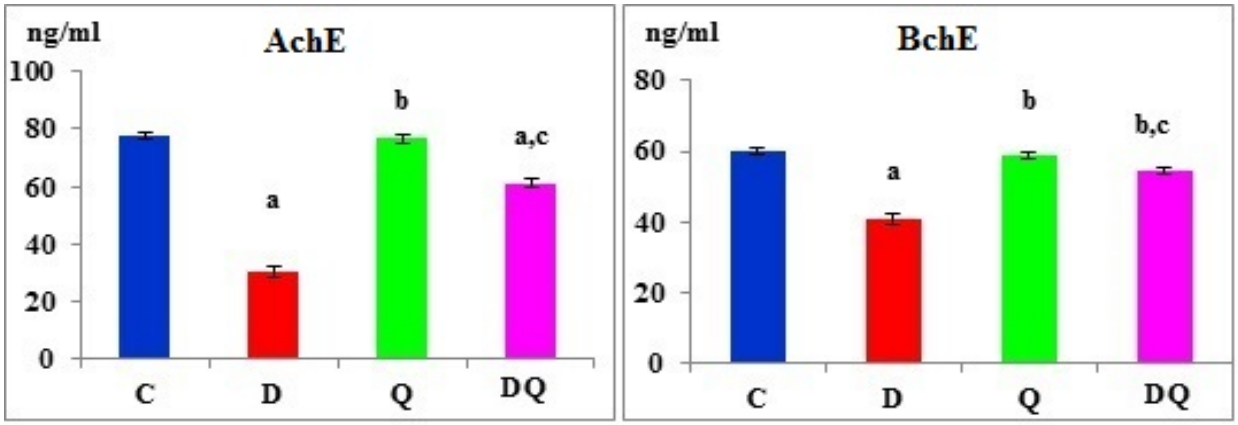

Figure 3: The activity of acetylcholinestrase (AchE) and butrylcholinestrase (BchE) in the testes of different experimental groups. The legend is as Table 1.

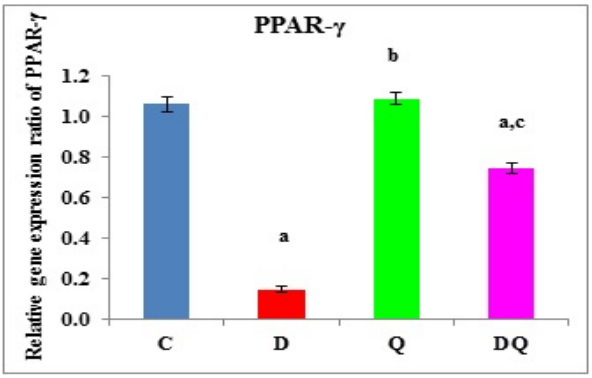

Figure 4: The relative ratio of Peroxisome Proliferator-Activated Receptor-γ (PPAR-γ) gene expression in the testes of different experimental groups. The legend is as Table 1.

Effect of quercetin on the relative ratio of Peroxisome Proliferator-Activated Receptor-γ (PPAR-γ) gene expression.

The relative ratio of Peroxisome Proliferator-Activated Receptor-γ (PPAR-γ) gene expressionshowed a significant (P<0.001) decrease in the T2DM group to 14%of the normal value. However, the T2DM group supplemented with quercetin showed a significant improvement of the relative ratio of the gene expression of PPAR-γ to 70% of the normal value.

Discussion

The present study demonstrated no toxicity symptoms with quercetin treatment (100 mg/kgb.wt.)were observed. Qtreated group showed non-significant changes in most of the studied biochemical parameters, as well in hematological profile and different organs' weight (unpublished data),as compared to the normal-non-diabetic animals, in concomitant with the previous study[1]. The administration of quercetin for 30 days suggested a markedimprovementin hyperglycemia and hyperlipidemia,whichcould be attributed to the quercetin stimulatory activity, which has nearly no effect on the glycemic status and oxidative stress markers in normal rats but have significant effects on the glucose level in

