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ORIGINAL CONTRIBUTION

Quail (*Coturnix japonica*) egg yolk bioactive components attenuate streptozotocin-induced testicular damage and oxidative stress in diabetic rats

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Abstract
Introduction The testicular milieu is the machinery for the metabolism of testosterone in the male reproductive system.
Purpose The dysfunction of this highly regenerating system is inevitable in the condition of glucose imbalance as a result of insulin machinery impairment. Therefore, it is imperative to recommend dietary intervention for attenuating the testicular dysfunction and oxidative stress resulting from STZ-induction of diabetes.
Methods STZ-induced diabetes (65 mg/kg, ip) was treated with QEYEM (50, 100 and 200 mg/kg/day) and quercetin (50 mg/kg/day) for 7weeks. In serum, glucose, testosterone, IL-6 and TNF-α levels were estimated, and in testis, tissues TBARS, sulfhydryl groups, nucleic acids and total protein (TP) levels were estimated. SOD, CAT and GST activities were also determined in testicular cells. Histopathological changes were evaluated in a cross-section of testis.
Results Testosterone concentration was decreased while pro-inflammatory markers were increased in STZ-assaulted rats. Treatment using QEYEM of diabetic rats corrected assaults and reverse significantly the diabetic conditions. QEYEM-treated groups showed significant inhibition of TBARS levels and elevation of testicular GSH, NP-SH, total protein (TP) and nucleic acids—DNA and RNA levels. The QEYEM administration reversed the inhibited activities of SOD, CAT and GST in testicular cells in diabetic rats. The characterization of the extract carried out through HPLC analytical techniques revealed vitamins A, D and E concentrations of 0.645, 0.012 and 6.3 mg/100 g, respectively.
Conclusion QEYEM supplementation to STZ-induced diabetic rats for seven (7) consecutive weeks is a potential intervention against testicular damage in adult diabetic rats, probably by decreasing oxidative stress.

Keywords Pro-inflammation markers · Diabetes · Oxidative stress · Testosterone dysfunction · Quail egg yolk

Background

Diabetes mellitus (DM) is a chronic, complicated metabolic disorder characterized by hyperglycemia, which often results from defects in insulin secretion, insulin action, or both. Moreover, DM is associated with severe disturbances of carbohydrate, fat, and protein metabolism [1]. A large number of studies, both in diabetic men and animal models indicate that DM causes male infertility based on impotency, retrograde ejaculation, and hypogonadism. DM may affect male reproductive functions at multiple levels including variation in sperm quality, altered spermatogenesis, morphological changes in testes, altered glucose metabolism in Sertoli–blood testis barrier, reduced testosterone, ejaculatory dysfunction, and a reduced libido. Several clinical and animal studies have focused on the molecular mechanism responsible for the alterations induced by DM in male reproductive potential including endocrine disorders, neuropathy, and increased oxidative stress [2]. Hyperglycemia is a disease condition that occurs in tissues with active redox cellular functions leading to generation of radical species that could progress the generation and proliferation by free radicals. Hyperglycemia increases the inflammatory markers'

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tumor necrosis factor (TNF)-α and IL-6 [3, 4].Oxidative stress is a pathological condition that sets in as a result of degenerative activities by free radicals which abstract electrons from biomolecules, possibly progressing to more highly reactive radicals, which can provoke irreversible cell injury in testicular cells. DM has adverse effects on the male sexual and reproductive functions [5]. Serum testosterone impairment and varying degrees of testicular lesions have also been demonstrated in streptozotocin (STZ)-induced diabetic animal models [6, 7]. Enhanced oxidative stress and changes in antioxidant capacity are considered to play an important role in the pathogenesis of chronic diabetes mellitus [7].

Male reproductive alterations have been widely reported in diabetic-induced animal models [8, 9]. STZ-induced diabetes in male rats resulted in atrophy of sex organs [10], changes in histoarchitecture of ventral prostate [11], diminution in sperm count [12], along with low levels in plasma gonadotrophins [13] and testosterone [12] resulting from apoptosis. Apoptosis is important for physiological development and homeostasis of an organism during embryogenesis and through adulthood [14]. Oxidative damage is the initial event, followed by inflammation and apoptosis [15]. It has been demonstrated that under diabetic status, oxidative stress is a major cause for loss of male germ cells since diabetic induction of testicular apoptotic cell death was forbidden by either antioxidant treatment with N-acetyl-L-cysteine or low-level ionizing radiation that induces up-regulation of testicular antioxidants [16].

Quail bird is a game bird that has been recognized and first mentioned by biblical record during the journey of the Israelites from Egypt thousands of years BC. The egg has been adopted as dietary intervention to diseases and disorders. Reports have shown the bioactivity of quail egg against physiological stress [17]. In Nigeria and some regions of the world where traditional medicine is relied on for health intervention as a result of economic decadence, quail egg has served in traditional and pharmacological medicine. The quail egg yolk had been reported to contain vitamins such as A, D and E. Vitamin E is a fat soluble vitamin with antioxidant properties. Vitamin E exists in eight different forms (isomers): alpha, beta, gamma and delta tocopherol; and alpha, beta, gamma and delta tocotrienol, but alpha is the most active form in humans. It has been proposed for the conditions, often based on its antioxidant properties [18]. Polyunsaturated fatty acids (PUFAs) have been reported to be present in the egg yolk with considerable amount of omega-3 PUFA [18]; these bioactive compounds have been described to intervene against tumor progression and mitigation against the oxidative stress.

The purpose of this study was to determine the protective and therapeutic potential of QEYEM on STZ-induced testicular damage and to elicit the role of antioxidant effect.

