Journal of Animal and Veterinary Advances 12 (6): 747-753, 2013
ISSN: 1680-5593

Effects of Resveratrol on Testis Damage in Streptozotocin Induced Diabetic Rats

[1]Esin Yulug, [1]Sibel Tured, [2]Ahmet Alver, [3]Omer Kutlu,
[3]Ersagun Karaguzel and [2]Cemil Kahraman
[1]Department of Histology and Embryology,
[2]Department of Medical Biochemistry, [3]Department of Urology,
Faculty of Medicine, Karadeniz Technical University, 61080 Trabzon, Turkey

Abstract: Oxidative stress is known to play an important role in the pathogenesis of diabetes. Resveratrol has antioxidant and antidiabetic effects. This study purposed to evaluate the protective effect of resveratrol against testicular injury in streptozotocin-induced diabetic rats. Twenty four male Sprague Dawley rats were randomly divided into four groups: control, Resveratrol (RSV), Diabetes (DM) and DM+RSV groups. DM group was induced by a single intraperitoneal (i.p.) injection of streptozotocin (60 mg kg^{-1}). RSV and DM+RSV groups were administered 20 mg kg^{-1} resveratrol i.p. per day. All rats were sacrificed at the end of the 14th day. Testis tissues and blood samples were collected for biochemical and histopathological examination. At the end of the 1st and 2nd weeks, blood sugar levels rose significantly in the DM and DM+RSV groups. These levels also decreased significantly in the DM+RSV group compared to the DM group. Tissue malondialdehyde levels rose significantly in the DM and DM+RSV groups, compared to the control and RSV groups and decreased in the DM+RSV group compared to the DM group but this decrease was not significant. According to Johnsen's tubular biopsy scoring, spermatogenesis decreased in the DM group, increased in the DM+RSV group compared to the DM group significantly. Apoptotic index in seminiferous tubule cells increased in the DM and DM+RSV groups compared to the control group and decreased in the DM+RSV group compared to the DM group significantly. The findings show that DM increases oxidative stress and testicular damage. RSV protects DM-induced oxidative testicular damage at the histopathological and apoptotic level, in particular by reducing blood sugar levels.

Key words: Diabetes mellitus, resveratrol, apoptosis, testis, rat

INTRODUCTION

Diabetes Mellitus (DM) is a common metabolic disease associated with severe functional and physiological complications (Sadik *et al*., 2011). DM is characterized by high blood glucose levels due to defects of the release of insulin from the a cells of the islets of Langerhans in the pancreas or insulin hormone sensitivity or defects in both (Eiselein *et al*., 2004). Increase of blood glucose levels causes structural and functional changes in various target tissues and organs (Kianifard *et al*., 2012). DM has side-effects on male reproductive functions in diabetic patients and animal models (Sainio-Pollanen *et al*., 1997; Meyer *et al*., 2000; Kanter *et al*., 2012; Kianifard *et al*., 2012). Decreases in testis and body weights, a rise in abnormal spermatogenesis with a decrease in sperm numbers have been observed in studies on diabetic rats. Also, vacuolization in Sertoli cells and a rise in apoptotic cells in germ cells (particularly spermatogonia and spermatocytes) in the seminiferous tubules have been reported in diabetic rats (Kanter *et al*., 2012; Sainio-Pollanen *et al*., 1997; Amaral *et al*., 2006). Although, the mechanism has not been fully explained increased blood sugar affecting oxidative defense mechanisms and increasing oxidative stress are thought to play a significant role in the pathogenesis (Armagan *et al*., 2006; Ricci *et al*., 2009).

