

## Quercetin Attenuates Testicular Damage and Oxidative Stress in Streptozotocin-induced Diabetic Rats

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**Abstract:** The present study aims to examine the influence of Quercetin (QR) in testis of Streptozotocin (STZ)-induced diabetic rats. Diabetes was induced by a single injection of STZ (65 mg/kg, ip). Quercetin (25 and 50 mg/kg/day) was treated to normal and diabetic rats for 5 weeks. In serum, glucose, testosterone, Interleukin-6 (IL-6), Interleukin-1beta (IL-1 $\beta$ ) and Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) levels were estimated and in testis tissues Thiobarbituric Acid Reactive Substances (TBARS), sulfhydryl groups, nucleic acids and Total Protein (TP) levels were estimated. Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione-S-Transferase (GST) activities were also determined in testicular cells. In penile tissue cyclic Guanosine Monophosphate (cGMP) levels were measured. Histopathological changes were evaluated in a cross-section of testis. Testosterone levels were decreased while pro-inflammatory markers were increased in diabetic rats. QR treatment to diabetic rats corrected these changes. In penile tissues cGMP content was markedly inhibited and normalized by the QR treatment. In STZ-induced diabetic rats, TBARS levels were significantly increased while T-GSH, NP-SH, DNA, RNA and TP levels were decreased and in QR treated groups showed significant inhibition in increased TBARS levels and decreased T-GSH and NP-SH levels. The inhibited activities of SOD, CAT and GST in testicular cells of diabetic rats were increased after QR treatment. The reduced levels of nucleic acids and TP in diabetic rats were markedly corrected in QR treated groups. Histopathological evaluation revealed damage in testicular cells of diabetic rats and the treatment with QR showed protection. These results suggest that, QR supplementation to STZ-induced diabetic rats for five consecutive weeks is a potentially beneficial agent to reduce testicular damage in adult diabetic rats, probably by decreasing oxidative stress.

**Keywords:** Diabetes, oxidative stress, quercetin, streptozotocin, testicular damage

### INTRODUCTION

Diabetes Mellitus (DM), a life threatening as well as life style modifying metabolic disorder is characterized by hyperglycemia or diminished insulin secretion, or both. Symptoms of DM include polyuria, polydipsia, weight loss, polyphagia and blurred vision (VanZandt *et al.*, 2004). According to the International Diabetes Federation (IDF), there were 151 million diabetic patients in 2000, this number reached to 194 million in 2003 and to 221 million in 2010 and is expected to be 334 million in 2025 (Mehuys *et al.*, 2008). DM has adverse effects on the male sexual and reproductive functions (Kanter *et al.*, 2012). Serum testosterone impairment and varying degrees of testicular lesions have also been demonstrated in Streptozotocin (STZ) induced diabetic animal models (Cai *et al.*, 2000; Baynes and Thorpe, 1999). Enhanced oxidative stress and changes in antioxidant capacity are considered to play an important role in the pathogenesis of chronic diabetes mellitus (Baynes and Thorpe, 1999). Hyperglycemia increases the inflammatory

markers' Tumor Necrosis Factor (TNF) - $\alpha$ , Interleukin (IL) -1 and IL-6 (Dhindsa *et al.*, 2004; Arya *et al.*, 2012).

Male reproductive alterations have been widely reported in diabetic-induced animal models (Mallidis *et al.*, 2009; Tsounapi *et al.*, 2012). STZ-induced diabetes in male rats resulted in atrophy of sex organs (Navarro-Casado *et al.*, 2010), changes in histoarchitecture of ventral prostate (Soudamani *et al.*, 2005), diminution in sperm count (Scarano *et al.*, 2006), along with low levels in plasma gonadotrophins (Baccetti *et al.*, 2002) and testosterone (Scarano *et al.*, 2006). 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 (Zhang *et al.*, 2010).

Antioxidants have been found beneficial to palliate DM-induced oxidative damage (Armagan *et al.*, 2006; Aybek *et al.*, 2008). Fruits and vegetables contain a vast

array of antioxidant component such as polyphenols (Potter, 1997). Flavonoids possess several physiological properties including antioxidant, antibactericidal, antiviral, anti-inflammatory, antimutagenic, anticancer and activation or inactivation of certain enzymes (Rice-Evans *et al.*, 1996). The powerful antioxidant activity of flavonoids suggests that these compounds could play a protective role in oxidative stress-mediated diseases and recent attention has focused on the potential uses of flavonoids-based drugs for the prevention and treatment of these pathologies (Kamalakkannan and Stanelly Mainzen Prince, 2006; Mirshekar *et al.*, 2010; Lu *et al.*, 2010). The antioxidant activity has been described to result from a combination of metal chelation via the ortho-dihydroxy phenolic structure and free radical-scavenging activities (Moridani *et al.*, 2003). As consequence of their polyphenolic structure, these compounds may act as hydrogen donors and are able to suppress free radical processes at three stages: the formation of superoxide ion, the generation of hydroxyl radicals in the Fenton reaction and the formation of lipid radicals (Moridani *et al.*, 2003; Lien *et al.*, 1999). They may also suppress lipid peroxidation by recycling other antioxidants, such as  $\alpha$ -tocopherol, through reduction of  $\alpha$ -tocopheroxyl radicals (Rice-Evans *et al.*, 1996). Quercetin (3, 3', 4', 5, 7-pentahydroxy flavones) is one of the most common native flavonoids occurring mainly in glycosidic forms such as rutin (5, 7, 3', 4'-OH, 3-rutinoside) (Havsteen, 1983). In Western diets, the richest sources of Quercetin glycosides are onions (347 mg/kg), apples (36 mg/kg), tea (20 mg/kg) and red wine (11 mg/kg) (Hertog *et al.*, 1993). Furthermore, quercetin is widely used in many countries as vasoprotectants and is ingredients of numerous multivitamin preparations and herbal remedies (Erlund *et al.*, 2000).

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

## MATERIALS AND METHODS

**Animals:** Adult male Wistar albino rats, weighing 250-280 g were received from experimental Animal Care Center (College of Pharmacy, King Saud University, Riyadh). All animals were maintained under controlled conditions of temperature ( $22\pm 1^\circ\text{C}$ ), humidity (50-55%) and light (12 h light/12 h dark cycle). They were acclimatized to the laboratory conditions for 7 days before the start of the experiment. Animals had free access to Purina rat chow (Manufactured by Grain Silos and Flour Mills Organization, Riyadh, Saudi Arabia) and drinking water. All experimental procedure including euthanasia was conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research (NIH Publications No. 80-23; 1996)

as well as Ethical Guidelines of the Experimental Animal Care Centre, College of Pharmacy, King Saud University (KSU), Riyadh, Kingdom Saudi Arabia (KSA).