Materials and methods

Materials

Streptozotocin (*N*-(methyl nitroso carbamoyl)-*α*-D-glucosamine) and Quercetin was purchased from Sigma Chemical Co., USA. All chemicals used in the present study were of highest analytical grade. Analytical kits were manufactured by Randox Laboratory Limited.

Sample collection and preparation of extract and characterization

Extraction of bioactive components is involved [19]; approximately 100 g of freeze-dried quail egg yolk was extracted with 500 ml of 80% methanol and 500 ml of 80% ethanol (80: 20, vol/vol) adjusted to pH 1.5 with 1 mol/l HCl. The samples were then mixed thoroughly using a vortex mixer for 2 min and centrifuged at 6000*g* for 10 min at 4 °C. The supernatant was evaporated under vacuum at 35 °C using a rotary evaporator, collected, freeze-dried, and stored at 4 °C.

The bioactive compounds of some fat soluble vitamins (A, D and E) were analyzed using saponification, and liquid extraction of organic solvents with HPLC–Fluorescence detection [20].

In vitro antioxidant assays

Since the determination of antioxidant activities is a quantitative analysis, all assays were performed in triplicate. For each test performed, various concentrations (25, 50, 100, 200 and 400 µg/ml) of the extract and standards were prepared with reconstitution in equal volume of methanol and ethanol.

Assay for total antioxidant activity

To the reagent solution containing sulphuric acid (0.6 M), sodium phosphate (28 mM) and ammonium molybdate (4 mM), 0.3 ml of sample was added and incubated at 95 °C in a water bath for 90 min. After cooling to room temperature, absorbance was recorded at 765 nm against reagent blank. The absorbance of the sample was extrapolated on the ascorbic acid standard curve to obtain the concentration of the sample in mg/ml, then the total antioxidant activity (mg/g ascorbic acid equivalence) was calculated.

Assay of reducing power activity

The reducing power of the extracts was determined according to the method [21]. Extract (0.5 ml) was mixed with 1.25 ml each of phosphate buffer and potassium ferricyanide ($C_6N_6FeK_3$). The mixture was incubated at 50 °C for 20 min.

Trichloroacetic acid (1.25 ml) was then added and the mixture centrifuged at 3000 rpm for 10 min. Thereafter, 1.25 ml of the upper layer of the solution was mixed with 1.25 ml of distilled water and 0.25 ml of $FeCl_3$. The absorbance was read at 700 nm. Higher absorbance of the reaction mixture indicates greater reductive potential.

Assay of DPPH radical scavenging (1,1-diphenyl-2-picrylhydrazyl) activity

The DPPH radical scavenging activity of the extracts was evaluated according to the method [22]. Exactly 1 ml of 0.3 mM DPPH prepared in methanol was added to 1 ml of extract of various concentrations and allowed to react at room temperature for 30 min. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm and the percentage scavenging activity was calculated using the following formula:

$$\%\ \text{Scavenging activity} = \left(\left(A_c - A_s\right)/A_c\right) \times 100,$$

where A_c is the absorbance of control and A_s is the absorbance of the extract.

Assay of 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity

ABTS radical scavenging activity of the plant extract was determined according to the method [23]. The stock solutions were of 7 mM $ABTS^+$ and 2.4 mM potassium persulfate. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 16 h at room temperature in the dark. The solution was diluted by mixing 5 ml $ABTS^+$ solution with 145 ml of distilled water to obtain an absorbance of 0.076 ± 0.001 units at 734 nm. Extracts (1 ml) at various concentrations were allowed to react with 1 ml of $ABTS^+$ solution, and the absorbance was measured at 734 nm after 30 min using a spectrophotometer and the percentage scavenging activity was calculated using the following formula:

$$\%\ \text{Scavenging activity} = \left(\left(A_c - A_s\right)/A_c\right) \times 100,$$

where A_c is the absorbance of control and A_s is the absorbance of the extract.

Assay of metal chelating activity

The metal chelating activity was determined according to the method [24]. Extract (1 ml) was added to 100 µl of 1 mM $FeSO_4 \cdot 7H_2O$. The reaction mixture was left at room temperature for 2 min. After which 0.5 ml of 0.5 mM 1, 10-phenanthroline was added and the mixture was incubated for 10 min at room temperature. The absorbance was read at 510 nm. The Fe^{2+} chelating capacity was calculated using the following formula:

$$Fe^{2+}\text{chelating activity } (\%) = \left(\left(A_c - A_s\right)/A_c\right) \times 100.$$

Assay of hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe^{2+}/ascorbate/EDTA/H_2O_2 system according to the method [25]. The reaction mixture contained 1 ml (3.0 mM deoxyribose, 0.1 mM EDTA, 2 mM H_2O_2, 0.1 mM L-ascorbic acid, 0.1 mM $FeCl_3.6H_2O$ in 10 mM phosphate buffer, pH 7.4) and various concentrations of the extracts. The reaction mixtures were incubated at 37 °C for 1 h, followed by the addition of 1 ml of 1% (w/v) TBA (in 0.25 N HCl) and 1.0 ml 10% (w/v) TCA. The reaction mixtures were heated in boiling water bath at 100 C for 20 min and the pink chromogen (malondialdehyde–TBA adduct) was extracted into 1.0 ml of butan-1-ol and the absorbance was read at 532 nm against reagent blank. The hydroxyl radical scavenging activity calculated using the following formula: Hydroxyl radical (OH^-) scavenging activity $(\%) = ((A_c - A_s)/A_c) \times 100$.

