Troxerutin affects the male fertility in prepubertal type 1 diabetic male rats

Zohreh Zavvari Oskuye 1, Fariba Mirzae Bavil 2, Gholam Reza Hamidian 3, Keyvan Mehri 1, Afsaneh Qadiri 1, Mahdi Ahmadi 4, Hajar Oghbæi 1, Amir Mansour Vatankhah 2, Rana Keyhanmanesh 2*

1 Department of Physiology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
2 Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
3 Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
4 Tuberculosis and Lung Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

ABSTRACT

Objective(s): Diabetes can gradually cause damage to the function and structure of male gonads. This survey was conducted to investigate the effect of troxerutin on hormonal changes, serum oxidative stress indices, and testicular function and structure in prepubertal diabetic rats.

Materials and Methods: Fifty prepubertal (6 weeks old) male Wistar rats were divided into five groups including Control, Troxerutin, Diabetic, Diabetic+Troxerutin, and Diabetic+Insulin. Type I diabetes was induced by 55 mg/kg of streptozotocin intraperitoneally. The groups were treated with 150 mg/kg/day troxerutin via oral gavage or 4-6 IU/day insulin via subcutaneous injection for 4 consecutive weeks. Blood sugar (BS) and serum levels of insulin, FSH, LH, testosterone, glutathione peroxidase (GPX), superoxide dismutase (SOD), malondialdehyde (MDA), and total antioxidant capacity (TAC) were analyzed. Testis and epididymis were removed for histopathologic study and analysis of sperm parameters.

Results: Troxerutin significantly reduced the BS in the diabetic group similar to insulin but could not affect insulin, FSH, or LH significantly. Troxerutin caused a significant increase in testosterone and GPX but had no significant effect on serum MDA, TAC, and SOD levels. In addition, troxerutin had a better effect than insulin on diabetes-induced testicular structural damage. Sperm analysis results also revealed that troxerutin and insulin could improve sperm number, motility, and viability in diabetic rats.

Conclusion: According to these results, it can be derived that administration of troxerutin is a suitable protective strategy for side effects of diabetes in testis of prepubertal diabetic male rats.

Introduction

Diabetes mellitus is a great endocrine and metabolic problem nowadays (1, 2). Diabetes mellitus Type 1 results from severe insulin deficiency while diabetes mellitus type 2 is characterized by insulin resistance, which may be merged with relatively reduced insulin secretion (3, 4). The report of the International Diabetes Federation in 2015 showed that the global prevalence of diabetes was estimated to be 415 million in adults and predicted that this figure will reach over 600 million in 2035 (5).

In diabetes mellitus type 1, pancreatic beta cells are damaged by the immune system, therefore, patients must use exogenous insulin to control blood sugar and inhibit risk of developing long-term complications (6, 7). Good glycemic control can avoid its complications (8).

One of the most common complications of diabetes is sexual dysfunction (9). It has been proposed that the reproductive complications of diabetes mellitus are caused by at least two different mechanisms including endocrine disorders (10) and oxidative stress (11-13). Some of sexual dysfunctions in diabetic men are disorders in ejaculation, libido (14), erection (15), testicular tissue structure (16), sperm quality (17, 18), and testosterone and gonadotropins secretion (19, 20).

Since chemical drugs have many side effects, herbal drugs today are considered for control of diabetes complications. Herbal nutrition and major pharmaceutical companies are currently doing research on natural materials to find new herbal subjects with the least side effects (21, 22). One of these herbal subjects is troxerutin also known as vitamin P4. It is a tri-hydroxyethylated derivative of natural flavonoids and can be found in tea, coffee, cereal grains, and some fruits and vegetables (23). This substance can be easily absorbed by the gastrointestinal system (24) and has many biological and pharmacological activities such as anti-oxidative (25), anti-inflammatory (26), anti-fatigue (27), anti-thrombolytic (28), and anti-hypoglycemic (29) properties. Previous experiments confirmed that troxerutin has protective effects on the kidneys (23), liver (30), brain (1), and vascular injuries (24); and chronic venous insufficiency (CVD) disease could be treated by this flavonoid (31). Moreover, troxerutin could prevent nickel-induced testicular toxicity in Wistar rats (32).

Although there are reports for the anti-hypoglycemic effects of troxerutin, we did not find any studies about...
the protective effects of troxerutin on the reproductive system in diabetic cases. Hence, this survey was designed to investigate the effect of troxerutin on testicular function and structure in type 1 diabetic male rats.