**Materials:** Streptozotocin (N- (methyl nitroso carbamoyl) - $\alpha$ -D-glucosamine) and Quercetin was purchased from Sigma Chemical Co., USA and Riedel-de Haën Co., USA respectively. All chemicals used in the present study were highest analytical grade.

**Diabetic induction:** Experimental diabetes was induced by a single dose of STZ (55 mg/kg, i.p.) in overnight fasted rats by dissolving in freshly prepared 5 mmol/L citrate buffer, pH 4.5 (Abuhashish *et al.*, 2013). 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. Forty-eight hours after the STZ injection, animals were fasted overnight and a drop of blood samples were analyzed for glucose levels (mg/dL) by using strips on glucometer (ACCU-CHEK ACTIVE, Roche, Germany). Individual glucose levels reached above 250 mg/dL is considered as diabetic.

**Experimental design:** In control group, normal healthy rats were taken and used as vehicle (Control). Diabetic-induced rats randomly divided into three groups (six rats in each group); untreated diabetic group (STZ), quercetin (25 mg/kg/day) treated to diabetic rats (QR25 + STZ) and quercetin (50 mg/kg/day) treated to diabetic (QR50 + STZ). Vehicle and drug treatments were continued for five consecutive weeks. At the end of the treatment, animals were fasted overnight, blood samples were obtained under light anesthesia and finally they were sacrificed. Testis of each rat was dissected a small portion of it was dipped in liquid nitrogen for one minute then kept in freezer at  $-80^\circ\text{C}$  till analysis. The blood samples were allowed to stand for 30 min at room temperature and then centrifuged at 3000 rpm for 10 min to separate the serum. Serum samples were kept in a freezer at  $-20^\circ\text{C}$  till analysis. A cross-section of testis from each group was preserved in 10% formalin for histopathology.

**Serum parameters:** Serum glucose levels were estimated by using commercially available diagnostic kits (Randox Lab Limited, U.K, Human GmbH and Germany). Serum pro-inflammatory cytokines including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6) and interleukin-1 $\beta$  (IL-1 $\beta$ ) concentrations were assayed by an enzyme-linked immunosorbent assay kit (Shanghai Senxiong Science and Technology Company, China). Testosterone levels in serum were estimated by using EIA-kit (Cayman Chemicals, USA).

**Tissue parameters:** The testes were homogenized in 50 mM phosphate buffered saline (pH 7.4) by using a

glass homogenizer (Omni International, Kennesaw, GA, USA). Half of the homogenates were centrifuged at 1000 g for 10 min at 4°C to separate nuclei and unbroken cells. The pellet was discarded and a portion of supernatant was again centrifuged at 12000 g for 20 min to obtain post-mitochondrial supernatant. In homogenate, MDA, T-GSH and NP-SH levels were estimated. In post-mitochondrial supernatant, SOD, CAT and GST activities were measured.

**Estimation of cGMP in penile tissue:** A cyclic GMP Immunoassay kit (R & D Systems, USA) was used to measure cGMP in penile homogenate samples.

**Estimation of MDA levels in testis:** A Thiobarbituric Acid Reactive Substances (TBARS) assay kit (ZeptoMetrix) was used to measure the lipid peroxidation product MDA equivalent. One hundred microliters 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 nmoles MDA/mg protein using molar extinction coefficient of MDA-thiobarbituric chromophore ( $1.56 \times 10^5$  M/cm).

**Estimations of T-GSH and NP-SH levels in testis:** The concentration of T-GSH was measured using the method described by Sedlak and Lindsay (1968). 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-nitro-benzoic acid)) (DTNB). Each sample tube was centrifuged at 3000 g at room temperature for 15 min. The absorbance of the clear supernatants was measured using spectrophotometer at 412 nm in one centimeter quartz cells. For NP-SH estimation, homogenate was mixed in 15.0 mL test tubes with 4.0 mL distilled H<sub>2</sub>O 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 of Habig *et al.* (1974). 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 by using Shimadzu spectrophotometer UV-1601 and enzyme activity was calculated as nmol of CDNB conjugate formed min<sup>-1</sup> mg<sup>-1</sup> protein using molar extinction coefficient of  $9.6 \times 10^3$  M/cm.

**Estimations of SOD activity in testicular cells:** The activity of SOD in testicular cells was estimated using

the method described by Kono (1978) 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 described by Aebi (1974). 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<sub>2</sub>O<sub>2</sub>. CAT activity was estimated spectro photometrically 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 and total protein levels in testicular cells:** The method described by Bregman (1983) 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 acids extract was treated either with diphenylamine or orcinolreagent for quantification of DNA and RNA levels, respectively. The modified Lowry method by Schacterle and Pollack (1973) was used to estimate levels of total protein in testes using bovine plasma albumin as a standard.

**Histopathological examination of testis:** Testes were fixed in 10% neutral buffered formalin, embedded in paraffin wax and sectioned at 3 µm. Sections were then stained with Hematoxylin and Eosin (H&E) stain and placed in slides for light microscopic examination. Slides were evaluated by a histopathologist who was blinded to the treatment groups to avoid any kind of bias.

**Statistical analysis:** All data were expressed as mean ± Standard Deviation (S.D.). Data were statistically analyzed using one-way ANOVA followed by Student-Newman-Keuls multiple comparisons test. The differences were considered statistically significant at  $p < 0.05$ . Graph Pad prism program (version 5) was used as analyzing software.