Assay of lipid peroxidation inhibition activity

The inhibition of lipid peroxidation was ascertained according to the method [26]. Albino rats were killed by cervical dislocation. The liver was carefully excised and washed in ice-cold 1.15% potassium chloride solution, blotted with filter paper and weighed. The liver homogenized in four volumes of phosphate buffer (pH 7.4) using a Teflon homogenizer. The resulting homogenate was centrifuged at 3000 rpm for 10 min to obtain the supernatant. A 200 µl aliquot of each of the supernatant was mixed with 60 µl 0.1M Tris HCl buffer (pH 7.4) and the extracts of various concentrations followed by the addition of 50 mM $FeSO_4$ and 240 µl of distilled water. The resultant mixture were incubated at 37 °C for 1 h. 600 µl of 8.1% SDS was added followed by the addition of 1200 µl of 1.3 M acetic acid buffer (pH 3.4) and 1200 µl 0.8% TBA. The mixture was heated at 100 °C for 1 h to complete the reaction. Then the samples were cooled and centrifuged at 3000 rpm for 10 min. The intensity of pink colored complex was measured at 532 nm and the inhibition of lipid peroxidation was calculated using the following formula:

$$\text{Inhibition } (\%) = \left(\left(A_c - A_s\right)/A_c\right) \times 100.$$

Biochemical evaluations

Animals

Adult male Wistar albino rats, weighing 210–230 g were received from the experimental Animal Care Center

(University of Ilorin, Kwara State, Nigeria). All animals were maintained under controlled conditions of temperature (22 ± 1 °C), humidity (50–55%) and light (12 h light/12 h dark cycle). They were acclimatized to the laboratory conditions for 14 days before the start of the experiment. Animals had free access to rat chow and drinking water. All the experimental procedures including euthanasia were conducted in accordance with the Ethical Regulation and Guide for the Care and Use of Laboratory Animals.

Diabetic induction

Experimental diabetes was induced by a single dose of STZ (65 mg/kg, i.p.) in overnight fasted rats by dissolving in freshly prepared 5 mmol/l citrate buffer, pH 4.5 [27]. After STZ injection, the rats had free access to glucose solution (5%) for 24 h to avoid and/or attenuate subsequent inevitable hyperinsulinemia and hypoglycemic shock. 48 h after the STZ injection, animals were fasted overnight and a drop of blood samples were analyzed for glucose levels (mg/dl) using strips on glucometer (ACCU-CHEK ACTIVE, Roche, Germany). The individual glucose levels reached above 250 mg/dl is considered as diabetic.

Experimental design

Diabetic-induced rats randomly divided into five groups (five rats in each group); untreated diabetic group (STZ), quercetin (50 mg/kg/day i.p) treated (QR50 i.p + STZ) and Quail egg yolk ethanolic–methanolic extract (50 mg/kg/day oral route) treated (QEYEM 50 oral route + STZ), Quail egg yolk ethanolic–methanolic extract (100 mg/kg/day oral route) treated (QEYEM 100 oral route + STZ) and Quail egg yolk ethanolic–methanolic extract (200 mg/kg/day oral route) treated (QEYEM 200 oral route + STZ). Groups that were not subjected to diabetes were Quail egg yolk ethanolic–methanolic extract (50 mg/kg/day oral route) (QEYEM 50), Quail egg yolk ethanolic–methanolic extract (100 mg/kg/day oral route) (QEYEM 100) and Quail egg yolk ethanolic–methanolic extract (200 mg/kg/day oral route) (QEYEM 200). Extract treatments were sustained for seven consecutive weeks. At the end of the experiment, animals were weighed and fasted overnight, blood samples were obtained under light anesthesia and finally they were killed.

Serum parameters

Serum levels of pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) concentrations were assayed by an enzyme-linked immunosorbent assay (ELISA) kit. The concentrations of testosterone in the serum were estimated using EIA-kit (Cayman Chemicals, USA).

Tissue parameters

The animals were quickly dissected and the testes removed and rinsed with ice-cold 1.15% potassium chloride. Testes were weighed and homogenized in 0.1 M phosphate buffered (pH 7.4) using a Teflon homogenizer. Half of the homogenated testes were centrifuged at 1000 g for 10 min at 4 °C to separate nuclei and unbroken cells. Another half of the homogenates was centrifuged at 12,000*g* for 20 min to obtain post-mitochondrial supernatant. MDA, T-GSH and NP-SH levels were estimated. In post-mitochondrial supernatant, SOD, CAT and GST activities were measured.

Estimation of MDA levels in testis

A thiobarbituric acid reactive substances (TBARS) assay kit (Randox) was used to measure the lipid peroxidation product MDA equivalent. 100 µl of homogenate was mixed with 2.5 ml reaction buffer (provided by the kit) and heated at 95 °C for 60 min. After the mixture had cooled, the absorbance of the supernatant was measured at 532 nm using a spectrophotometer. The lipid peroxidation product MDA levels are expressed in terms of n moles MDA/mg protein using molar extinction coefficient of MDA–thiobarbituric chromophore (1.56×10^5/M/cm).

Estimations of T-GSH level in testis

The concentration of T-GSH was measured using the method [28]. Homogenate was mixed with 0.2 M Tris buffer, pH 8.2 and 0.1 ml of 0.01 M Ellman's reagent, (5,5′-dithiobis-(2-nitrobenzoic acid)) (DTNB). Each sample tube was centrifuged at 3000g at room temperature for 15 min. The absorbance of the clear supernatants was measured using spectrophotometer at 412 nm in 1 cm quarts cells.