Materials and Methods

Animal and experimental design

Fifty prepubertal (6 weeks old, weighing 90–115 g) male Wistar rats were attained from animal center of Tabriz University of Medical Sciences and transported to Drug Applied Research Center. Animals were kept in standard laboratory conditions; 12 hr light/12 hr dark cycle, 20–22 °C, 45–55% moisture, and water and food ad libitum. A week after transportation and adaptation, the animals were randomly divided into 5 groups (n=10);

1. Control group (C).
2. Troxerutin group (T) which received troxerutin (Merck, Germany) 150 mg/kg/day via oral gavage for 4 weeks (24).
3. Diabetic group (DM) (1).
4. Diabetic group treated with troxerutin 150 mg/kg/day via oral gavage for 4 weeks (DT) (33).
5. Diabetic group treated with neutral protamine Hagedorn (NPH) insulin 4–6 IU/day subcutaneously (DI) (34).

For induction of diabetes, 55 mg/kg of liquefied streptozotocin (Sigma-Aldrich, Germany) in 10 mM sodium citrate (pH= 4.5) was injected intraperitoneally in DM, DI, and DT groups (35). Three days after streptozotocin injection, a blood sample of the tail vein was obtained and blood sugar (BS) was measured by means of a digital glucometer (Norditalia Elettromedicali S.r.l., Italy). If BS was more than 250 mg/dl, that animal was considered diabetic. The Ethics Committee of Tabriz University of Medical Sciences has supported all experimental procedures (No: IR.TBZMED.REC.1395.564).

Sampling

On the last day of experiments, animals were deeply anesthetized with intraperitoneal injection of a combination of ketamine and xylazine (80 and 12 mg/kg, respectively). Five milliliters of blood samples, taken from the inferior vena cava, were centrifuged at 3000 rpm for 10 min at room temperature. Then, serum aliquots were isolated and were kept at -80 °C for later hormonal and oxidative stress analysis. Finally, animals were sacrificed by decapitation and the testes were removed. Length (longitudinal radius), width (transversal radius), and height (perpendicular to the transversal radius) of each testis was measured by caliper and after macroscopic evaluation tissue samples were assessed after fixation in 10% buffered neutral formalin.

Hormonal analysis of serum

The serum levels of insulin (Shanghai crystal Day Biotech Co, LTD, China, for insulin with 0.05 ng/ml sensitivity), FSH (Bioassay Technology Laboratory, China, with 0.12 mlu/ml sensitivity), LH (Bioassay Technology Laboratory, China, with 0.051 mlu/ml sensitivity) and testosterone (Diametra Co, Italy, with 0.07 ng/ml sensitivity) were measured using enzyme-linked immuno-absorbent assay according to the manufacturer's instructions (8).

Oxidative stress measurement

Serum level of glutathione peroxidase (GPX), superoxide dismutase (SOD), malondialdehyde (MDA), and total antioxidant capacity (TAC) were analyzed. The activity of GPX was measured using Randox kit, United Kingdom. SOD was evaluated using a spectrophotometric method on the basis of the inhibition of a superoxide-induced reduced nicotinamide adenine dinucleotide (NADH) oxidation and MDA was measured using the thiobarbituric acid (TBARS) and colorimetric method (33). TAC was measured using the Randox Kit according to the protocols of the manufacturer.

Sperm parameters

Immediately after the last intervention, animals were anesthetized and the testes, epididymis and vas deferens were removed for determination of the spermatozoa parameters. To calculate the epididymal sperm storage, epididymis were isolated from the testes. Accordingly, epididymis was cut from the tail, and the sperm content of epididymis was aspirated into a pre-weighed pipette. Then the pipette was re-weighed to measure the weight of the aspirated fluid (36).

A 1 ml sample of the suspension containing sperm extracted from the epididymal tail was diluted with 20 ml of Ham’s F10 solution and the sperm count and motility were determined at ×400 magnification. Subsequently, the total mean of motile sperms in ten fields was expressed as a percentage of motility according to the World Health Organization (WHO) guidelines (37).

Determination of the spermatozoa viability percentage was also performed according to the WHO guidelines, using eosin (1%) and nigrosin (10%) staining. Briefly, 1 ml sperm suspension was mixed with 2 ml of eosin. After incubation at 37 °C for 30 sec, an equal volume of nigrosin was added to this suspension. Then the percentage of viable sperms was calculated in different groups under a light microscope (3).

Moreover, the total number of sperms was counted in a hemocytometer. Concisely, the number of sperms in diluted sperm solution was counted in five large squares of a hemocytometer. Then the sperm count per milliliter was calculated (38, 39).

For evaluation of sperm motility, at least 200 spermatozoa in 100 μl sperm suspension were evaluated under a light microscope and scored from A to D according to the WHO manual including (40, 41): grade A as fast progressive; grade B as slow progressive; grade C as non-progressive, and grade D as immotile sperm.

Histological analysis

Fixed testicular tissue samples were dehydrated in an ascending graded series of ethylc alcohol, cleared in xylol, and impregnated in paraffin. The testis was cut by a rotary microtome to 5 μm thin sections and was stained by hematoxylin and eosin (H&E) according to a previously described protocol. At least 10 sections were checked for each animal with a light microscope. Testis
Troxerutin improves reproduction in diabetic rats

Zavvari Oskuye et al.