diabetic rats. Similar results were observed in previous work[28,29]. The down-regulation of the hyperglycemia may be attributed to the potent antioxidant properties of quercetin[1,30].Healthy individuals sustain stable blood glucose levels through basal insulin secretion[31]. Diabetes showed significant increase in plasma glucose level, accompanied by a significant decrease in plasma insulin level[16,28]. Hyper-glycaemia in diabetes is the main symptom triggered by a lack of insulin and/or insulin resistance[2]. The diminished insulin levels reported in the current study is improved by quercetin treatment. Quercetin administration is beneficial for reducing the blood glucose concentration, thereby could promote the regeneration of pancreatic islets and increasing insulin release, thus exerting its beneficial anti-diabetic effects[1,31].Quercetin treatment protected and maintained the pancreatic tissues subjected to STZ-induced oxidative stress by directly quenching lipid peroxides and indirectly enhancing production of pancreatic antioxidants enzymes[1,9,32,33].It was observed that T2DM-induced physiological alterations, resulting in damage of differenttissues by increasing ALT, AST, ALP, GGT and LDH activities, cholesterol and triglycerides[34] levels.The activities of ALT, AST, ALP and GGT and LDH,thelevels of urea, creatinine, TC and TGwere significantly elevated in the T2DM group, which is in agreeing with the previous research studies[1,28,29,35,36]**.** Also, the data showed that the level of total protein (TP) and albumin was obviously reduced in the T2DM group, which could be attributed to the leakage of proteins and albumin in urine in diabetic patients due to glomerular basement membrane damage combined with an increase in trans-glomerular filtration pressure[1].

Otherwise, quercetin was effective to ameliorate these biochemical parameters towards normal levels. The quercetin treatment has the ability to attenuate the hepatorenal toxicity, which is associated with the decrease of oxidative and nitrosative stress in different tissues[9,37]**.**Quercetinadministration could protectthe cardiovascular system from damage via improving the marker enzymes ALT, AST, ALP, GGT and LDH activities and restoring the total protein levels[38], LDH activity[39] and TC and TG levels[1,40]. However, the Q treatmentcould significantly improve the serum albumin levels, which could be reflected in the reduction of kidney damage due to NA-STZ induced hyperglycemia, inconsistent with Arya*etal.*[1]**.**Quercetin declined the excretion of ALP and GGT concentration, suggesting its protective effect against renal tubular toxicity[41].Consequently, quercetin establishes beneficial biochemical ameliorative effects on diabetes, cardiac,hepatic, and renal functional markers.

In the current study, the acid phosphatase (ACP) activity was significantly increased in the T2DM group, however,administrationof quercetin showed a significant decline in ACPactivity, whichisin agreeing with Shafekco-workers[42]. Acid phosphatase was elevated in diabetes mellitus, whereas, there is a significant correlation of acid phosphatase with sugar level in the blood. Acid phosphatase could be used as a marker for the development, control of diabetes and prognosis of diabetic complications[43].

The data of the present study revealed a significant hormonal decrease in LH, FSH and testosterone levels in T2DM rats. Diabetes is associated with reproductive disorders[7]**.** Testicular function is predominantly regulated by pituitary hormones. The FSH and the LH regulate spermatogenesis and Leydig cell function, respectively[44]. Diabetes exhibited remarkable dimensioned levels of serum FSH and LH, which could be attributed to the physiological disturbance in insulin/glucose levels in the serum[33]. Declined levels of insulin for long time established intra-pituitary LH accumulation with disrupt terminal glycosylation Olivares co-workers[45].Moreover, the administration of high doses of streptozotocin (STZ) to male rats could induce a decline in the testicular testosterone production[46], which could be attributed to the impaired Leydig cell function and in the pattern of androgen biosynthesis[7]. Testosterone showed a critical role in the regulation of spermatogenesis metabolic support by Sertoli cells, alterations of testosterone in T2DM may be the starting point of male subfertility/infertility[47].In contrast, quercetintreatment to the diabetic animals significantly up regulated the level of these hormones in the serum.