Estimation of NP-SH levels in testis

NP-SH was measured using the method [2]. The homogenate was mixed in 15.0-ml test tubes with 4.0 ml of distilled H_2O and 1.0 ml of 50% trichloroacetic acid (TCA). The tubes were shaken intermittently for 10–15 min and centrifuged for 15 min at approximately 3000*g*. Two ml of supernatant was mixed with 4.0 ml of 0.4 M Tris buffer, pH 8.9, 0.1 ml DTNB added and the sample shaken. The absorbance was read within 5 min of the addition of DTNB at 412 nm against a reagent blank with no homogenate.

Estimation of GST activity testicular cells

The activity of GST was measured by the method [29]. The reaction mixture consisted of 1.0 mM GSH, 1.0 mM CDNB, 0.1 M phosphate buffer (pH 7.4) and 0.1 ml of PMS in a total volume of 3.0 ml. The change in absorbance was recorded at 340 nm using Shimadzu spectrophotometer UV-1601 and enzyme activity was calculated as nmol of CDNB conjugate formed min^{-1} mg^{-1} protein using molar extinction coefficient of 9.6×103/M/cm.

Estimations of SOD activity in testicular cells

The activity of SOD in testicular cells was estimated using the method [30], with the aid of nitroblue tetrazolium as the indicator. Superoxide anions are generated by the oxidation of hydroxylamine hydrochloride. The reduction of nitroblue tetrazolium to blue formazon mediated by superoxide anions was measured 560 nm under aerobic conditions. Addition of superoxide dismutase inhibits the reduction of nitroblue tetrazolium and the extent of inhibition is taken as a measure of enzyme activity. The SOD activity was expressed as units/mg protein as compared to a standard curve.

Estimation of CAT activity in testicular cells

Catalase activity in testicular cells was estimated by the method [31]. In brief, aliquot of 0.5 ml post-mitochondrial supernatant was mixed with 2.5 ml of 50 mM phosphate buffer (pH 7.0) and 20 mM H_2O_2. CAT activity was estimated spectrophotometrically following the decrease in absorbance at 240 nm. The specific activity of CAT was expressed in terms of units/mg protein as compared to a standard curve.

Determination of nucleic acids levels in testicular cells

The method [32] was used to estimate DNA and RNA levels in testis homogenate. Briefly, tissues were homogenized in ice-cold distilled water. The homogenates were then suspended in 10% ice-cold trichloroacetic acid (TCA). Pellets were extracted twice with 95% ethanol. The nucleic acid extract was treated either with diphenylamine or orcinol reagent for the quantification of DNA and RNA levels, respectively.

Assay of determination of total protein level in testicular cells

This was carried out using the manufacturer protocol of Randox Total Protein Kit [33]. 1 ml of reagent R1(Sodium hydroxide (100 mmol/l), sodium–potassium tartrate (16 mmol/l), Potassium iodide (15 mmol/l) and copper II sulfate (6 mmol/l)) was added to 0.02 ml of the test sample, the mixture was incubated at 25 °C and the absorbance was then measured against the reagent blank at a wavelength of 546 nm.

Total protein concentration

$$= (\text{Abs sample} / \text{Abs standard}) \times \text{standard concentration.}$$

Statistical analysis

All values are expressed as mean ± standard deviation. Statistical evaluation was done using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). The significance level was set at $p < 0.05$.

Results

Biochemical evaluations

Figure 1 consists of results which revealed that QEYEM has antioxidant activities through various antioxidant mechanisms. These activities could be as a result of the finding that the yolk extract contains families of fat soluble vitamins A, D, and E in concentrations 0.645, 0.012 and 6.3 mg/100 g, respectively, revealed through high performance chromatographic techniques (Table 1).

Animals subjected to diabetic conditions by STZ induction were significantly reduced in weight, but increase in the weight of the testes was observed compared to the normal group ($p < 0.05$). The mitigation against the assault was demonstrated with significant difference at varying concentrations of the extract; 50, 100 and 200 mg/kg/day BW ($p < 0.05$) (Fig. 2). In diabetic rats, TBARS levels were significantly ($p < 0.05$) increased while the T-GSH ($p < 0.05$) and NP-SH ($p < 0.05$) levels decreased when compared to normal animals. Treatment with quercetin dose (50 mg/kg/day) for 7 weeks significantly ($p < 0.05$) inhibited the testicular TBARS concentration compared to untreated diabetic rats, and treatment with varying concentrations of quail egg yolk extract which revealed a concentration dependent effects significantly ($p < 0.05$). The inhibition of T-GSH and NP-SH levels in diabetic rats was significantly ($p < 0.05$) reversed after 7 weeks of treatment with quail egg yolk extract 50, 100 and 200 mg/kg/day treatment to diabetic rats by slight significant difference ($p < 0.05$).

Enzymatic activities of SOD, CAT and GST in testicular cells were significantly inhibited in diabetic rats. Quail egg yolk extract treatment with 200 mg/kg/day dose to diabetic rats significantly enhanced the activities of SOD, CAT and GST in testicular cells compared to 50 and 100 mg/kg/day quail egg yolk treatment as well as untreated diabetic animals.