## RESULTS

Serum glucose and testosterone levels were significantly ( $p < 0.001$ ) increased in diabetic rats compared to control animals. QR treatment with both

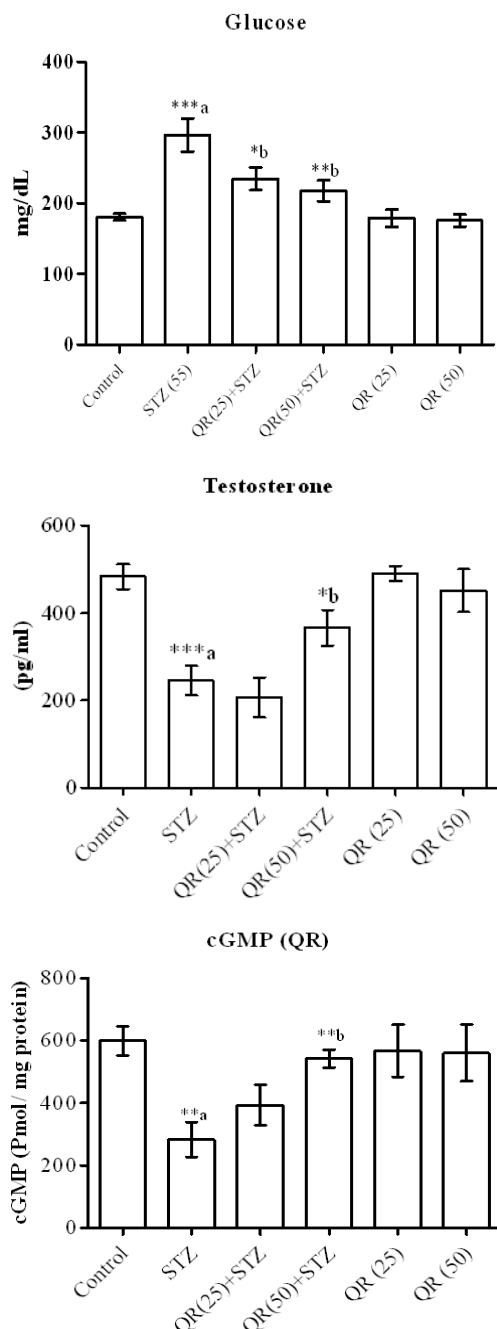


Fig. 1: Effect of quercetin on serum glucose and testosterone levels and penile cGMP levels of normal and diabetic rats

Data were expressed as Mean±S.E.M. and analyzed using one-way ANOVA followed by student-newman-keuls multiple comparisons test; \*: p<0.05; \*\*: p<0.01; \*\*\*: p<0.001 (n = 6); <sup>a</sup>: STZ, QR (25) and QR (50) groups were compared with control; <sup>b</sup>: QR (25) + STZ and QR (50) + STZ groups were compared with STZ group

the doses to diabetic rats significantly inhibited the hyperglycemic affect. Serum testosterone and penile cGMP levels in diabetic rats were significantly

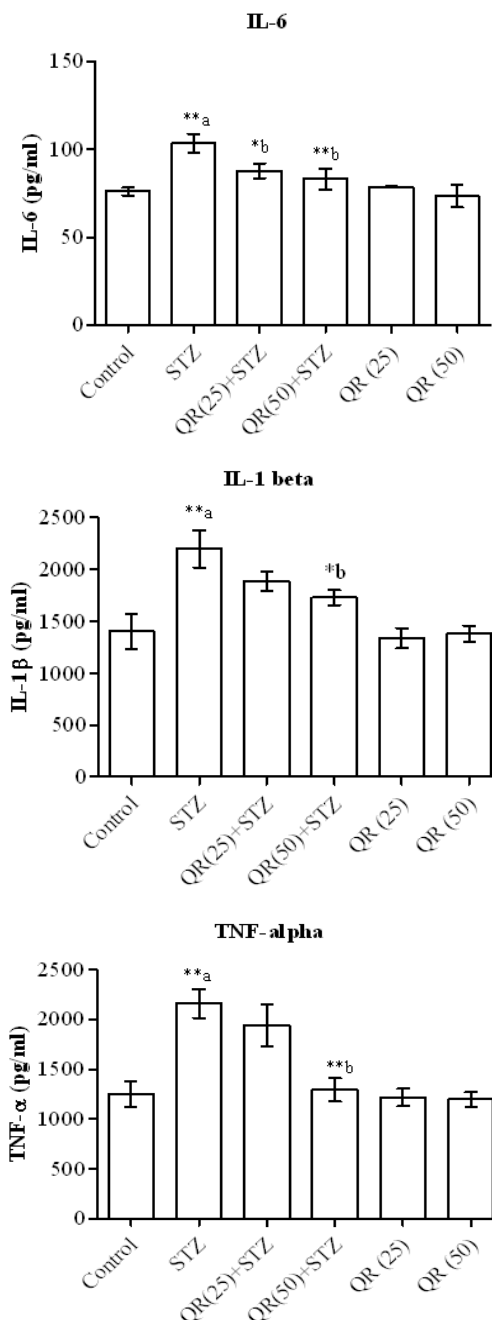


Fig. 2: Effect of quercetin on serum levels of IL-6, IL-1β and TNF-α of normal and diabetic rats

Data were expressed as Mean±S.E.M. and analyzed using one-way ANOVA followed by Student-Newman-Keuls multiple comparisons test; \*: p<0.05, \*\*: p<0.01; \*\*\*: p<0.001 (n = 6); <sup>a</sup>: STZ, QR (25) and QR (50) groups were compared with control; <sup>b</sup>: QR (25) + STZ and QR (50) + STZ groups were compared with STZ group

inhibited compared to controls. Treatment with QR (50 mg/kg/day) to diabetic rats for 5 weeks significantly enhanced to testosterone and cGMP levels compared to their respective controls (Fig. 1).

Pro-inflammatory biomarkers (IL-6, IL-1 $\beta$  and TNF- $\alpha$ ) in serum of diabetic rats were markedly ( $p < 0.01$ ) increased compared to control animals. These markers were significantly ( $p < 0.05$ ) inhibited after 5 weeks QR (50 mg/kg/day) treatment to diabetic rats (Fig. 2).

In diabetic rats, TBARS levels were significantly ( $p < 0.01$ ) increased while T-GSH ( $p < 0.05$ ) and NP-SH ( $p < 0.01$ ) levels decreased when compared to control animals. Treatment with higher dose (50 mg/kg/day) of QR to diabetic rats for 5 weeks significantly ( $p < 0.01$ ) inhibited the testicular TBARS levels compared to untreated diabetic rats. The reduced T-GSH and NP-SH levels in diabetic rats were significantly ( $p < 0.05$ ) enhanced after 5 weeks QR (50 mg/kg/day) treatment to diabetic rats (Fig. 3).