Under physiological conditions of glucose and insulin deregulation, testicular cells could be undergo metabolic adaptations and use alternative substrates, mainly via lactate production that expected to overproduction of reactive oxygen species and induce oxidative stress[48].Oxidativestress induced in diabetescould be contributed towards the development of testicular dysfunctionthat could be attributed to the insulin deficiency[33,49]. Excessive production of free radicals or reactive oxygen species (ROS) cause attenuation of the endogenous antioxidant enzymes, induction of inflammatory responses, the decline of the testicular function, and hence evoked apoptosis and cell death[48]. SOD protects the cells against oxidative stress by catalyzing the dismutation of the highly reactive superoxide radical $O_2^{\bullet-}$ to O_2 and H_2O_2[50]. Then, CAT catalyzed the removal of H_2O_2 via its reduction to water and molecular oxygen**,** while, GSH-Px removed hydrogen peroxide by converting reduced glutathione into oxidized glutathione[51]. Thus, the decrease in the activities of SOD and CAT could be attributed to the feedback inhibition or oxidative inactivation of the enzymes' proteins due to the excess ROS generation. The observed depletion of GSH could be attributed to the excess utilization during the detoxification process demanding the glutathione enzymes system, which evoked enhancement of the lipid peroxidation and inactivate GSH-Px in the testicular tissues[51]. The data of this study revealed alterations in antioxidant defense system ascertained by elevation of MDA, NO levels, and declines in GSH content and the activity of the antioxidant enzymes; SOD, CAT and GSH-Px. The results of the present investigation are consistent with the findings reported byArya*et al*.[52]. However, quercetin ameliorated the antioxidant status in the diabetic rats by improving the antioxidant enzymes activity and GSH content accompanied by significant decreases in MDA and NO levels in the testicular tissues.Quercetinshowed protective effects in the diabetic rats, possibly by decreasing oxidative stress[53,54].

Oxidative stress induced by hyperglycemia in T2DM is recognized to be associated with activation of inflammatory signaling pathways, including transcription factors like nuclear factor kappa B (NF-κB). NF-κB induces the release of the pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6[55]. The results of the present work demonstrated marked elevations in NF-κB, TNF-α and IL-6 levels in the testicular tissues of T2DM group. These upraised proinflammatory cytokines acquire antagonistic characters to insulin due to their ability to augment Insulin Receptor Substrate (IRS) phosphorylation, causing insulin resistance[56]. Quercetin down-regulated the levels of TNF-α and IL-6, which could be attributed to the attenuation and the scavengingof the free radicals induced NF-κB translocation and ameliorating the oxidative stress in T2DM rats[54].

Moreover, the AchE and BchE activity found to be significantly decreased in the T2DM group in the current study. This result is in agreement withSebai*et al.*[57], they reported a significant decrease in the activity of serum AchE and BchE in alloxan induced diabetic rats, due to the inhibition of their synthesis.AchE and BchE are present in neuronal and non-neuronal cells, including testis[58]. Acetylcholine is a neurotransmitter that synthesized by choline acetyltransferase, while its action is terminated by AchE and BchE. AchE and BchE cleave acetylcholine into choline and acetate. The functional action acetylcholine requires binding to specific receptors: the nicotinic acetylcholine receptor (nAChR) and the muscarinic acetylcholine receptor (MR). Acetylcholine is able to regulate the cytokines production by binding to AChR[59]. However, the T2DM group supplemented with quercetin showed a significant enhancement of AchEandBchE activities, in the present work. Moreover, BChE may play a role in the development of hypertriglyceridemia in DM2, associated with a positive correlation with impaired glucose tolerance, resistance to or deficiency of insulin[60,61].Quercetin modulates the alterations induced by Cd in AChE activity in different brain regions, suggesting that the antioxidant property of quercetin may contribute to the prevention of cholinergic dysfunction[62].

Peroxisome proliferator–activated receptors (PPARs) are members of the nuclear hormone receptor superfamily, which can modulate gene expression by physically interacting with other transcription factors as nuclear factor kappa B (NF-κB)[63], therefore regulate the inflammatory and apoptotic signaling pathway. Also, its expression in adipose tissues improves the terminal differentiation of adipose and lipid storage[64]**.**PPAR-

γ is also involved in glucose metabolism, whereas its activation decreases the insulin resistance in muscle and adipose tissue, increases the production of proteins involved in glucose uptake[65]. Also, its expression in adipose tissues improves the terminal differentiation of adipose and lipid storage[65,66]. PPAR-γis recognized as the main regulator of gene transcription and plasma concentrations of adiponectin; proteins secreted by adipose tissues that have been exposed to be involved in obesityand insulin sensitivity or resistance. Whereas, PPAR-γ binds directly to a functional PPAR-responsive element (PPRE) in adiponectin promoter, leading to improvement of adiponectin gene transcription[67,68]. Further, isoforms of the PPAR, including PPAR-γ have been distinguished in testicular tissues, advocating a role for insulin sensitization in testicular function in diabetes[69].PPAR-γ found to plays criticalfunctions in spermatogenesis[70], and improvethe antioxidant status, whereas its antagonist; pioglitazone enhanced the testicular antioxidant defense system in alloxan-induced diabetic rabbits without affecting blood glucose levels[71,72].The present study demonstrated a remarkable decline in the relative ratio of PPAR gene expression in testicular tissues of T2DM group. Meanwhile, quercetin administration to T2DM animals showed up-regulation of the PPAR gene expression in testicular tissues, which could be attributed to its potent antioxidant activity.