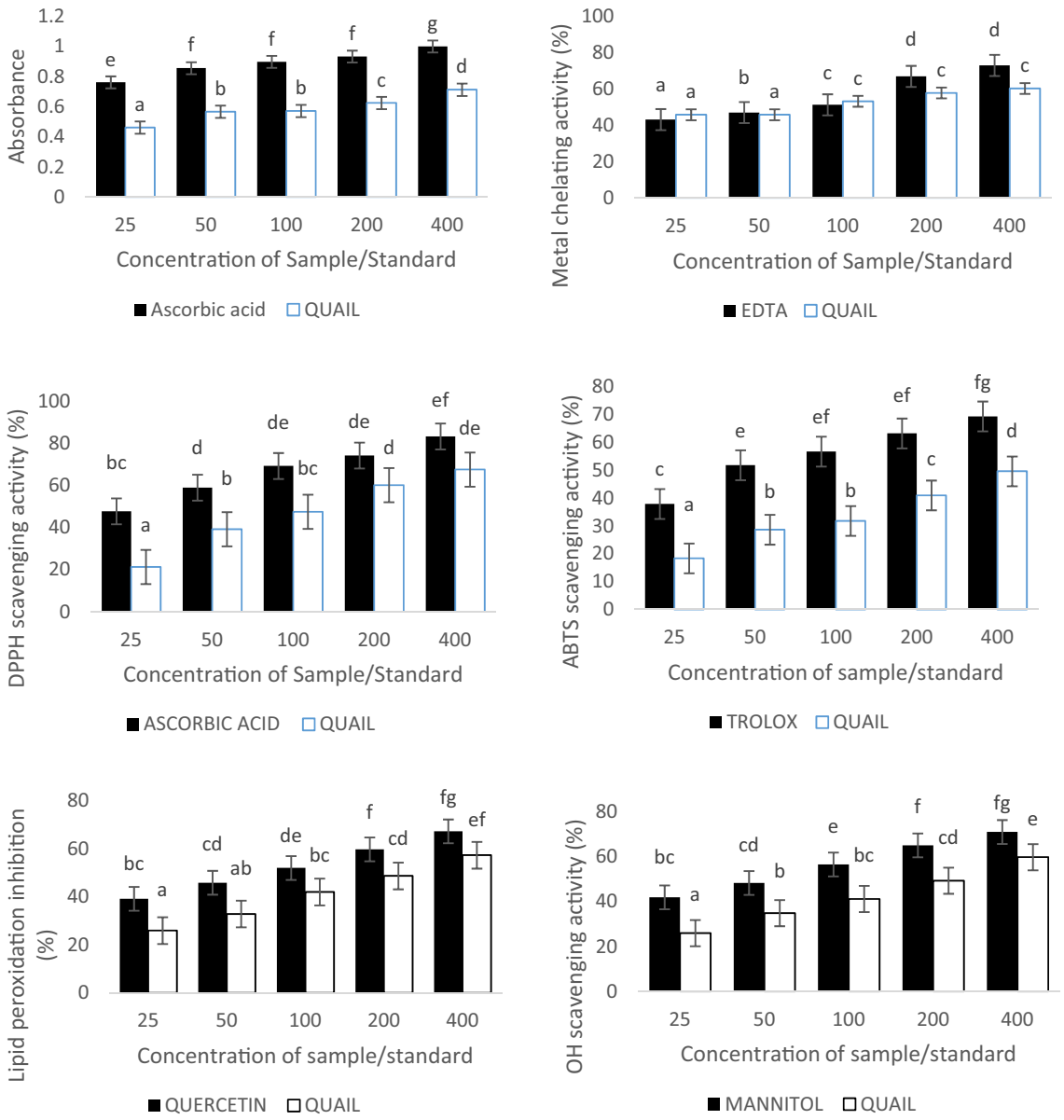

Fig. 1 Analyses of in vitro antioxidant activities of ethanolic–methanolic quail egg yolk. Data were expressed as Mean ± SEM and analyzed using one-way ANOVA followed by Duncan's multiple range test (DMRT) ($n = 3$). Values with different superscript are significantly different ($p < 0.05$)

Table 1 HPLC analyses of QEYEM using vitamins A, D andE

	Concentrations mg/100 g
Vitamin A	0.645
Vitamin D	0.012
Vitamin E	6.3

STZ-induced diabetes reduced the concentrations of total protein and nucleic acids (DNA and RNA) in testicular cells significantly compared to control rats. The treatment with doses of quail egg yolk extract significantly elevated the total protein, DNA and RNA levels when compared to untreated diabetic animals.

Pro-inflammatory markers IL-6 and TNF-α in serum of diabetic rats were markedly ($p < 0.01$) increased compared to control animals. These markers were significantly ($p < 0.05$) inhibited after 7 weeks of 50, 100 and 200 mg/kg/day quail egg yolk treatment of diabetes. The 50 mg/kg/day had significantly least activity than 100 and 200 mg/kg/day administration.

Plate (a) of Fig. 3 shows normal testicular cells of rat, highlighting complete germinal cells with full spermatogenesis. In STZ-induced diabetic rat shown on plate (b) in Fig. 3, showed thickening of the basement membrane with elongation of the