Enzymatic activities including SOD, CAT and GST in testicular cells were significantly diminished in

diabetic rats as compared to control animals. QR treatment with higher dose to diabetic rats significantly enhanced the activities of SOD, CAT and GST in testicular cells compared to untreated diabetic animals (Fig. 4).

In testicular cells of STZ-induced diabetic rats, total protein and nucleic acid (DNA and RNA) levels were significantly inhibited compared to control rats. The treatment with higher taken dose to diabetic rats was significantly elevated the total protein, DNA and RNA levels when compared to untreated diabetic animals (Fig. 5).

In control rat, plate (a) in Fig. 6, shows normal testicular cells of rat, highlighting complete germinal cells with full spermatogenesis. In STZ-induced diabetic rat, plate (b) in Fig. 6, shows marked thickening of the basement membrane and abnormal configurations with elongation of the tubules. They are

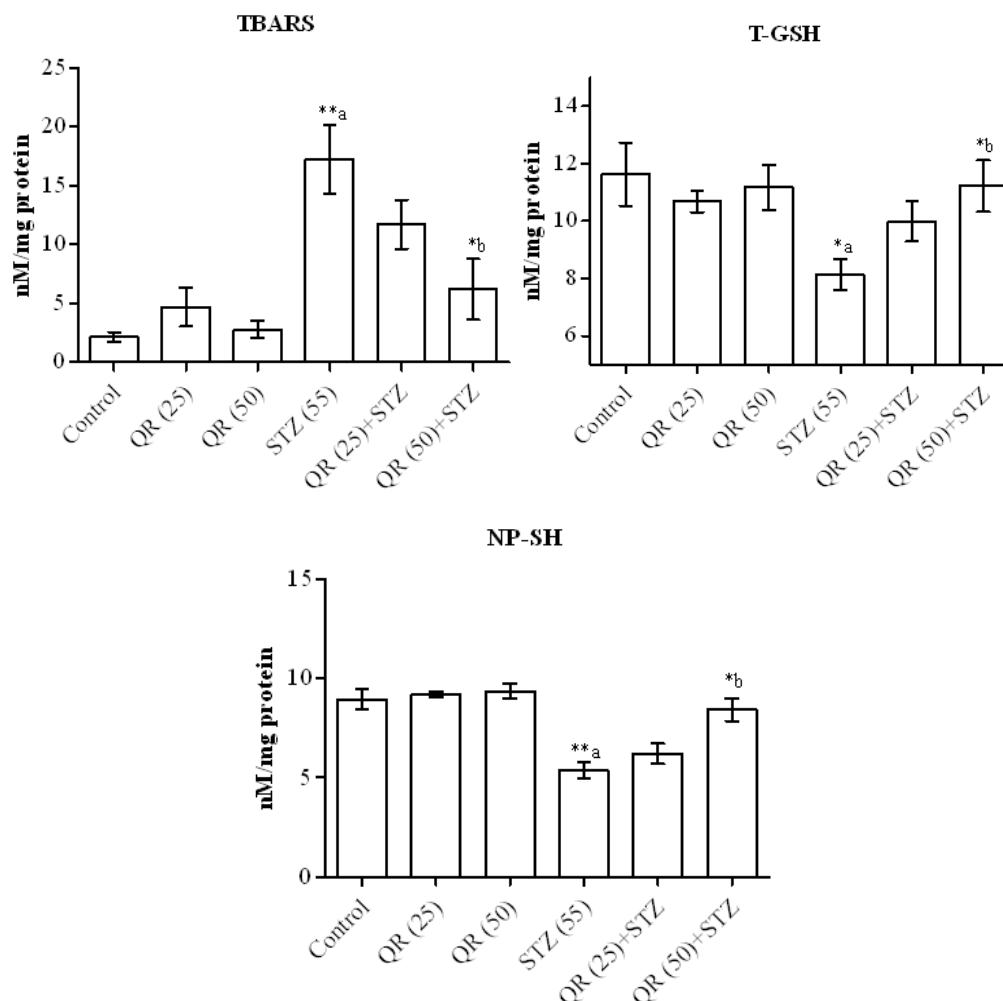


Fig. 3: Effect of quercetin on TBARS, T-GSH and NP-SH levels of testes in diabetic rats

Data were expressed as Mean $\pm$ S.E.M. and analyzed using one-way ANOVA followed by student-newman-keuls multiple comparisons test; \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$  ( $n = 6$ ); <sup>a</sup>: STZ, QR (25) and QR (50) groups were compared with control; <sup>b</sup>: QR (25) + STZ and QR (50) + STZ groups were compared with STZ group

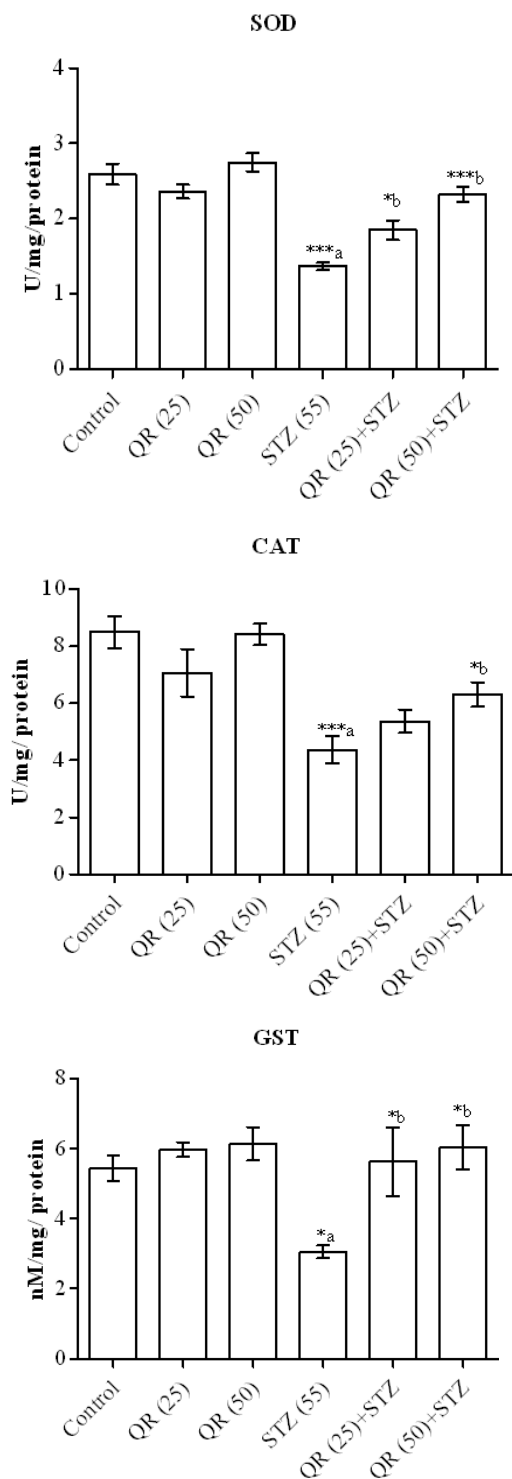


Fig. 4: Effect of quercetin on SOD, CAT and GST activity of testes in diabetic rats