Resveratrol (3,5,4'-trihydroxy-trans-stilbene) (RSV) is a natural phytoalexin (Jeandet *et al*., 1991; Oktem *et al*., 2010) which has been found in at least 72 plant species especially in grape (Jeandet *et al*., 1991). Researches has shown that RSV has anti-inflammatory, anti-oxidant and anti-diabetic properties (Su *et al*., 2006; Collodel *et al*., 2011). One study investigating the effects of RSV in diabetes reported that it reduced plasma glucose concentrations and at the same time had improving effects on various symptoms of diabetes (such as a fall in body

Corresponding Author: Esin Yulug, Department of Histology and Embryology, Faculty of Medicine, Karadeniz Technical University, 61080 Trabzon, Turkey

weight, polydipsia and polyphagia (Su *et al*., 2006). RSV's lipid peroxidation reducing effect has been shown to be more potent that of other phenols such as epicatechin, catechin and quercetin (Shingai *et al*., 2011). RSV has been also shown to reduce DNA breaks with its scavenging of hydroxyl radicals. Another study showed that it significantly reduced germ cell apoptosis in the testis (Uguralp *et al*., 2005). On the basis of this information, researchers hypothesized that RSV may cause lower plasma glucose concentrations and reduce testis injury with its antioxidant property. Researchers encountered no studies in the literature investigating the effects of RSV on testis injury in Streptozotocin (STZ) induced diabetes.

In the present study, researchers aimed to investigate the effect of DM on rat testicular tissue and to determine the effects of resveratrol in STZ induced diabetic rat testes.

MATERIALS AND METHODS

Animals and experimentals design: This was a randomized, controlled animal study. All animals received human care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. This experiment was approved by the Karadeniz Technical University Animal Care and Ethical Committee. All animals were kept at room temperature (22±2°C) in a controlled 12/12 h light/dark cycle. Standard laboratory animal chow and water were givenduring the whole period of experiment. Twenty four male Spraque Dawley rats (6 weeks old) were randomly divided into four groups.

Control group (n = 6): A single intraperitoneal (i.p.) injection of 0.1 molar citrate buffer solution (pH 4.5) was applied.

RSV group (n = 6): The 20 mg kg^{-1} RSV (R5010-500 mg, Sigma-Aldrich, St. Louis, MO, USA) was administered i.p. every day throughout the course of the experiment (14 days).

STZ-induced DM group (n = 6): Diabetes was induced by a single i.p. injection of STZ (S0130-500MG, Sigma-Aldrich, Co., St. Louis, USA) (60 mg kg^{-1}, freshly dissolved in 0.1 molar citrate buffer pH 4.5). The 48 h after administration, rats were fasted for 18 h and their blood sugar levels were measured by tail blood using a glucometer (Optium Xceed, Abbott, UK). Rats with blood sugar levels above 250 mg dL^{-1} were regarded as having DM.

DM+RSV group (n = 6): The 20 mg kg^{-1} RSV was administered i.p. every day (14 days) of the experiment to STZ-induced DM rats.

Fasting blood sugar levels of rats were measured at the end of the 1st and 2nd weeks following induction of diabetes. At the end of the 14th day the weight of each animal was recorded, a midline incision was performed from the level of the genital swelling under 50 mg kg^{-1} ketamine hydrochloride (Ketalar, Pfizer, Turkey) anesthesia and the right testes were extracted. Each testis was divided in two pieces. One half was fixed in Bouin's solution for histopathological examination. The other half of each testis was placed in an Eppendorf tube for biochemical examination and stored at -20°C.

Determination of tissue MDA: A piece of testis tissue was used to measure Malondialdehyde (MDA) levels. The samples were minced and homogenized in an ice-cold 1. The 15% KCl solution containing 0.50 mL L^{-1} Triton X-100 using an Ultra-Turrax T25 homogenizer. MDA levels in testis samples was determined as MDA concentration by the method of Uchiyama and Mihara (1978). Absorbance of the organic layer was evaluated at 532 nm. Tetramethoxypropane was used as a standard and MDA levels were assessed as nanomoles per gram wet tissue.