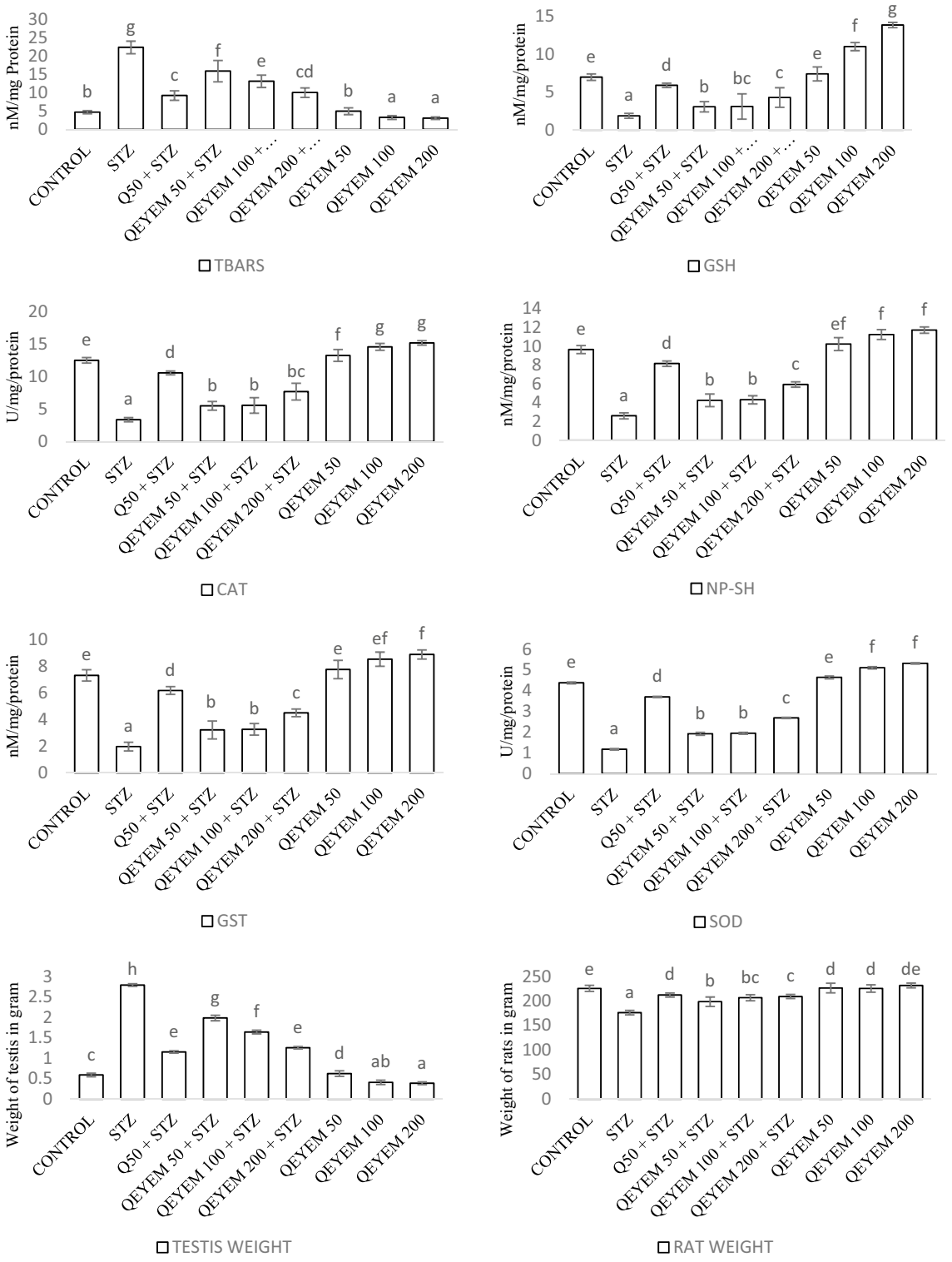

Fig. 2 Effect of ethanolic–methanolic quail egg yolk on levels of TBARS, GSH, CAT, NP-SH, GST, SOD, weight of rats and testes, GSR, glucose, DNA, RNA IL-6 and TNF-α, testosterone of normal and diabetic rats. Data were expressed as Mean ± SEM and analyzed using one-way ANOVA followed by Duncan's multiple range test (DMRT) ($n = 5$). Values with different superscript are significantly different ($p < 0.05$)