Results

The levels of blood glucose and serum insulin

The blood glucose levels in DM, DT, and DI groups increased significantly compared to the control and troxerutin groups (P<0.001). Both insulin and troxerutin treatment significantly diminished the blood glucose levels in comparison with the diabetic group (P<0.001) (Figure 1a).

Injection of streptozotocin in the DM group reduced insulin level compared to C and T groups (P<0.01 to P<0.05). There was an increased insulin level in the DI group compared to diabetic rats (P<0.001), however, administration of troxerutin nonsignificantly increased the insulin level compared to the DM group (Figure 1b).

Statistical analysis

The results were expressed as mean ± SEM (standard error of the mean). Statistical analysis was performed by SPSS software version 22 (IBM company, SPSS Inc., 2010). One-way analysis of variance (ANOVA) followed by post hoc Tukey’s test was used to assess the statistical significance of data between different groups. It was considered significant if P<0.05.

The levels of serum glutathione peroxidase (GPX) and malondialdehyde (MDA)

Induction of diabetes did not show any effect on the GPX level; however, in DI and DT groups, administration of insulin and troxerutin increased GPX level significantly (P<0.001). It must be mentioned that insulin and troxerutin did not have a significant difference in this regard (Figure 2a).

Although induction of diabetes increased the MDA level in comparison to the controls, this increment was not statistically significant. Treatment with insulin and troxerutin in DI and DT groups nonsignificantly decreased the level of MDA. There was no significant difference between DI and DT groups (Figure 2b).

The serum levels of total antioxidant capacity (TAC) and superoxide dismutase (SOD)

Induction of diabetes and administration of troxerutin and insulin did not change the TAC level significantly compared to the C group (Figure 3a).

Although induction of diabetes decreased the SOD level in comparison to the controls, it was not statistically significant. Treatment with insulin and troxerutin in DI and DT groups nonsignificantly increased the level of
The serum levels of LH, FSH, and testosterone

The serum testosterone level decreased significantly after diabetes induction compared to C and T groups ($P<0.001$), whereas LH and FSH serum levels decreased nonsignificantly. Treatment with insulin and troxerutin in DI and DT groups increased testosterone level significantly in comparison to diabetic rats ($P<0.001$), although the increments in the serum levels of LH and FSH in these groups were not statistically significant. There were not any significant differences between DT and DI groups (Figures 4a, b, and c).

Sperm parameters

The induction of diabetes reduced the total number, motility, and viability of sperms in comparison to C and T groups ($P<0.001$). Troxerutin could not affect these parameters in control rats. Administration of troxerutin and insulin significantly improved all of these parameters compared to the DM group ($P<0.001$). There were no significant differences between DT and DI groups (Figures 5a, b, and c).

Sperm motility grade

Induction of diabetes significantly decreased the percentages of fast progressive, slow progressive and non-progressive sperms ($P<0.001$ to $P<0.01$) but increased the percentage of immotile sperms ($P<0.001$) compared to control and troxerutin groups. Treatment
with troxerutin significantly increased the percentages of fast progressive and slow progressive sperms but decreased the percentage of immotile sperms (P<0.001). Moreover, in the DI group, insulin administration increased the percentages of fast progressive, slow progressive, and non-progressive sperms (P<0.01 to P<0.001) and decreased the percentage of immotile sperm (P<0.001) compared to the DM group. The percentage of non-progressive sperms of DT and DI groups was significantly different (P<0.01, Table 1).

**Histological analysis of testis**

Histological studies of testis tissue demonstrated that the structure of the testis and seminiferous tubule are completely normal in the control and troxerutin-treated groups (Figures 6A and B). Microscopical analysis revealed that diabetes induction resulted in structural disturbance of the animals’ testis including increased interstitial space and vascular congestion and destruction of germinal epithelium of seminiferous tubule. Existence of many vacuoles in germinal epithelium caused rupture in the cells integrity of this tissue. Irregular shape and shrinkage of seminiferous tubules basement membrane and disturbance of cellular arrangement and organization could be observed in diabetic rats (Figure 6C).

Histological studies of the testis in the diabetic group treated by troxerutin showed that the tissue structure was improved relatively compared to the diabetic group. The germinal epithelium of seminiferous tubule had proper structure and was similar to the control group. Increased interstitial space could be still observed in this group but vascular congestion was completely resolved (Figure 6D).