Data were expressed as Mean±S.E.M. and analyzed using one-way ANOVA followed by Student-Newman-Keuls multiple comparisons test; \*: p<0.05; \*\*: p<0.01; \*\*\*: p<0.001 (n = 6); <sup>a</sup>: STZ, QR (25) and QR (50) groups were compared with control; <sup>b</sup>: QR (25) + STZ and QR (50) + STZ groups were compared with STZ group

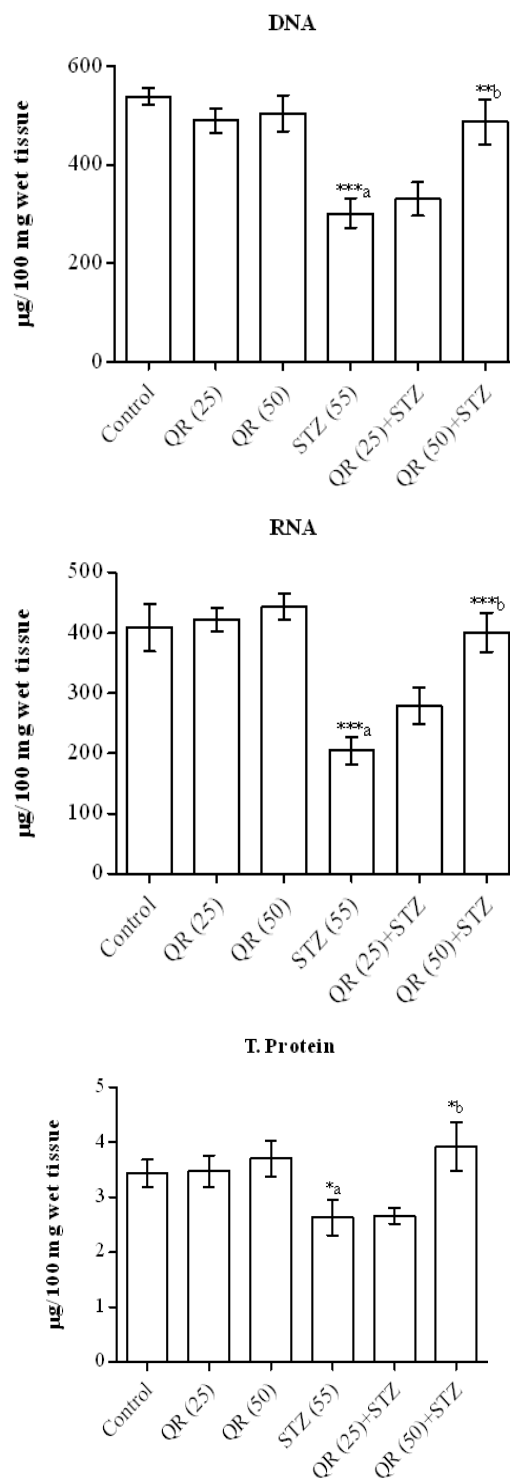


Fig. 5: Effect of quercetin on DNA, RNA and total protein levels of testes in diabetic rats

Data were expressed as Mean±S.E.M. and analyzed using one-way ANOVA followed by student-newman-keuls multiple comparisons test; \*: p<0.05; \*\*: p<0.01; \*\*\*: p<0.001 (n = 6); <sup>a</sup>: STZ, QR (25) and QR (50) groups were compared with control; <sup>b</sup>: QR (25) + STZ and QR (50) + STZ groups were compared with STZ group