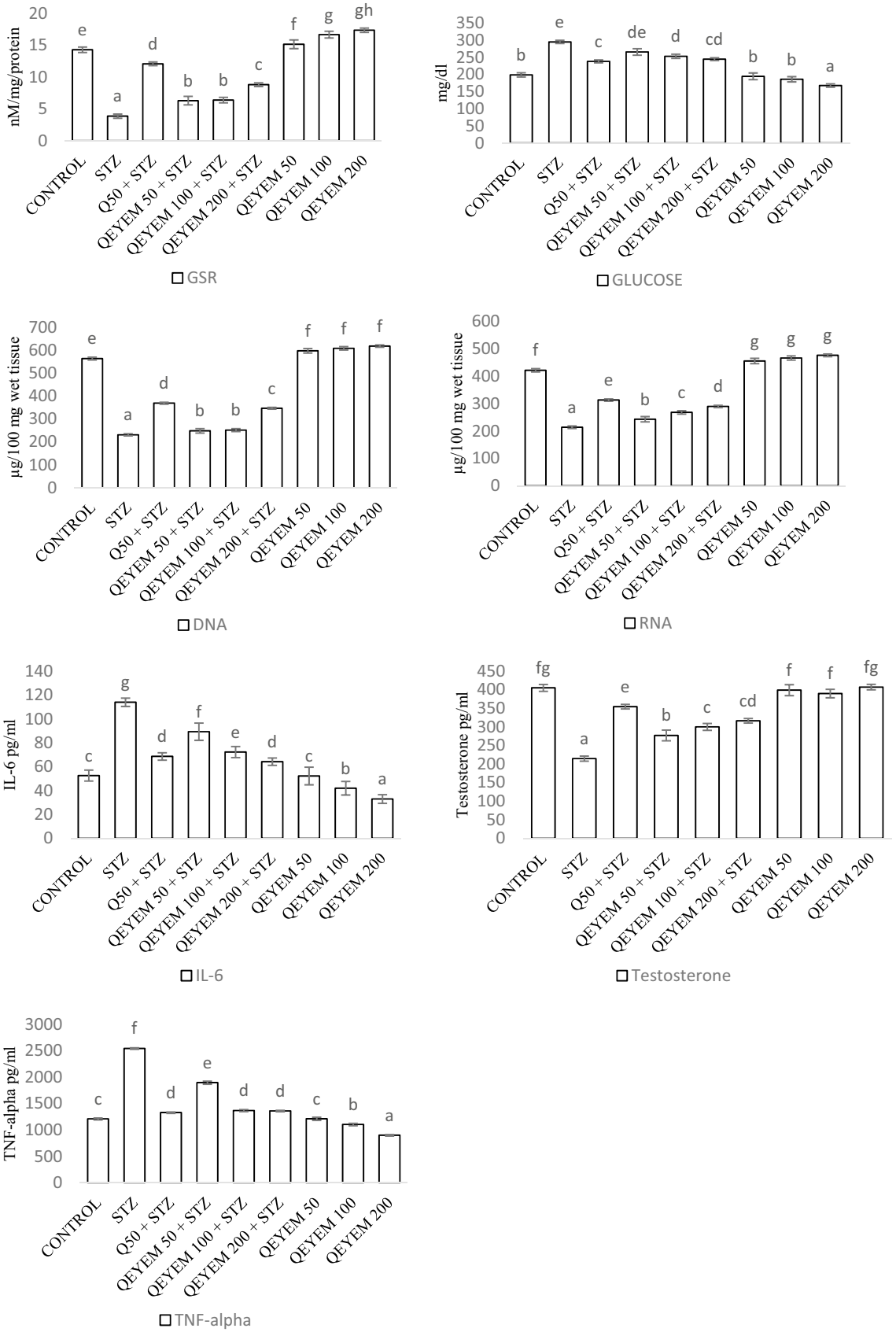

Fig. 2 (continued)

tubules. The spermatogonia cells show degeneration in most of the tubules while they are proliferating in others. The interstitial tissue is edematous and contains numerous thickened wall dilated and congested blood vessels and found no spermatis or sperms. Quail egg yolk extract (50 mg/kg/day) treatment of diabetic rats for seven consecutive weeks revealed a thin basement membrane in the seminiferous tubules with few abnormal configurations with variable size and contour (Fig. 3c). The treatment with higher doses (100 and 200 mg/kg/day) of quail egg yolk extract showed numerous seminiferous tubules, with few scattered abnormal convolutions. The spermatogonia in most of the tubules are degenerated. Some tubules show focal proliferations of the spermatogonia. Complete germinal cells with full spermatogenesis were seen.

Discussion

The vitamin E is the most prominent among the vitamins of the egg yolk with finding showing reduced vitamins A and

