Veterinary Research Forum
Vol: 2, No: 1, March, 2011, 13 - 24

Original Article

The Histological, Histomorphometrical and Histochemical Changes of Testicular Tissue in the Metformin Treated and Untreated Streptozotocin-Induced Adult Diabetic Rats

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Received: 1 December 2010, Accepted: 26 January 2011

Abstract

In this investigation, diabetes was induced in adult male Sprague-Dawley rats by single intraperitoneal injection of streptozotocin (STZ) at 45 mg kg^{-1} of body weight. A group comprised of 8 diabetic rats was treated with metformin at 100 mg kg^{-1} of body weight for reducing the elevated blood glucose level. The results revealed that, in the untreated diabetic rats, the body and testicular weight reduced in comparison with the control rats ($P < 0.05$) , the metformin treated diabetic rats showed body weight loss in comparison with the control group ($P < 0.05$). In the untreated diabetic rats, the blood glucose level significantly increased in comparison with control and metformin treated diabetic rats. Histomorphological examinations revealed a reduction in testicular capsule diameter, seminiferous tubules (STs) and germinal epithelium height, increase of amorphous material of interstitial tissue, germ cell depletion, decrease in cellular population and activity and disruption of spermatogenesis in the untreated diabetic rats in comparison with control group. In metformin treated diabetic rats, the histomorphological alterations were seen in lesser part in comparison with untreated diabetic group. The results from this study proved that, there was a direct relationship between increased levels of blood glucose as a result of STZ-induced diabetes and the histomorphological changes of testicular tissue.

Key words: Diabetes, Streptozotocin, Metformin, Histomorphology of testis, Rat

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Introduction

Diabetes mellitus is a serious metabolic disorder with numerous complications.[1] It is well known that, increase of blood glucose levels leads to structural and functional changes in various target tissues and organs.[2] Experimentally induced diabetes in male rats is associated with altered functions of reproductive system.[3] Streptozotocin (STZ)-induction of diabetes in rats is used as a model for studying and evaluation of the effects of diabetes on various organs.[4, 5] In addition, the morphologic alterations observed in the testes of STZ-induced diabetic rats are not caused by a direct effect of the drug, but rather by diabetes.[6] As a result, this method for induction of diabetes is applicable for the study of diabetes-related alterations of target organs. The effects of diabetes on testicular function have been reported to the lack of insulin and subsequently the impairment of regulatory action of this hormone on both Leydig and Sertoli cells.[6] In this regard, it was well known that gonadal dysfunction and decrease in testosterone production lead to insufficient production of spermatozoids.[7, 8, 9] The change of the structure of reproductive system in diabetic conditions has been reported in several studies.[2, 10, 11] Also, the effect of diabetes on the changes of body and testicular weight has been reported in several investigations but, there is a very limited data about the alterations of the diameter and/or the length of testis in diabetic conditions. It has been reported that, in diabetic rats testicular weight is decreased around 20 percent compared to healthy and streptozotocin resistant rats.[12] While, other authors reported an increase in testicular weight at short time and decrease in testicular weight at long time, similar to these data the reduction of body weight has also been reported.[1, 12] Metformin (dimethylbiguanide) is one of the blood glucose lowering drugs that lowers the fasting blood plasma glucose concentration and improves the glucose tolerance without altering the plasma insulin profile.[13, 14] In this study metformin was used for the evaluation of the change of blood glucose levels on the structure and function of male reproductive system. It was reported that, acute phase of diabetes develops during eight days till three weeks and chronic phase of diabetes occurs at three weeks or more after induction of diabetes.[9, 15]

The aims of this study were to investigate the long time effects of diabetes on the histology and morphology of testicular tissue, as an important endocrine organ of the reproductive system which involve in fertility/infertility; and to find out the relationships between the changes of blood glucose levels with the histological annotations of testicular tissue in adult male diabetic rats.

Materials and Methods

Animal procedure. Adult male Sprague-Dawley rats with a body weight 200 ± 20 g were used in this study. They were provided by the Center of Animal Housing and Breeding of the Faculty of Veterinary Medicine. The animals were placed in standard cages (2 animals per cage) for two month under 12-hour light: dark cycle with 23˚C-25˚C room temperature until reached desired body weight for beginning the study. All animals were received standard laboratory animal's chow and water ad labitum during the whole period of experiment. In this study, all animal procedures were carried out in accordance with the standards for human care and use of laboratory animals which has been approved by the university animal welfare committee.