Histopathological analysis: Testis tissues fixed in Bouin's solution. For histopathological examination tissues were embedded in paraffin blocks after routine preparation procedures. Sections 4 μm in thickness were taken from the paraffin blocks and stained with Haematoxylin-Eosin (H&E). Testis tissue from each group was assessed under a light microscope (Olympus BX-51; Olympus, Tokyo, Japan). Seminiferous tubule diameter and thickness of the germinal epithelium were measured using the Analysis 5 Research Program (Olympus Soft Imaging Solutions, Münster, Germany). Spermatogenic cells in the seminiferous tubule and the interstitial space were examined. Johnsen's Tubular Biopsy Scores (JTBS) were used in the assessment of spermatogenesis and testicular injury in the seminiferous tubule (Johnsen, 1970). A score of 0-10 was given to each tubule according to epithelial maturation. Mean JTBS in each section of testis tissue was calculated by dividing total value by number of seminiferous tubules. Apoptotic cells in testis of all groups were identified by the terminal deoxynucleotidyl Transferase-mediated dUTP Nick End-Labeling (TUNEL) staining in this study. TUNEL technique was performed with an *in situ* Cell Death Detection kit, POD, (ROCHE, Mannheim, Germany) with the manufacturer's instructions. Color was then developed with a 3, 3'-diaminobenzidine including kit (DAB, Sigma, St. Louis,

MO, USA). The TUNEL technique was used to evaluate apoptosis in the seminiferous tubule cells. Cells with a homogeneously stained brown nucleus were defined as TUNEL (+) and apoptotic. TUNEL (+) and TUNEL (-) cell numbers in 10 seminiferous tubules in slides from each group were counted using the Analysis 5 Research Program. Apoptotic Index (AI) was evaluated as TUNEL (+) cell number/total cell number×100 (Karaguzel *et al*., 2012).

Statistical analysis: All data were reported as mean±SEM. To evaluate the significant differences, Kruskal Wallis analysis of variance and Mann-Whitney U test with corrected Bonferroni test were used. Differences were considered to be statistically significant if $p<0.05$.

RESULTS AND DISCUSSION

Changes in the body weight: The mean body weights, histopathologic analysis results and tissue MDA levels of all groups are given in Table 1. In comparison of study groups body weights revealed a significant decrease in the DM and DM+RSV groups compared to the control and RSV groups. Body weight in the DM+RSV group increased significantly compared to that in the DM group.

Tissue MDA levels: Testis tissue MDA levels increased significantly in the DM and DM+RSV groups compared to the control and RSV groups. A decrease in MDA levels was observed in the DM+RSV group compared with the DM group, though this was not statistically significant.

Histopathological findings: Under light microscopy, the testes had a normal morphological architecture in the control and RSV groups (Fig. 1A and B). In the DM group, researchers observed a decrease in the amount of spermatozoa in the lumen of the seminiferous tubule, accumulation toward the lumen of the germinal epithelium, increase giant multinuclear cell number, degeneration and vacuolization in germinal cells (Fig. 1C). Although, spermatozoa and occasional other germinal cells that had not completed maturation were observed in the lumen of the seminiferous tubule in the DM+RSV group, the morphological architecture of the seminiferous tubule was close to normal (Fig. 1D). According to JTBS scoring, a significant decrease was observed in spermatogenesis in the DM and DM+RSV groups compared to the control and RSV groups. This was significantly higher in the DM+RSV group compared to the DM group. Diameter of the seminiferous tubule and thickness of the germinal epithelium were significantly lower in the DM and DM+RSV groups compared to the control and RSV groups; they were significantly higher in the DM+RSV group compared to the DM group.

Only a few TUNEL-positive cells in the spermatogonia was observed in the control and RSV

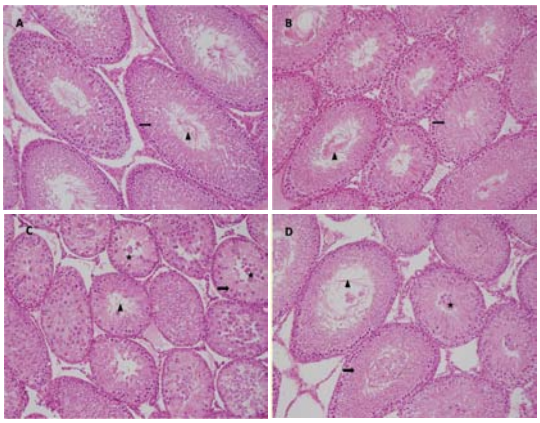

Fig. 1: Photomicrograph of testicular tissue in different groups; A) Control; B) RSV; C) DM; D) DM+RSV groups) (H&E, original magnification x200); A and B) Normal germinal epithelium (arrow) architecture, spermatozoon (arrow head) in the lumen; C) Degenerative changes in the germinal cells in the seminiferous tubule (arrow), loss or decrease of spermatozoon (arrow head), the giant cell formation with two or three nucleus (star) and spermatogenetic cells in the lumen (star); D) Nearly normal germinal epithelium architecture (arrow), spermatozoon in the lumen (arrow head) and occasional germinal epithelial cells in the lumen (star)