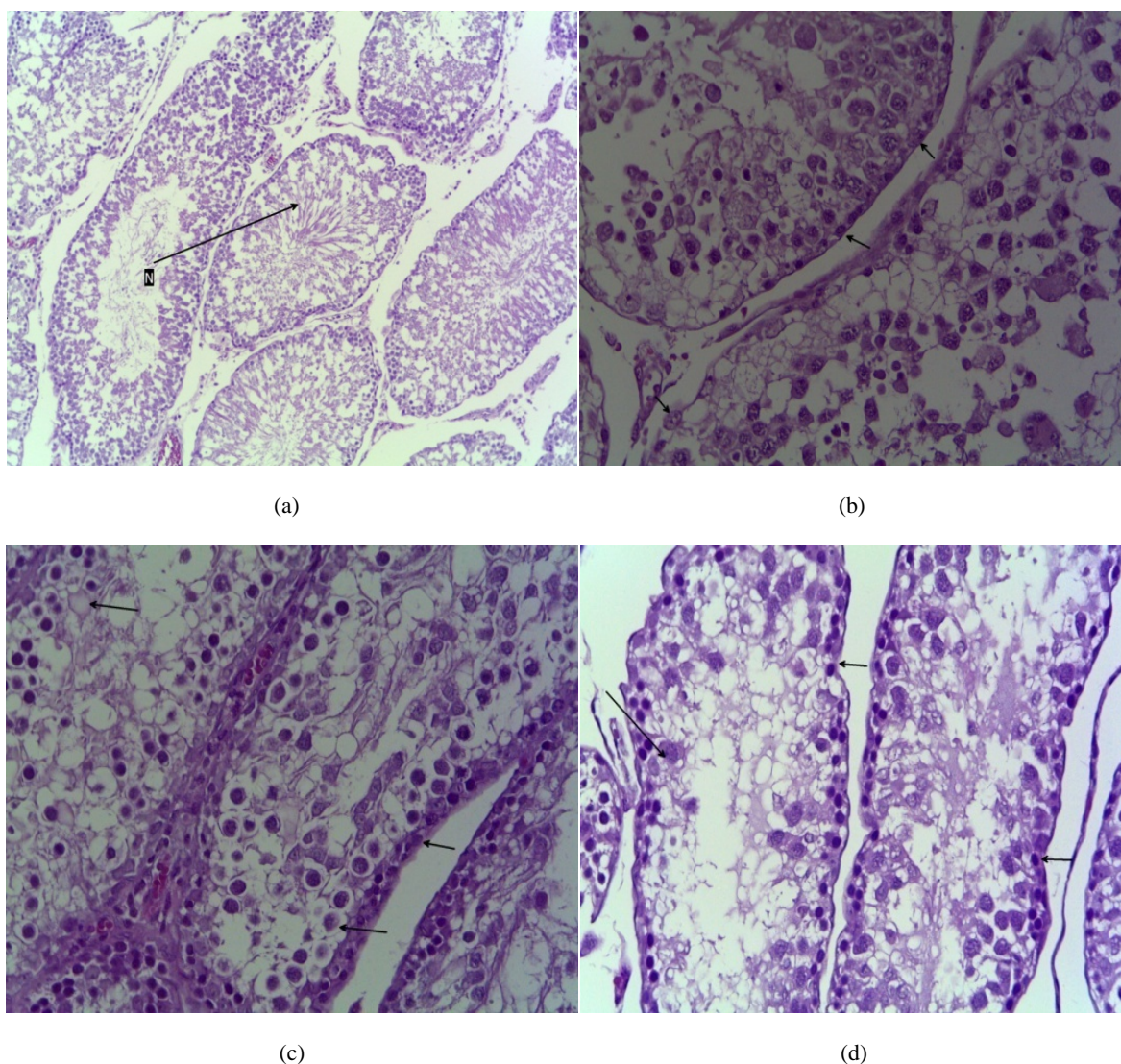


Fig. 6: Light microscopy of testicular tissue in different groups, (a) in control, normal testicular architecture was seen, (b) in STZ-induced diabetic rats, severe testicular damage was noted, (c) after QR (25 mg/kg/day) treatment to diabetic rats, mild improvement in the seminiferous tubule structure was seen and (d) in high dose (50 mg/kg/day) of QR treated group, the testicular tissue almost look like normal

variable in size and contour. The spermatogonia cells show degeneration in most of the tubules while they are proliferating in others. The maturation is up to primary spermatogonia. The interstitial tissue is edematous and contains numerous thickened wall dilated and congested blood vessels and found no spermatids or sperms. QR (25 mg/kg/day) treatment to diabetic rats for 5 consecutive weeks shows the histopathological changes in Fig. 6c. It observed numerous seminiferous tubules with thin basement membrane, abnormal configuration are few and the tubules are slightly variable in size and contour. They are lined by spermatogonia cells up to primary spermatogonia. No significant degeneration was seen and the sertoli cells were present. Spermatids and sperms were seen. The interstitial tissue shows no edema or congestion. The

treatment with higher dose (50 mg/kg/day) of QR shows 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 (Fig. 6d).

## DISCUSSION

Present study was designed to elucidate the relationship between diabetic-induced oxidative stresses by following the parameters related to testicular function in the rats. Our results revealed that STZ-injection causes significant alterations in the male reproductive milieu during the 5-weeks diabetic period

by markedly inhibiting serum testosterone and penile cGMP levels. Diabetes induced significant oxidative impairments as evidenced by LPO biomarkers such as MDA levels increased and sulfhydryl groups decreased in the testicular cells. Antioxidant enzyme activities including SOD, CAT and GST were significantly inhibited in testicular cells of diabetic rats and in contrast, pro-inflammatory biomarker like IL-6, IL-1 $\beta$  and TNF- $\alpha$  level were markedly elevated. Furthermore, cytotoxic effects of STZ-induced diabetes showed significantly decreased as DNA and RNA levels in testicular cells. The above diabetic-induced changes were significantly ameliorated by the QR treatment.

Present data revealed the significant decreased in serum testosterone levels of diabetic rats compared to control animals. Treatment with QR (50 mg/kg/day) to diabetic rats for 5 consecutive weeks corrected the testosterone levels compared to untreated diabetic rats. These results are in agreement with earlier reports that, QR treatment enhanced the testosterone levels in diabetic animals (Khaki *et al.*, 2010; Tang *et al.*, 2008). cGMP levels in penile tissues of diabetic rats were significantly inhibited in present study as compared to control animals. In earlier experimentally-induced diabetic animals, similar inhibition of cGMP levels was observed in penile tissue (Yang *et al.*, 2008, 2013). Flavonoids are known to improve erectile function by their antioxidant and anti-inflammatory properties (Zhang *et al.*, 2013; Yang *et al.*, 2012). In present study, treatment with QR to diabetic rats improved the cGMP levels in penile tissue as compared to untreated diabetic rats, this may justify by its pharmacological potentials.

Several researchers showed the oxidative impairments in the male reproductive system following experimental studies (Agbaje *et al.*, 2007; Shrilatha, 2007; Amaral *et al.*, 2006) however the knowledge is still inadequate. Elevated LPO biomarker like MDA in the testicular milieu is demonstrated to have fundamental implications on testicular physiology and sperm function (Agarwal and Said, 2005). 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- $\kappa$ B), which mediate vascular Smooth Muscle cell (SMC) growth/migration and the expression of pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$  and IL-6 (Touyz, 2004). 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 (Emanuelli *et al.*, 2000; Senn *et al.*, 2003). Therefore, inhibition of H<sub>2</sub>O<sub>2</sub>-induced NF- $\kappa$ B translocation in b-TC6 cells and ameliorating oxidative stress in diabetic rats explains an associative relationship between the inflammatory cytokines and DM. In present study, it is shown that the elevated

levels of TNF- $\alpha$ , IL-1 $\beta$  and IL-6 in the serum significantly inhibited in QR treated diabetic rats, suggesting beneficial anti-inflammatory effect of QR on insulin resistance in DM.

Present data showed the significant increase in estimated LPO biomarker as MDA in testicular cells of untreated diabetic rats. Our results are consistent with increased cellular oxidative stress and accumulation of lipid peroxides and MDA levels demonstrated in experimental type-I diabetes mellitus (Zhao *et al.*, 2010). Our QR treatment to diabetic rats showed significant decrease the elevated testicular MDA levels compared to the untreated diabetic group. These findings demonstrate the beneficial effects which are wielded by the antioxidant treatment on the diabetic testis during the oxidative insult. It is well documented 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 (Fang *et al.*, 2002). In this regard, an increased level of GSH implicates 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 (Pastore *et al.*, 2003).