**Table 1. The effect of troxerutin on sperm motility grade in prepubertal type 1 diabetic male rats (n=10) for 4 weeks**

| Motility Grades | Scores in groups | % (Mean±SEM) |
|-----------------|------------------|--------------|
|                 | Control          | T            | DM            | DT            | DI            |
| Grade A (fast progressive) | 48.6±0.68        | 49.6±0.24    | 20.4±0.93     | 30.6±0.87     | 29.4±0.60     |
| Grade B (slow progressive)   | 22.0±0.55        | 25.0±0.63    | 12.2±0.58     | 28.0±0.89     | 25.6±1.07     |
| Grade C (non-progressive)   | 8.6±0.24         | 8.2±0.37     | 5.8±0.37      | 5.4±0.24      | 8.2±0.20      |
| Grade D (immotile)          | 20.8±0.86        | 17.2±0.58    | 61.6±1.32     | 36.0±1.00     | 36.8±1.24     |

Data are expressed as mean±SEM. Statistical differences between control and different groups: +++; P<0.001, ++; P<0.01, Statistical differences between troxerutin and different groups: ***; P<0.001, **; P<0.01, Statistical differences between diabetic and different groups: !!!; P<0.001, !!; P<0.01, Statistical differences between DT and DI groups: ##; P<0.01. Control (C), troxerutin (T), diabetic (DM), diabetes+troxerutin (DT), and diabetes+insulin (DI)
Microscopic studies also revealed that treatment by insulin in the diabetic group resulted in evident structural improvement but increased volume in interstitial space could be still observed. Vascular congestion was less than in the diabetic rats but it was not totally eliminated. The effect of insulin administration on the improvement of structural changes of testis tissue was less compared with the group treated by troxerutin for 4 weeks (Figure 6E).

Johnsen’s score analysis illustrated a significant decrease in all diabetic rats (D, DT, and DI groups) compared to C and T groups (P<0.001). Treatment with troxerutin and insulin in DT and DI groups induced a significant increment compared to the DM group (P<0.001) (Figure 7).

Discussion

Our results indicated that treatment of diabetic animals by insulin and troxerutin resulted in significant increase of GPX, testosterone, total number, viability, and motility of sperm and Jonson’s score, although it reduced the blood sugar. The previous studies have shown that high blood sugar levels can affect fertility (42, 43). The results of a study have proven that hyperglycemia increased the testicular inflammatory cytokines (44). Other studies also suggested that hyperglycemia caused excessive production of ROS (45, 46) followed by the destruction of the testicular membrane (47).

Nowadays various drugs are used to treat diabetes, which may have a negative effect on other organs in addition to lowering blood glucose levels. One study reported that the use of sulfonylureas has led to apoptosis in beta cells and the failure of long-term treatment (48). Researchers also found that metformin and glibenclamide caused a significant reduction in the number and motility of the sperm and testicular damage, due to elevated lipid peroxidation and decreased antioxidant status in the testes (49).

Because of these side effects, scientists have tried to use alternative herbal drugs for controlling complications of diabetes. One of these medicines is troxerutin. Regarding the positive effects of insulin on fertility, we decided to compare the drug with insulin. Insulin stimulates various functions of Sertoli cells, such as transferrin secretion, DNA and protein synthesis, glycine metabolism, lactate production (50, 51), and differentiation of spermatogonia through insulin-like growth factor receptor (IGF-1) (52).

In a study in 2014, increased sensitivity to insulin in the presence of troxerutin has been reported as a major finding (53). Another study has also shown that routine supplementation effectively relieves symptoms of metabolic syndrome. In addition, they showed that the administration of troxerutin in high-cholesterol-fed type 2 diabetic rats reduced the blood glucose levels, consistent with our results (54).

Kawamura et al. reported that SOD glycosylation percentage was significantly elevated in diabetic people compared with controls. The activity of glycosylated SOD is less than natural SOD (55). A study showed that MDA level of seminal plasma in diabetic men with normal sperm is more than that of non-diabetic men. Also, it has been shown that diabetic men have lower levels of TAC compared to non-diabetic men (56). However, the current study showed that administration of troxerutin (150 mg/kg) in immature diabetic rats had no significant effect on SOD, MDA, and TAC levels of serum in comparison to the diabetic group, but led to increment in serum level of GPX in comparison to the diabetic group. Previous study has also revealed that administration of troxerutin to diabetic rats will not have a significant impact on the increase of SOD in comparison to those which had not received troxerutin, although the serum level of GPX significantly increased (24), which is consonant with the results of our study. However, the current results did not coincide with the findings of Fan et al who investigated the effect of troxerutin on D-galactose-induced renal injury in mice. These results indicated that it could increase the activity of antioxidant enzymes and reduce the lipid peroxidation products (23). The reasons for such differences can be attributed to the duration and severity of diabetes, method, and dosage of drug administration and method of diabetes induction.