Table 1: Body weight, histopathological scores and biochemical parameters in all groups

	Groups			
Parameters	Control	RSV	DM	DM+RSV
Body weight (g)	215±12.67	222.1±14.07	140.1±12.850[a]	197.7±7.97[ab]
Diameter size (μm)	264.2±4.746	257.4±5.820	181.4±27.320[a]	231.1±9.78[ab]
Germinative cell thickness (μm)	109.5±7.45	104.7±7.590	58.04±10.46[a]	89.7±11.16[ab]
Jonnsen tubuler biopsy scors	9.26±0.30	9.13±0.16	5.8±0.3200[a]	7.97±0.15[ab]
Testes apoptotic index (%)	5.31±1.39	6.31±2.07	56.67±6.280[a]	18±2.44[ab]
Tissue MDA (nmol g^{-1} tissue)	21.25±0.83	22.82±0.95	26.64±0.790[a]	24.71±0.84[a]

[a]$p<0.05$ compared with control and RSV groups; [b]$p<0.05$ compared with DM group; RSV: Resveratrol; DM: Diabetes Mellitus; MDA: Malondialdehyde

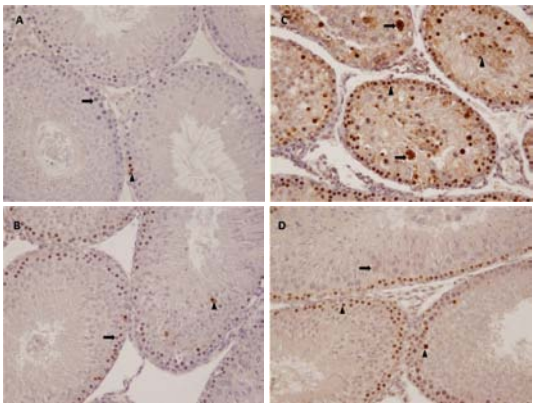

Fig. 2: Photomicrograph of testicular tissue in different groups. A) Control; B) RSV; C) DM; D) DM+RSV groups) (TUNEL staining, original magnification x400); A, B and D) Normal (arrow) and TUNEL (+) apoptotic (arrow head) spermatogenetic cells; C) TUNEL (+) apoptotic spermatogenetic cells (arrow head) and multinucleated giant cells (arrow)

Table 2: Blood glucose levels in all groups

	Groups			
Levels	Control	RSV	DM	DM+RSV
Initial (mg dL^{-1})	84.17±4.11	84.00±5.96	419.7±48.91[a]	414.8±49.24[a]
1st week (mg dL^{-1})	80.50±5.71	69.83±2.31	456.0±24.46[a]	294.0±29.86[ab]
2nd week (mg dL^{-1})	81.50±5.28	69.33±4.50	449.7±20.37[a]	241.8±47.47[ab]

[a]$p<0.05$ compared with control and RSV groups; [b]$p<0.05$ compared with DM group; RSV: Resveratrol; DM: Diabetes Mellitus

groups (Fig. 2A and B) at analysis of testicular apoptosis. In the DM group, widespread apoptosis was present in spermatogenic cells in almost all seminiferous tubule epithelia (Fig. 2C). Apoptosis decreased in the DM+RSV group compared to the DM group being particularly limited to the spermatogonia and primary spermatocytes (Fig. 2D). At statistical analysis, the AI in germinal epithelial cells rose significantly in the DM and DM+RSV groups compared to the control and RSV groups. Apoptosis decreased significantly in the DM+RSV group compared to the DM group.

Blood glucose levels: The mean blood glucose levels of all groups are given in Table 2. Initial blood glucose levels, at the end of the 1st week's and the 2nd week blood glucose levels increased significantly in the DM and DM+RSV groups compared to the control and RSV groups. Blood glucose levels at the end of the 1st week and 2nd week decreased significantly in the DM+RSV group compared to the DM group.