In conclusion, present data revealed that the hyperglycemia leads to testicular dysfunction involving significant oxidative stress in testes of experimentally-induced diabetic rats. QR rehabilitated the activities of the antioxidant enzymes, down-regulated the levels of LPO biomarker MDA and inhibiting the nucleic acids levels in the testis of diabetic rats.

## REFERENCES

- Abuhashish, H.M., S.S. Al-Rejaie, K.A. Al-Hosaini, M.Y. Parmar and M.M. Ahmed, 2013. Alleviating effects of morin against experimentally-induced diabetic osteopenia. *Diabetol. Metab. Syndr.*, 5: 1-8.
- Aebi, H., 1974. Catalase. In: Bergmeyer, H.U. (Ed.), *Methods in Enzymatic Analysis*. Academic Press, New York, pp: 674-684.
- Agarwal, A. and T.M. Said, 2005. Oxidative stress, DNA damage and apoptosis in male infertility: A clinical approach. *BJU Int.*, 95: 503-507.
- Agbaje, I.M., D.A. Rogers, C.M. McVicar, N. McClure, A.B. Atkinson, C. Mallidis and S.E. Lewis, 2007. Insulin dependant diabetes mellitus: implications for male reproductive function. *Hum. Reprod.*, 22: 1871-1877.
- Amaral, S., A.J. Moreno, M.S. Santos, R. Seica and J. Ramalho-Santos, 2006. Effects of hyperglycemia on sperm and testicular cells of Goto-Kakizaki and streptozotocin-treated rat models for diabetes. *Theriogenol.*, 66: 2056-2067.



- Armagan, A., E. Uz, H.R. Yilmaz, S. Soyupek, T. Oksay and N. Ozcelik, 2006. Effects of melatonin on lipid peroxidation and antioxidantenzymes in streptozotocin-induced diabetic rat testis. *Asian J. Androl.*, 8: 595-600.
- Arya, A., S.C. Cheah, C.Y. Looi, H. Taha, M.R. Mustafa and M.A. Mohd, 2012. The methanolic fraction of *Centratherum anthelminticum* seed downregulates pro-inflammatory cytokines, oxidative stress and hyperglycemia in STZ-nicotinamide-induced type 2 diabetic rats. *Food Chem. Toxicol.*, 50: 4209-4220.
- Aybek, H., Z. Aybek, S. Rota, N. Sen and M. Akbulut, 2008. The effects of diabetes mellitus, age and vitamin E on testicular oxidative stress. *Fertil. Steril.*, 90: 755-760.
- Baccetti, B., A. La Marca, P. Piomboni, S. Capitani, E. Bruni, F. Petraglia and V. De Leo, 2002. Insulin-dependent diabetes in men is associated with hypothalamo-pituitary derangement and with impairment in semen quality. *Hum. Reprod.*, 17: 2673-2677.
- Baynes, J.W. and S.R. Thorpe, 1999. Role of oxidative stress in diabetic complications: A new perspective on an old paradigm. *Diabetes*, 48: 1-9.
- Bregman, A.A., 1983. *Laboratory Investigation and Cell Biology*. John Wiley and Sons, New York, pp: 51-60.
- Cai, L., S. Chen, T. Evans, D.X. Deng, K. Mukherjee and S. Chakrabarti, 2000. Apoptotic germcell death and testicular damage in experimental diabetes: Prevention by endothelin antagonism. *Urol. Res.*, 28: 342-347.
- Dhindsa, S., D. Tripathy, P. Mohanty, H. Ghanim, T. Syed, A. Aljada and P. Dandona, 2004. Differential effects of glucose and alcohol on reactive oxygen species generation and intranuclear nuclear factor-kappaB in mononuclear cells. *Metabolism*, 53(3): 330-334.
- Emanuelli, B., P. Peraldi, C. Filloux, D. Sawka-Verhelle, D. Hilton and E. Van Obberghen, 2000. SOCS-3 is an insulin-induced negative regulator of insulin signaling. *J. Biol. Chem.*, 275: 15985-15991.
- Erlund, I., T. Kosonen, G. Alfthan, J. Mäenpää, K. Perttunen, J. Kenraali, J. Parantainen and A. Aro, 2000. Pharmacokinetics of quercetin from quercetin aglycone and rutin in healthy volunteers. *Eur. J. Clin. Pharmacol.*, 56: 545-553.
- Fang, Y., S. Yang and G. Wu, 2002. Free radicals, antioxidants and nutrition. *Nutrition*, 18: 872-879.
- Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.*, 249: 7130-7139.
- Havsteen, B., 1983. Flavonoids: A class of natural products of high pharmacological potency. *Biochem. Pharmacol.*, 32: 1141-1148.
- Hertog, M.G., P.C. Hollman, M.B. Katan and D. Kromhout, 1993. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands. *Nutr. Cancer*, 20: 21-29.
- Kamalakkannan, N. and P. Stanely Mainzen Prince, 2006. Rutin improves the antioxidant status in streptozotocin-induced diabetic rat tissues. *Mol. Cell. Biochem.*, 293: 211-219.
- Kanter, M., C. Aktas and M. Erboğa, 2012. Protective effects of quercetin against apoptosis and oxidative stress in streptozotocin-induced diabetic rat testis. *Food Chem. Toxicol.*, 50: 719-725.
- Khaki, A., F. Fathiazad, M. Nouri, A.F. Khaki, N.A. Maleki, H.J. Khamnei and P. Ahmadi, 2010. Beneficial effects of quercetin on sperm parameters in streptozotocin-induced diabetic male rats. *Phytother. Res.*, 24: 1285-1291.
- Kono, Y., 1978. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. *Arch. Biochem. Biophys.*, 186: 189-195.
- Lien, E.J., S. Ren, H.H. Bui and R. Wang, 1999. Quantitative structure-activity relationship analysis of phenolic antioxidants. *Free Radical Bio. Med.*, 26: 285-294.
- Lu, Y.X., Q. Zhang, J. Li, Y.X. Sun, L.Y. Wang, W.M. Cheng and X.Y. Hu, 2010. Antidiabetic effects of total flavonoids from *Litsea Coreana* leave on fat-fed, streptozotocin-induced type 2 diabetic rats. *Am. J. Chin. Med.*, 38: 713-725.
- Mallidis, C., I. Agbaje, J. O'Neill and N. McClure, 2009. The influence of type 1 diabetes mellitus on spermatogenic gene expression. *Fertil. Steril.*, 92: 2085-2087.
- Mehuys, E., L. De Bolle, L. Van Bortel, L. Annemans, I. Van Tongelen, J.P. Remon and M. Giri, 2008. Medication use and disease management of type 2 diabetes in Belgium. *Pharm. World Sci.*, 30: 51-56.
- Mirshekar, M., M. Roghani, M. Khalili, T. Baluchnejadmojarad and S. Arab Moazzen, 2010. Chronic oral pelargonidin alleviates streptozotocin-induced diabetic neuropathic hyperalgesia in rat: Involvement of oxidative stress. *Iran Biomed. J.*, 14: 33-39.
- Moridani, M.Y., J. Pourahmad, H. Bui, A. Siraki and P.J. O'Brien, 2003. Dietary flavonoid iron complexes as cytoprotective superoxide radical scavengers. *Free Radical Bio. Med.*, 34: 243-253.
- Navarro-Casado, L., M.A. Juncos-Tobarra, M. Chafer-Rudilla, L.I. de Onzono, J.A. Blazquez-Cabrera and J.M. Miralles-Garcia, 2010. Effect of experimental diabetes and STZ on male fertility capacity. Study in rats. *J. Androl.*, 31: 584-592.
- Pastore, A., G. Federici, E. Bertini and F. Piemonte, 2003. Analysis of glutathione: implication in redox and detoxification. *Clin. Chim. Acta*, 333: 19-39.