Ballester et al. observed that induction of type 1 diabetes by streptozotocin for 3 months disturbed the function of Leydig cells and decreased the serum level of testosterone. This could be due to lack of stimulation effect of insulin on these cells. They also showed that the serum level of FSH and LH would also decrease in such conditions (57). In our study, the level of testosterone decreased significantly, although the levels of LH and FSH were decreased nonsignificantly. It seems that the duration of the experimental period of our study (4 weeks) can be the reason for these results, as the work of Ballester et al. (57) was conducted for 3 months. On the other hand, the age of rats could also make a difference. They worked on adult rats whereas our study was performed on prepubertal rats.

Previous study has revealed that diabetes can cause severe abnormalities in sperm by increasing oxidative stress in testis and epididymis tissues (58). It has been shown that sperm cells of mammals contain high levels of lipids with high unsaturated fatty acids. On the other hand, spermatogenesis use lipids as the main material for the peroxidation process. This can make the testis, epididymis, and released sperms in the seminiferous tubule a suitable site for production of free radicals as the result of lipid peroxidation during diabetes. The high rate of cell proliferation in germinal epithelium of the seminiferous tubule and reduction of anti-oxidative defense during diabetes can even intensify this issue (59). It has been also shown that hyperglycemia can increase the production of free radicals by increased glycolysis, activation of the sorbitol pathway in the cell, glucose self-oxidation, and proteins non-enzymatic glycation (45, 46), which is compatible with our findings in this investigation.

The results of qualitative and quantitative analysis of sperms in our study revealed that induction of diabetes by streptozotocin not only can affect the viability and the total number of sperms but also it can reduce the quality of sperm motility. Previous studies also suggested that diabetes and its consequent hyperglycemia can cause a reduction in quality and quantity parameters of sperm (3), disturb the spermatogenesis process (60), and also
Troxerutin improves reproduction in diabetic rats

Zavvari Oskuye et al.

Troxerutin improves reproduction in diabetic rats

Zavvari Oskuye et al.

decrease the sperm motility (57) by decreasing the production rate of testosterone.

Troxerutin has numerous biological protective effects against oxidative, fibrinolytic, inflammatory, γ-radiation, and diabetic damage and cancer (28, 54). It acts by affecting reactive oxygen species (ROS) and enzyme activities, probably indirectly by affecting the antioxidants acting on enzyme activities (61). The present study showed that troxerutin administration, similar to insulin, could relatively improve the diabetes-induced decrease in quantity and quality parameters of sperms. It seems that troxerutin can decrease the oxidative stress and blood sugar and relatively inhibit the side effects of diabetes on quality and quantity indices of sperm.

The results of this survey showed that induction of diabetes caused severe damage to the testicular tissue structure. Microscopical study showed that diabetes induced vascular hyperemia in the interstitial tissue of testes and increased the interstitial space of the seminiferous tubules due to interstitial edema. Kolahian et al. also reported these events in their study (3). The outcomes of our study displayed that induction of diabetes caused severe degeneration of spermatogenic cells as a result of structural changes in the germinal epithelium of seminiferous tubules. The disappearance of spermatogenic cells leads to the observation of many vacuoles in the germinal epithelium. In another study these reports were also observed (62). The results of the sperm analysis indicated that diabetes reduced testicular function and normal sperm production. The results of present investigation also showed that insulin or troxerutin therapy can importantly inhibit structural changes of the testis, surprisingly the effect of troxerutin was better than insulin and decreased the vascular congestion in testis, which was also consonant with the results of sperm analysis. So possible mechanisms that may be proposed for these effects of troxerutin include anti-inflammatory drug effect (28, 54), reduction of blood glucose (54, 63), improved insulin sensitivity (53), and indirect effect on antioxidants (61) because oxidative stress disrupts fertility (47).

Conclusion

According to these results, it can be concluded that administration of troxerutin is a suitable protective strategy for side effects of diabetes in testis of prepubertal diabetic male rats.

Acknowledgment

This report is based on a database from the thesis entitled “Effect of Troxerutin on blood levels of stress oxidative, testosterone, LH, FSH, insulin, and histological and stereological changes in type 1 diabetic adult and prepubertal male rat” registered in Drug Applied Research Center of Tabriz University of Medical Sciences, Tabriz, Iran. Moreover, the authors appreciate Professor S. Babri for giftng troxerutin.

Conflicts of Interest

The authors declare that they have no conflicts of interest to disclose.