DM today very commonly seen in young males, is often accompanied by sexual dysfunction. Infertility complications are seen in approximately 90% of diabetic patients (Meyer *et al*., 2000; Sadik *et al*., 2011). It is therefore important for patients' fertility to be ensured. This study was evaluated the probable curative effects of RSV in an experimental model of STZ induced diabetes as biochemical and histopathological. STZ is a synthetic antitumor antibiotic. It is used to induce an experimental model of diabetes in laboratory animals such as rats, guinea pigs and mice (Pitkin and Reynolds, 1970; Losert *et al*., 1971; Lazarus and Shapiro, 1972). This alkylating agent causes pancreatic β-cell death (Kianifard *et al*., 2012). A decrease in pancreatic β cells leads to development of diabetes (Kuhn-Velten *et al*., 1982; Kianifard *et al*., 2012). To investigate the side-effects of diabetes, researchers used a model of diabetes induced with STZ in rats (Losert *et al*., 1971; Kuhn-Velten *et al*., 1982; Kianifard *et al*., 2012). Diabetes was induced with a single dose of STZ (Kanter *et al*., 2012; Kianifard *et al*., 2012).

Reproduction system dysfunctions in diabetes have been reported as being related to oxidative stress. Oxidative stress is associated with a rise in free oxygen radical production or an imbalance in the oxidant-antioxidant system (Agarwal *et al*., 2006; Kanter *et al*., 2012). Free oxygen radical production is determined quantitatively with the measurement of MDA (a product of lipid peroxidation, Neilsen *et al*., 1997). Lipid peroxidation is induced with protein glycation and glucose oxidation in diabetic rats. Hyperglycemia has been reported to increase the production of mitochondrial reactive oxygen species and to cause diabetes complications (Kiritoshi *et al*., 2003; Ricci *et al*., 2009). Oxidative stress increases in hyperglycemia since various reducing sugars are produced (through glycolysis and the multipath route). These reducing sugars easily increase the production of reactive oxygen species (non-enzymatic glucose reaction) (Armagan *et al*., 2006). Diabetes has been shown to lead to reproductive system function compromise and oxidative stress has been shown to impair spermatogenesis and to cause a rise in MDA levels in the testis (Fushimi *et al*., 1989; Lucesoli *et al*., 1999). Researchers also determined a significant increase in MDA levels in testicular tissue in STZ-induced diabetic rats. An increased level of MDA in diabetic rats shows lipid peroxidation damage (Kanter *et al*., 2012). In the study, tissue MDA levels in the DM+RSV group was lower than that in the DM group. However, the difference was not statistically significant. Researchers think that MDA levels decreasing with RSV therapy may associated with RSV's inhibition of lipid peroxidation and reactive oxygen products (Su *et al*., 2006; Kasdallah-Grissa *et al*., 2006).

A decrease in body weight has been described in STZ induced diabetes in earlier studies (Armagan *et al*., 2006; Kanter *et al*., 2012). This decrease has been emphasized as being associated with STZ's impairment of the body's anabolic activity and its adverse effect on somatic cells by raising blood glucose (Soudamani *et al*., 2005; Tsounapi *et al*., 2012). In the study, body weights decreased significantly in the group with induced diabetes and increased significantly in the diabetic group administered RSV. Researchers attribute this condition may be related to RSV reducing the effects of STZ (Su *et al*., 2006; Hamadi *et al*., 2012).

Earlier studies have reported that blood glucose levels rise significantly in diabetic rats (Amaral *et al*., 2006; Kanter *et al*., 2012). In the study, blood glucose levels in the diabetic rats also rose significantly in the 1st and 2nd weeks. Earlier studies have also reported that the administration of RSV increased insulin secretion from pancreatic beta cells (Chen *et al*., 2007; Bagul *et al*., 2012) or caused a rise in insulin sensitivity (Bagul *et al*., 2012). In the study, blood glucose levels decreased significantly in DM+RSV group compared to the DM groups in the 1st and 2nd weeks. This supports the previously described idea that RSV reduces glucose levels in STZ induced diabetic rats (Chen *et al*., 2007; Bagul *et al*., 2012).