- Potter, J.D., 1997. Cancer prevention: Epidemiology and experiment. *Cancer Lett.*, 114: 7-9.
- Rice-Evans, C.A., N.J. Miller and G. Paganga, 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Bio. Med.*, 20: 933-956.
- Scarano, W.R., A.G. Messias, S.U. Oliva, G.R. Klinefelter and W.G. Kempinas, 2006. Sexual behaviour, sperm quantity and quality after short-term streptozotocin-induced hyperglycaemia in rats. *Int. J. Androl.*, 29: 482-488.
- Sedlak, J. and R.H. Lindsay, 1968. Estimation of total, protein-bound and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal. Biochem.*, 25: 192-205.
- Senn, J.J., P.J. Klover, I.A. Nowak, T.A. Zimmers, L.G. Koniaris, R.W. Furlanetto and R.A. Mooney, 2003. Suppressor of cytokine signaling-3 (SOCS-3): A potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. *J. Biol. Chem.*, 278: 13740-13746.
- Shrilatha, B., 2007. Early oxidative stress in testis and epididymal sperm in streptozotocin-induced diabetic mice: Its progression and genotoxic consequences. *Reprod. Toxicol.*, 23: 578-587.
- Soudamani, S., S. Yuvaraj, T. Malini and K. Balasubramanian, 2005. Experimental diabetes has adverse effects on the differentiation of ventral prostate during sexual maturation of rats. *Anat. Rec. Part A*, 287: 1281-1289.
- Tang, X.Y., Q. Zhang, D.Z. Dai, H.J. Ying, Q.J. Wang and Y. Dai, 2008. Effects of strontium fructose 1,6-diphosphate on expression of apoptosis-related genes and oxidative stress in testes of diabetic rats. *Int. J. Urol.*, 15: 251-256.
- Touyz, R.M., 2004. Reactive oxygen species, vascular oxidative stress and redox signaling in hypertension: What is the clinical significance? *Hypertension*, 44: 248-252.
- Tsounapi, P., M. Saito, F. Dimitriadis, S. Koukos, S. Shimizu and K. Satoh, 2012. Antioxidant treatment with edaravone or taurine ameliorates diabetes-induced testicular dysfunction in the rat. *Mol. Cell. Biochem.*, 369: 195-204.
- VanZandt, M.C., E.O. Sibley, E.E. McCann, K.J. Combs, B. Flam, D.R. Sawicki, A. Sabetta, A. Carrington, J. Sredy, E. Howard, A. Mitschler and A.D. Podjarny, 2004. Design and synthesis of highly potent and selective (2-arylcarbamoyl-phenoxy)-acetic acid inhibitors of aldose reductase for treatment of chronic diabetic complications. *Bioorgan. Med. Chem.*, 12: 5661-5675.
- Yang, Y., X. Gao, X. Wang, L. Su and H. Xing, 2012. Total flavonoids of fructus chorspondiatis inhibits collagen synthesis of cultured rat cardiac fibroblasts induced by angiotensin II: Correlated with NO/cGMP signaling pathway. *Eur. J. Pharm. Sci.*, 47: 75-83.
- Yang, R., J. Wang, Y. Chen, Z. Sun, R. Wang and Y. Dai, 2008. Effect of caffeine on erectile function via up-regulating cavernous cyclic guanosine monophosphate in diabetic rats. *J. Androl.*, 29: 586-591.
- Yang, J., T. Wang, J. Yang, K. Rao, Y. Zhan, R.B. Chen, Z. Liu, M.C. Li, L. Zhuan, G.H. Zang, S.M. Guo, H. Xu, S.G. Wang, J.H. Liu, Z.Q. Ye, 2013. S-allyl cysteine restores erectile function through inhibition of reactive oxygen species generation in diabetic rats. *Andrology*, 1: 487-494.
- Zhang, J., A.M. Li, B.X. Liu, F. Han, F. Liu, S.P. Sun, X. Li, S.J. Cui, S.Z. Xian, G.Q. Kong, Z.C. Xin and Z.L. Ji, 2013. Effect of icarid II on diabetic rats with erectile dysfunction and its potential mechanism via assessment of AGEs, autophagy, mTOR and the NO-cGMP pathway. *Asian J. Androl.*, 15: 143-148.
- Zhao, H., S. Xu, Z. Wang, Y. Li, W. Guo, C. Lin, S. Gong, C. Li, G. Wang and L. Cai, 2010. Repetitive exposures to low-dose X-rays attenuate testicular apoptotic cell death in streptozotocin-induced diabetes rats. *Toxicol. Lett.*, 192: 356-364.