References

1. Baluchnejadmajordoom T, Jamali-Raeuuf N, Zabihnejad S, Rabiee N, Roghani M. Troxerutin exerts neuroprotection in 6-hydroxydopamine lesion rat model of Parkinson’s disease: Possible involvement of PI3K/ERβ signaling. Eur J Pharmacol 2017; 801:72-78.
2. Ding GL, Liu Y, Liu ME, Fan JX, Guo MX, Sheng JZ, Huang H. The effects of diabetes on male fertility and epigenetic regulation during spermatogenesis. Asian J Androl 2015; 17:948-53.
3. Kolahian S, Sadri H, Larijani A, Hamidian G, Davasaz A. Supplementation of diabetic rats with leucine, zinc, and chromium: effects on function and histological structure of testes. Int J Vitam Nutr Res 2015; 85:311-21.
4. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. β-Cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. Diabetes 2003; 52:102-110.
5. Thomas MC, Cooper ME, Zimmer P. Changing epidemiology of type 2 diabetes mellitus and associated chronic kidney disease. Nat Rev Nephrol 2016; 12:73-81.
6. Ashcroft FM, Rorsman P. Diabetes mellitus and the β cell: the last ten years. Cell 2012; 148:1160-1171.
7. Regnell SE, Lernmark A. Early prediction of autoimmune (type 1) diabetes. Diabetologia 2017; 60:1370-1381.
8. Zurita-Cruz JN, Nishimura-Meguro E, Villasis-Keever MA, Hernández-Méndez ME, Garrido-Magaña E, Rivera-Hernández AJ. Influence of the informal primary caretaker on glycemic control among prepubertal pediatric patients with type 1 diabetes mellitus. J Pediatr (Rio J) 2017; 93:136-141.
9. Sexton WJ, Jarow JP. Effect of diabetes mellitus upon male reproductive function. Urology 1997; 49:508-513.
10. Ali ST, Shaikh RN, Ashfaq Siddiqui N, Siddiqui PQ. Serum and urinary levels of pituitary-gonadal hormones in insulin-dependent and non-insulin-dependent diabetic males with and without neuropathy. Arch Androl 1993; 30:117-123.
11. Ramalho-Santos J, Amaral S, Oliveira PJ. Diabetes and the impairment of reproductive function: possible role of mitochondria and reactive oxygen species. Curr Diabetes Rev 2008; 4:46-54.
12. Glacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res 2010; 107:1058-1070.
13. Tiwari BK, Pandey KB, Abidi AB, Rizvi SI. Markers of oxidative stress during diabetes mellitus. J Biomark 2013; 2013:1-8.
14. Watcho P, Mbiakou UC, Jeugo HG, Wankeu M, Nguefack TB, Carro-Juarez M, Kamanyi A. Delay of ejaculation induced by Bersama engleriana in nicotinamide/streptozotocin-induced type 2 diabetic rats. J Vet Med Sci 2017; 79:5603-5609.
15. Byran JG, Gajraj J. Erectile dysfunction and its association with metabolic syndrome and endothelial function among patients with type 2 diabetes mellitus. J Diabetes Complications 2012; 26:141-147.
16. Abd El-Twab SM, Mohamed HM, Mahmoud AM. Taurine and pioglitazone attenuate diabetes-induced testicular damage by abrogation of oxidative stress and up-regulation of the pituitary–gonadal axis. Can J Physiol Pharmacol 2016; 94:651-661.
17. Pommjuna A, Rathananphart J, Fungfuang W. Effects of Vernonia cinerea on reproductive performance in streptozotocin-induced diabetic rats. Vet Med Sci 2017; 79:572-578.
18. Wankeu-Nya M, Florea A, Bâlici S, Watcho P, Matei H, Kamanyi A. Dracaena arborea alleviates ultrastructural spermatogenic alterations in streptozotocin-induced diabetic rats. BMC Complement Altern Med 2013; 13:71-79.
19. De A, Singh MF, Singh V, Ram V, Bisht S. Treatment effect of l-Norvaline on the sexual performance of male rats with streptozotocin induced diabetes. J Pharmacol 2016; 771:247-254.
20. Rovira-Llopis S, Bañuls C, de Marañon AM, Diaz-Morales...
N. Jover A, Garzon S, Rocha M, Victor VM, Hernandez-Mijares A. Low testosterone levels are related to oxidative stress, mitochondrial dysfunction and altered subclinical atherosclerotic markers in type 2 diabetic male patients. Free Radic Biol Med 2017; 108:155-162.

21. Patel DK, Kumar R, Laloo D, Hulagudatha S. Diabetes mellitus: an overview on its pharmacological aspects and reported medicinal plants having anti diabetic activity. Asian Pac J Trop Biomed 2012; 2:411-420.

22. Goyal M. Traditional plants used for the treatment of diabetes mellitus in Sur Sargas Constituency, Jodhpur, Rajasthan – An ethnomedical survey. J Ethnopharmacol 2015; 174:364-368.

23. Pan SH, Zhang ZF, Zheng YL, Lu J, Wu DM, Shan Q, Hu B, Wang YY. Troxerutin protects the mouse kidney from D-galactose caused injury through anti-inflammation and anti-oxidation. Int Immunopharmacol 2009; 9:91-96.

24. Badalzadeh R, Layeghzadeh N, Alihemmati A, Mohammad M. Beneficial effect of troxerutin on diabetes-induced vascular damages in rat aorta: histopathological alterations and antioxidation mechanism. Int J Endocrinol Metab 2015; 13:e25699.