The effects of diabetes on the microscopic structure of the testis have been reported in earlier studies (Kanter *et al*., 2012; Kianifard *et al*., 2012; Tsounapi *et al*., 2012). Some studies have determined degeneration in germinal cells, giant cell formation, changes in the interstitial area and a decrease in seminiferous tubule diameters (Kanter *et al*., 2012; Kushwaha and Jena, 2012). The genotoxic effects on the testis of excessive reactive oxygen radical production have been shown to lead to DNA damage and germ cell anomalies (Doreswamy *et al*., 2004; Kushwaha and Jena, 2012). In this study, testis structure in diabetic group was considerably compromised with widespread germinal cell loss. A significant decrease was observed in spermatogenesis in the DM group according to JTBS. Seminiferous tubule diameter decreased significantly in the DM group. This confirms that spermatogenesis was not completed. A pronounced improvement in spermatogenesis was observed in the DM+RSV group. Spermatogenesis and seminiferous tubule diameter increased significantly in this group compared to the DM group. Researchers think that the improvement findings in the study may have developed in association with the antioxidant property of RSV (Su *et al*., 2006; Collodel *et al*., 2011).

A pronounced increase in apoptotic cell death in the testis has been described in diabetic rats (Cai *et al*., 2000). This is emphasized as one of the factors responsible for infertility in diabetic males (Tsounapi *et al*., 2012; Agbaje *et al*., 2007). The mitochondrial pathway is reported to be involved and the Bax/Bcl-2 ratio has been shown to increase in the apoptotic mechanism in the rat testis (Zhao *et al*., 2010, 2011). Excessive reactive oxygen radical production induces lipid peroxidation and mitochondrial damage (Noriega-Cisneros *et al*., 2013). In the study, apoptosis was significantly increased in spermatogenetic cells of diabetic rats. A significant decrease in TUNEL (+) apoptotic cells was observed in the DM+RSV group. This suggests that the apoptosis-reducing effect of RSV in this study is associated with the antiapoptotic mechanism (Uguralp *et al*., 2005; Soufi *et al*., 2012).

CONCLUSION

This study showed significant testis damage in STZ induced diabetes and that this damage is accompanied by a rise in blood glucose and tissue MDA levels. RSV has beneficial effects against diabetes induced germinal cell damage, particularly by decreasing blood glucose levels and apoptosis. Furthermore, research is needed for the mechanism involved in these protective effects of RSV to be better understood.

REFERENCES

Agarwal, A., S. Gupta and S. Sikka, 2006. The role of free radicals and antioxidants in reproduction. Curr. Opin. Obst. Gynecol., 18: 325-332.

Agbaje, I.M. , D.A. Rogers, C.M. McVicar, N. McClure, A.B. Atkinson, C. Mallidis and S.E.M. Lewis, 2007. Insulin dependant diabetes mellitus: Implications for male reproductive function. Human Reprod., 22: 1871-1871.

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. Theriogenology, 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 antioxidant enzymes in streptozotocin-induced diabetic rat testis. Asian J. Androl., 8: 595-600.

Bagul, P.K., H. Middela, S. Matapally, R. Padiya and T. Bastia *et al*., 2012. Attenuation of insulin resistance, metabolic syndrome and hepatic oxidative stress by resveratrol in fructose-fed rats. Pharmacol. Res., 66: 260-268.

Cai, L., S. Chen, T. Evans, D.X. Deng, K. Mukherjee and S. Chakrabarti, 2000. Apoptotic germ-cell death and testicular damage in experimental diabetes: Prevention by endothelin antagonism. Urol. Res., 28: 342-347.

Chen, W.P., T.C. Chi, L.M. Chuang and M.J. Su, 2007. Resveratrol enhances insulin secretion by blocking K_{ATP} and K_V channels of beta cells. Eur. J. Pharmacol., 568: 269-277.

Collodel, G., M.G. Federicoa, M. Geminiania, S. Martini and C. Bonechi *et al*., 2011. Effect of trans-resveratrol on induced oxidative stress in human sperm and in rat germinal cells. Reprod. Toxicol., 31: 239-246.