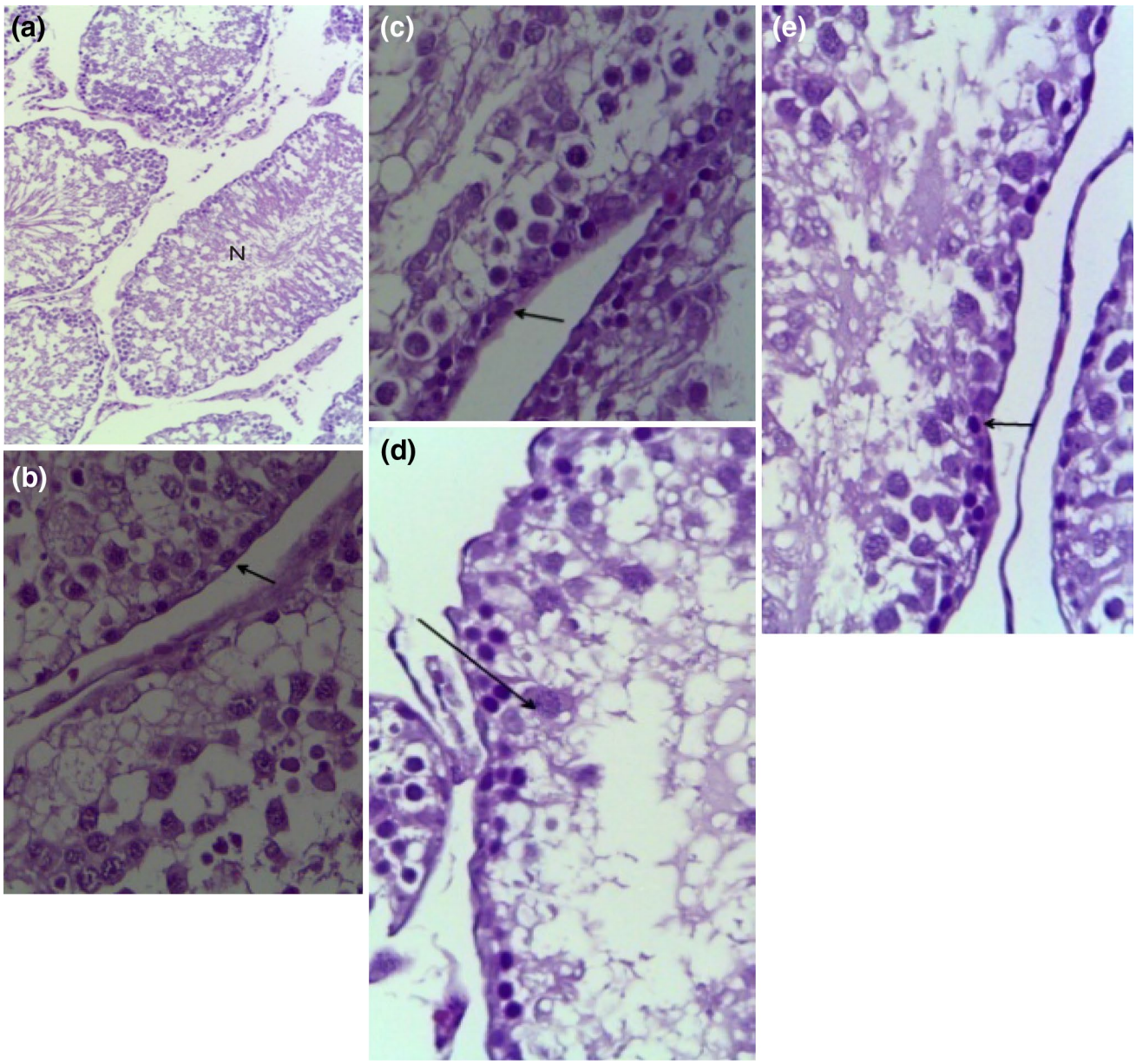

Fig. 3 Histopathology of testicular tissue under light microscopy in different groups: **a** in control, normal testicular architecture was seen, **b** in STZ-induced diabetic rats, severe testicular damage was revealed, **c** after quail egg yolk extract administration (50 mg/kg/day) treatment to diabetic rats, mild improvement in the seminiferous tubule structure was seen, and **d** in high dose (100 and 200 mg/kg/day) of quail egg yolk treated group, the testicular tissue almost look like normal

D, this was similar to findings by Tanarson et al. [18] which revealed higher concentration for vitamin E with reduced amount for A and D. Vitamin E is a fat soluble vitamin with antioxidant properties. These vitamins could be responsible for the antioxidant and the anti-inflammatory activities of the extract.

Oxidative stress may play a pathogenic role in diabetes-related male reproductive function abnormalities. Studies have shown that men suffering from diabetes have sperms with greater DNA fragmentation and an increase in advanced glycation end products and their receptors (RAGE) leading to deterioration of sperm quality, sperm functions coupled with changes in testicular metabolite levels and spermatogenic gene expression. Several studies have shown that antioxidant treatment improves glycemic index, reduces diabetic complications, and protects components from oxidative damage [2].

Spermatogenesis is a regeneration process which involves metabolic processes that generate free radical which causes male infertility through prolong effects leading to oxidative stress, results in germ cell apoptosis and subsequent hypospermatogenesis. This research study revealed the mitigating capacity of QEYEM on diabetes assault, resulting in oxidative stresses by monitoring majorly the parameters of testicular functions. The results revealed that STZ induction caused significant modifications in the testicular milieu during the period of the experiment. Serum testosterone secretion and activities of testicular antioxidant enzymes were markedly reduced as a result of the diabetic assault. There was a prominent rise in the concentration of MDA as result of lipid peroxidation induced by the activities of the free radicals generated by the progression in the diabetes state, similar effect was observed with testicular cells sulfhydryl groups, which experienced a significant decrease. These effects were reversed by the QEYEM treatment, which owing to bioactive compounds in a considerable amount, could pose as anti-oxidative to progressions of diabetes implications. Antioxidant enzyme activities including SOD, CAT and GST were significantly inhibited in testicular cells as a result of diabetic assault. The QEYEM treatment had also proved valid by enhancing the reduced activities of the enzymes. Furthermore, pro-inflammatory markers such as IL-6 and TNF-α levels were markedly elevated as a result of STZ injection. These have progressed inflammatory processes which were revealed to be inhibited by the treatment with QEYEM and quercetin [3]. Furthermore, cytotoxic effects of STZ-induced diabetes significantly decreased as DNA and RNA levels in testicular cells, resulting from proliferation by free radicals which would not stop at just lipid, carbohydrates and protein alone, but will migrate into the cell where they attacked the nuclear membrane and the composition of the nucleus compromising metabolic activities by protein metabolism disruption. The above diabetic-induced changes were significantly ameliorated by the QEYEM treatments.

It is also known that diabetes has strong correlation with oxidative stress resulting from free radicals/ROS where they act as intercellular second messengers downstream of many signaling molecules, including transcription factors (NF-jB), which mediate vascular smooth muscle cell (SMc) growth/migration and the expression of pro-inflammatory cytokines [3, 34]. These elevated pro-inflammatory cytokines possess antagonistic properties to insulin because of their ability to augment insulin receptor substrate (IRS) phosphorylation, leading to insulin resistance [3, 35, 36]. Therefore, inhibition of H_2O_2-induced NF-jB translocation in b-TC6 cells and ameliorating oxidative stress in diabetic rats explains an associative relationship between the inflammatory cytokines and DM [3].

These findings demonstrate the beneficial effects which are wielded by the antioxidant treatment on the diabetic testis during the oxidative assault. Previous reports had revealed that GSH is an intracellular antioxidant with several biological functions, such as cellular protection against oxidation, which is one of the more important GSH functions because its sulfhydryl group is a strong nucleophile that confers antioxidant protection and protects DNA, proteins and other biomolecules from ROS [37]. Increased level of endogenous antioxidant parameters implicated augmentation of the antioxidant capacity and reduced peroxidation of membrane lipids, whose principal end product is MDA, which is a marker of damage caused by oxidative stress [38].

In conclusion, these findings revealed that diabetes is related to testicular dysfunction resulting from significant oxidative stress in testes of diabetic rats. QEYEM enhanced the activities of the antioxidant enzymes, down-regulated the activities that proliferates the cells leading to MDA generation, sulfhydryl reduction and denaturation of proteins via biosynthesis of pro-inflammatory markers, leading to the inhibition of the disruption of nucleic acid biosynthesis in the testicular milieu of diabetic rats. The study had also revealed that the antioxidant and anti-inflammatory activities were contributed by the presence of fat soluble vitamins (A, D and E) that are present in physiologically amounts as well as other polar and non-polar bioactive compounds of the quail egg yolk which boost the antioxidant status, and could have variable functional properties such as scavenging of active oxygen species, inhibition of the generation of free radicals and chain breaking activity.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest among authors.

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