25. Panat NA, Maurya DK, Ghaskadbi SS, Sandur SK. Troxerutin, a plant flavonoid, protects cells against oxidative stress induced cell death through radical scavenging mechanism. Food Chem 2016; 194:32-45.

26. Lu J, Wu DM, Zheng YL, Hu B, Cheng W, Zhang ZF, Li MQ. Troxerutin counteracts domoic acid-induced memory deficits in mouse by inhibiting CCAAT/enhancer binding protein β-mediated inflammatory response and oxidative stress. J Immunol 2013; 190:3466-3479.

27. Zamanian M, Hajizadeh MR, Esmaeili Nadimi A, Shamsizadeh A, Allahavakoli M. Anti-fatigue effects of troxerutin on exercise endurance capacity, oxidative stress and matrix metalloproteinase-9 levels in trained male rats. Fund Clin Pharmacol 2017; 31:447-455.

28. Liu C-M, Ma J-Q, Lou Y. Chronic administration of troxerutin and matrix metalloproteinase-9 levels in trained male rats. J Sex Med 2015; 12:600-610.

29. Alipour MR, Khamaneh AM, Yousefzadeh N, Mohammad-Rajabi D, Soufi FG. Upregulation of microRNA-146a was not accompanied by downregulation of pro-inflammatory markers in diabetic kidney. Mol Biol Rep 2013; 40:6477-6483.

30. Amoral S, Moreno AJ, Santos MS, Seija R, Ramalho-Santos J. Effects of hyperglycemia on sperm and testicular cells of Goto-Kakizaki and streptozotocin-treated rat models for diabetes. Theriogenology 2006; 66:2056-2067.

31. Oger P, Yazebeck C, Gervais A, Dorphin B, Gout C, Jacquesson L, Ayel JP, Kahn V, Rougier N. Adverse effects of hepatitis B virus on sperm motility and fertilization ability during IVF. Reprod Biomed Online 2011; 23:207-212.

32. Keegan BR, Barton S, Sanchez X, Berkeley AS, Krey LC, Grifo J. Isolated teratozoospermia does not affect in vitro fertilization outcome and is not an indication for intracytoplasmic sperm injection. Fertil Steril 2007; 88:1583-1588.

33. Bahmanzadeh M, Abolhassani F, Amidi F, Ejtemaeimehr Sh, Salehi M, Abbasi M. The effects of nitric oxide synthase inhibitor (L-NNAME) on epidymal sperm count, motility, and morphology in varicoceleized rat. Daru 2008; 16:23-28.

34. Organization WH, WHO laboratory manual for the examination and processing of human semen 2010.

35. Johnsen SG. Testicular biopsy score count—a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. Horm Res Paediatr 1970; 1:2-25.

36. Ogbaii H, Alipour MR, Hamidian G, Ahmadi M, Ghorbanzadeh V, Keyhanmanesh R. Two months sodium nitrate supplementation alleviates testicular injury in streptozotocin-induced diabetic male rats. Exp Physiol 2018; doi: 10.1113/EP087198.

37. Keyhanmanesh R, Hamidian G, Alipour MR, Ranjbar M, Ogbaii H. Protective effects of sodium nitrate against testicular apoptosis and spermatogenesis impairments in streptozotocin-induced diabetic male rats. Life Sci 2018; 211:63-73.

38. Samir Zahikho, Nehal Abo-Elnaga, Amel FM Ismail, Esraa Moussa. Studies on fertility of diabetic male rats treated with olive leaves extract. J Biomed Pharm Res 2016; 5:18-27.

39. Ahmed, N., Advanced glycation end-products—role in pathology of diabetic complications. Diabetes Res Clin Pract 2005; 67:3-21.

40. Jakus, V. and N. Rietbrock, Advanced glycation end-products and the progress of diabetic vascular complications. Physiol Res 2004; 53:131-142.

41. Tremellen K. Oxidative stress and male infertility—a clinical perspective. Human Reproduction Update 2008; 14:243-258.

42. Maedler K, Carr RD, Bosco D, Zuelleg RA, Berney T, Donath MY. Sulfonylurea induced β-cell apoptosis in cultured human islets. J Clin Endocrinol Metab 2005; 90:501-506.

43. Adaramoye O, Akanni O, Adesanoye O, Labo-Popoola O, Olaremi O. Evaluation of toxic effects of metformin hydrochloride and glibenclamide on some organs of male rats. Niger J Physiol Sci 2012; 27:137-144.

44. Alves MG, Socorro S, Silva J, Barros A, Sousa M, Cavaco JE, Alipour MR. In vitro cultured human Sertoli cells secrete high amounts of acetate that is stimulated by 17β-estradiol and suppressed by insulin deprivation. Biochim Biophys Acta 2012; 1820:84-89.