Doreswamy, K., B. Shrilatha, T. Rajeshkumar and Muralidhara, 2004. Nickel-induced oxidative stress in testis of mice: Evidence of DNA damage and genotoxic effects. J. Androl., 25: 996-1003.

Eiselein, L., H.J. Schwartz and J.C. Rutledge, 2004. The challenge of type 1 diabetes mellitus. ILAR J., 45: 231-236.

Fushimi, H., H. Horie, T. Inoue, M. Kameyama and K. Kanao *et al*., 1989. Low testosterone levels in diabetic men and animals: A possible role in testicular impotence. Diabetes Res. Clin. Pract., 6: 297-301.

Hamadi, N., A. Mansour , M.H. Hassan, F. Khalifi-Touhami and O. Badary, 2012. Ameliorative effects of resveratrol on liver injury in streptozotocin-induced diabetic rats. J. Biochem. Mol. Toxicol., 26: 384-392.

Jeandet, P., R. Bessis and B. Gautheron, 1991. The production of resveratrol (3,5,4'-trihydroxystilbene) by grape berries in different developmental stages. Am. J. Enol. Viticult., 42: 41-46.

Johnsen, S.G., 1970. Testicular biopsy score count: A method for registration of spermatogenesis in human testes: Normal values and results in 335 hypogonadal males. Hormones, 1: 2-25.

Kanter, M., C. Aktas and M. Erboga, 2012. Protective effects of quercetin against apoptosis and oxidative stress in streptozotocin-induced diabetic rat testis. Food Chem. Toxicol., 50: 719-725.

Karaguzel, E., O. Kutlu, E. Yulug, S. Mungan, I.O. Kazaz, D.S. Tok and G.K. Ozgur, 2012. Comparison of the protective effect of dipyridamole and acetylsalicylic acid on long-term histologic damage in a rat model of testicular ischemia-reperfusion injury. J. Pediatr. Surg., 47: 1716-1723.

Kasdallah-Grissa, A., B. Mornagui, E. Aouani, M. Hammami, N. Gharbi, A. Kamoun and S. El-Fazaa, 2006. Protective effect of resveratrol on ethanol-induced lipid peroxidation in rats. Alcohol Alcoholism, 41: 236-239.

Kianifard, D., R.A. Sadrkhanlou and S. Hasanzadeh, 2012. The ultrastructural changes of the sertoli and leydig cells following streptozotocin induced diabetes. Iran J. Basic Med. Sci., 15: 623-635.

Kiritoshi, S., T. Nishikawa, K. Sonoda, D. Kukidome and T. Senokuchi *et al*., 2003. Reactive oxygen species from mitochondria induce cyclooxygenase-2 gene expression in human mesangial cells: Potential role in diabetic nephropathy. Diabetes, 52: 2570-2577.

Kuhn-Velten, N., D. Waldenburger and W. Staib, 1982. Evaluation of steroid biosynthetic lesions in isolated Leydig Cells from the testes of streptozotocin-diabetic Rats. Diabetology, 23: 529-533.

Kushwaha, S. and G.B. Jena, 2012. Enalapril reduces germ cell toxicity in streptozotocin-induced diabetic rat: Investigation on possible mechanisms. Naunyn Schmiedebergs Arch. Pharmacol., 385: 111-124.

Lazarus, S.S. and S.H. Shapiro, 1972. Streptozotocin-induced diabetes and islet cell alterations in rabbits. Diabetes, 21: 129-137.

Losert, W., A. Rilke, O. Loge, and K.D. Richter, 1971. Comparative biochemical study on the diabetogenic effect of streptozotocin in mice, rats, Chinese hamsters and guinea pigs. Arzneimittelforschung, 21: 1643-1653.

Lucesoli, F., M. Caliguri, M.F. Roberti, J.C. Perazzo and C.G.Fraga, 1999. Dose-dependent increase of oxidative damage in the testes of rats subjected to acute iron overload. Arch. Biochem. Biophys., 372: 37-43.