The data of the present study showed that diabetes induced by NA-STZ declined the animals' body and testis weight, which could be attributed to protein consumption, instead of the unavailable carbohydrates for energy production[73]. Our result is in consent with the previous studies that concluded a decrease in the testicular and body weight of diabetic animals[9]. Further, the present study established a remarkable decrease in the count and viability of spermsbesides enhancement of the sperm abnormality in diabetic rats. Instantaneous administration of NA and STZ induce achronicdiabetic situation like type-2 diabetes, but NAcan protectLangerhansislets from cytotoxicdamages induced by STZ. Furthermore, earlier research works indicate that administration of STZ could trigger sperm depletion and abnormalities, which could be attributed to ROS overproduction, whereas, the oxidative stress has a critical task in the pathogenesis of diabetes-induced male reproductive impairment[7,9]. Overproductions of ROS evoke testicular oxidative injury, germ cell apoptosis, and sperm count and viability depletion. In addition, the spermatozoa membrane, which contains high polyunsaturated fatty acid constituents, is very sensitive to oxidative stress[74]. The observed disturbance in the antioxidant status in the testicular tissues in this study could evokedepletion in the adenosine tri-phosphate formationandsperm motility[9]. On the other hand, the data indicate that quercetin treatment improved the total and testis weights, sperm count, and viabilityas well reduced the sperms abnormality in T2DM animals.

The results of the current study demonstrate that quercetin showed antidiabeticactivity and therapeutic effects on the complications of induced type-2 Diabetes (T2DM) nicotinamide-streptozotocin in male rats,owingto its potent antioxidant, scavenging of ROS, and anti-inflammatory actions[11]. The therapeutic effect of quercetin on the reproductive dysfunction is mediated by regulation of glucose/insulin metabolism, maintenance of the pituitary hormones levels, amelioration of different testicular biochemical functions, which is mediated by improving the testicular antioxidant, anti-inflammatory status, AchE, BchE activities andupregulation of the PPAR-γ gene expression. Up-regulation of the PPAR-γ gene expression could be responsible for the down-regulation of NF-κB; and consequently down-regulation of TNF-α and IL-6 levels and a decline of the oxidative stress.

In conclusion

This study was performed in order to investigate the therapeutic; biochemical effects of the flavonoid compound quercetin to treat the complications, with a glance at the testicular dysfunction of type-2 diabetes induced by nicotinamide-streptozotocin (NA–STZ)in male rats. Oral supplementation of quercetinshowedanti-diabetic;hypoglycemic activity in T2DM rats. Further, quercetin treatment of T2DM rats dramatically reducedhepato-renal and cardiac toxicity as evidenced by ameliorating serum total cholesterol, triglycerides, albumin, urea, creatinine levels, liver transaminases (ALT, AST), alkaline phosphatase (ALP) and gamma glutamyl

transpeptidase (GGT) activities.Furthermore,quercetin supplementation enhanced the levels of insulin, testosterone (T), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in serum. Additionally, quercetin administration improved the antioxidant status in the testicular tissues of T2DM rats, by enhancing the SOD, GSH-Px, CAT, AchE andBchEactivities in parallel with decline the MDA, NO, IL-6, TNF-α levels and up-regulated the relative ratio of PPAR-γ gene expression. In conclusion:Quercetin has potential therapeutic effects on different complications of T2DM induced bynicotinamidestreptozotocin in male rats.Quercetinimproved testicular dysfunctions in T2DM, which is mediated by improving the testicular antioxidant, anti-inflammatory status, AchE, BchE activities and up-regulation of the PPAR-γ gene expression.Werecommend the regular use of the flavonoids and quercetin rich dietary plants, fruits, vegetables, and onion that could have advantageous effects on diabetic persons and decrease risk of infertility in men.

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