45. Nakayama Y, Yamamoto T, Abé SI. IGF-I, IGF-II and insulin promote differentiation of spermatogonia to primary spermatocytes in organ culture of newt testes. Int J Dev Biol 1999; 43:343-347.

46. Geetha R, Yogalakshmi B, Sreeja S, Bhavani K, Anuradha CV. Troxerutin suppresses lipid abnormalities in the heart of high-fat–high-fructose diet-fed mice. Mol cell biochem 2014; 387:123-134.

47. Lu J, Wu DM, Zheng ZH, Zheng YL, Hu B, Zhang ZF. Troxerutin protects against high cholesterol-induced cognitive deficits in mice. Brain 2011; 134:783-797.

48. Oliveira PF, Alves MG, Rato L, Laurentino S, Silva J, Sá R, Barros A, Sousa M, Carvalho RA, Cavaco JE, Socorro PF. In vitro cultured human Sertoli cells secrete high amounts of acetate that is stimulated by 17β-estradiol and suppressed by insulin deprivation. Biochim Biophys Acta 2012; 1823:1389-1394.

49. Oliveira PF, Alves MG, Rato L, Laurentino S, Silva J, Sá R, Barros A, Sousa M, Carvalho RA, Cavaco JE, Socorro PF. In vitro cultured human Sertoli cells secrete high amounts of acetate that is stimulated by 17β-estradiol and suppressed by insulin deprivation. Biochim Biophys Acta 2012; 1823:1389-1394.

50. Barros A, Sousa M, Carvalho RA, Cavaco JE, Socorro PF. In vitro cultured human Sertoli cells secrete high amounts of acetate that is stimulated by 17β-estradiol and suppressed by insulin deprivation. Biochim Biophys Acta 2012; 1823:1389-1394.

51. Oliveira PF, Alves MG, Rato L, Laurentino S, Silva J, Sá R, Barros A, Sousa M, Carvalho RA, Cavaco JE, Socorro PF. In vitro cultured human Sertoli cells secrete high amounts of acetate that is stimulated by 17β-estradiol and suppressed by insulin deprivation. Biochim Biophys Acta 2012; 1823:1389-1394.

52. Nakayama Y, Yamamoto T, Abé SI. IGF-I, IGF-II and insulin promote differentiation of spermatogonia to primary spermatocytes in organ culture of newt testes. Int J Dev Biol 1999; 43:343-347.

53. Geetha R, Yogalakshmi B, Sreeja S, Bhavani K, Anuradha CV. Troxerutin suppresses lipid abnormalities in the heart of high-fat–high-fructose diet-fed mice. Mol cell biochem 2014; 387:123-134.

54. Lu J, Wu DM, Zheng ZH, Zheng YL, Hu B, Zhang ZF. Troxerutin protects against high cholesterol-induced cognitive deficits in mice. Brain 2011; 134:783-797.
56. Karimi J, Goodarzi MT, Tavilani H, Khodadadi I, Amiri I. Relationship between advanced glycation end products and increased lipid peroxidation in semen of diabetic men. Diabetes Res Clin Pract 2011; 91: 61-66.
57. Ballester J, Muñoz MC, Domínguez J, Rigau T, Guinovart JJ, Rodríguez-Gil JE. Insulin-dependent diabetes affects testicular function by FSH-and LH-linked mechanisms. J Androl 2004; 25: 706-719.
58. La Vignera S, Condorelli R, Vicari E, D’Agata R, Calogero AE. Diabetes mellitus and sperm parameters. J Androl 2012; 33: 145-153.
59. Kim ST, Moley KH. Paternal effect on embryo quality in diabetic mice is related to poor sperm quality and associated with decreased glucose transporter expression. Reproduction 2008; 136: 313-322.
60. Baccetti B, La Marca A, Piomboni P, Capitani S, Bruni E, Petraglia F, De Leo V. Insulin-dependent diabetes in men is associated with hypothalamic-pituitary derangement and with impairment in semen quality. Hum Reprod 2002; 17: 2673-2677.
61. Vinothkumar R, Vinoth Kumar R, Sudha M, Viswanathan P, Balasubramanian T, Nalini N. Modulatory effect of troxerutin on biotransforming enzymes and preneoplastic lesions induced by 1,2-dimethylhydrazine in rat colon carcinogenesis. Exp Mol Pathol 2014; 96: 15-26.
62. Kanter M, Aktas C, Erboga M. Protective effects of quercetin against apoptosis and oxidative stress in streptozotocin-induced diabetic rat testis. Food Chem Toxicol 2012; 50: 719-725.
63. Lu J, Wu DM, Hu B, Cheng W, Zheng YL, Zhang ZF, Ye Q, Fan SH, Shan Q, Wang YJ. Chronic administration of troxerutin protects mouse brain against D-galactose-induced impairment of cholinergic system. Neurobiol Learn Mem 2010; 93: 157-164.