Meyer, K., J. Deutscher, M. Anil, A. Berthold, M. Bartsch and W. Kiess, 2000. Serum androgen levels in adolescents with type 1 diabetes: Relationship to pubertal stage and metabolic control. J. Endocrinol. Invest., 23: 362-368.

Neilsen, F., B.B. Mikkelsen, J.B. Neilsen, H.R. Andersen and P. Grandjean, 1997. Plasma malondialdehyde as biomarker for oxidative stress: Reference interval and effects of life-style factors. Clin. Chem., 43: 1209-1214.

Noriega-Cisneros, R., C. Cortes-Rojo, S. Manzo-Avalos, M. Clemente-Guerrero and E. Calderon-Cortes *et al*., 2013. Mitochondrial response to oxidative and nitrosative stress in early stages of diabetes. Mitochondrion. 10.1016/j.mito.2013.05.012.

Oktem, G., A. Uysal, O. Oral, E.D. Sezer and M. Olukman *et al*., 2010. Resveratrol attenuates doxorubicin-induced cellular damage by modulating nitric oxide and apoptosis. Exp. Toxicol. Pathol., 64: 471-479.

Pitkin, R.M. and W.A. Reynolds, 1970. Diabetogenic effects of streptozotocin in rhesus monkeys. Diabetes, 19: 85-90.

Ricci, G., A. Catizone, R. Esposito, F.A. Pisanti, M.T. Vietri and M. Galdieri, 2009. Diabetic rat testes: Morphological and functional alterations. Andrologia, 41: 361-368.

Sadik, N.A.H., M.M. El-Seweidy and O.G. Shaker, 2011. The antiapoptotic effects of sulphurous mineral water and sodium hydrosulphide on diabetic rat testes. Cell Physiol. Biochem., 28: 887-898.

Sainio-Pollanen, S., K. Henriksen, M. Parvinen, O. Simell and P. Pollanen, 1997. Stage-specific degeneration of germ cells in the seminiferous tubules of non-obese diabetic mice. Int. J. Androl., 20: 243-254.

Shingai, Y., A. Fujimoto, M. Nakamura and T. Masuda, 2011. Structure and function of the oxidation products of polyphenols and identification of potent lipoxygenase inhibitors from fe-catalyzed oxidation of resveratrol. J. Agric. Food Chem., 59: 8180-8186.

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. Record A Discov. Mol. Cell. Evol. Biol., 287: 1281-1289.

Soufi, F.G., M. Vardyani, R. Sheervalilou, M. Mohammadi and M.H. Somi, 2012. Long-term treatment with resveratrol attenuates oxidative stress pro-inflammatory mediators and apoptosis in streptozotocin-nicotinamide-induced diabetic rats. Gen. Physiol. Biophys., 31: 431-438.

Su, H.C., L.M. Hung and J.K. Chen, 2006. Resveratrol, a red wine antioxidant, possesses an insulin-like effect in streptozotocin-induced diabetic rats. Am. J. Physiol. Endocrinol. Metab., 290: E1339-E1346.

Tsounapi, P., M. Saito, F. Dimitriadis, S. Koukos and S. Shimizu *et al*., 2012. Antioxidant treatment with edaravone or taurine ameliorates diabetes-induced testicular dysfunction in the rat. Mol. Cell. Biochem., 369: 195-204.

Uchiyama, M. and M. Mihara, 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem., 86: 271-278.

Uguralp, S., U. Usta and B. Mizrak, 2005. Resveratrol may reduce apoptosis of rat testicular germ cells after experimental testicular torsion. Eur. J. Pediatr. Surg., 15: 333-336.

Zhao, H., S. Xu, Z. Wang, Y. Li and W. Guo *et al*., 2010. Repetitive exposures to low- dose X-rays attenua tetesticular apoptotic cell death in streptozotocin-induced diabetes rats. Toxicol. Lett., 192: 356-364.

Zhao, Y., Y. Tan, J. Dai, B. Li and L. Guo *et al*., 2011. Exacerbation of diabetes-induced testicular apoptosis by zinc deficiency is most likely associated with oxidative stress, p38 MAPK activation and p53 activation in mice. Toxicol. Lett., 200: 100-106.