Experimental Design. In this study, the animals were divided into three different experimental groups of 8 animals each: **(1)** *control:* normal and apparently healthy rats that did not receive any type of treatment; **(2)** *diabetic:* in the animals of this group the experimental diabetes was

induced by a single intraperitoneal injection of STZ with a dose of 45 mg kg^{-1} of body weight; **(3)** *diabetic + metformin:* this group consisted of STZ-induced diabetic rats that treated by metformin with a dose of 100 mg kg^{-1} of body weight per day by oral gavages method from two weeks after induction of diabetes till end of study. In the control group, instead of streptozotocin, same volume of citrate buffer was injected intraperitoneally. The animals in groups 1 and 2 received normal saline daily by oral gavages equal to group 3 from two weeks after induction of diabetes. The duration of experiment was 10 weeks after induction of diabetes, but metformin was given two weeks after induction of diabetes, and continued till end of study.[16, 17]

Treatments and chemicals. In this study, the streptozotocin (Sigma, ST. Louis, MO, USA) was used for induction of diabetes in rats. The STZ dissolved in 0.1 M citrate sodium buffer (pH = 4.5) and injected to overnight fasting animals. Diabetes was confirmed 48 hours after injection of STZ. For this aim, the blood of fasting animals collected from tail vein by use of 24 gauge needle and glucose levels determined with an automated glucose analyzer device (Glucometer, On Call EZ, SD, USA). The animals with blood glucose levels above 200 mg/dl were considered diabetic and were used in this study.[18] Two weeks after induction of diabetes, the animals of group 3 received freshly prepared metformin HCL (GLUCOPHAGE, Merck Sante s.a.s., LYON - FRANCE) dissolved in distilled water until to the end of study.

Body weight and blood collection. At the end of study, the weight of each animal was recorded. The overnight fasting rats were anesthetized with diethyl ether. For measurement of plasma glucose levels, the blood was immediately collected by cardiac puncture and plasma separated from the blood cells by centrifugation. Then, all blood plasma samples immediately stored at -20˚C until further analyses. Blood glucose levels were determined by spectrophotometery using the glucose-oxidase method (Unico 1200, Japan).

Physical parameters of gonads. After weighing and sacrificing of animals, the right and left gonads of each rat were separated from the body and their weight were recorded. Each testis was separated from its adjacent epididymis and the diameter and length of each testis was measured.

Tissue preparation for histological and histochemical study. For histological study, testicular tissues were immediately fixed in 10 % formaldehyde in buffered solution containing 54 mM NaH_2PO_4 and 28 mM Na_2HPO_4 (pH 7.4) and kept at 4˚C.[19] After 48 hours of the fixation period, the transverse section was made on the middle part of each testis and kept immersed in the fixative for the completion of tissue fixation. Then, formaldehyde-fixed samples were embedded in paraffin and sliced 6-7 micrometer thick and were mounted onto albumin-precoated glass slides. The mounted tissue samples were deparaffined with xylol and stained with the hematoxylin and eosin (H&E) method for histological observations using light microscopy. For histochemistry, the fixed frozen tissue specimens from the middle part of right and left testes sectioned with cryostate microtome then, sections were stained with Oil-Red-O and Sudan Black B techniques for detection of lipid accumulation in the cells of the seminiferous tubules and interstitial connective tissue.

Morphometeric analysis. For morphometeric assessment of seminiferous tubules, the slides were studied at 200 × magnification. To obtain more correct results, the seminiferous tubules that sectioned transversely were studied and the shortest diameter of them was considered for measurement. The analyses were performed from images obtained and digitalized using an Olympus DP70 digital

camera (Olympus Europe, Hamburg, Germany). The images were then processed by the computerized image analysis system software cell* (Olympus Soft Imaging Solutions GmbH, Münster, Germany). The scale bar was 200 μm and twenty tubules from each specimen were measured in different fields of tissue.

Evaluation of spermatogenesis in Testicular Tissue. For the estimation of spermatogenesis in testicular tissue, three different indices were used. Tubular differentiation index (TDI), repopulation index (RI) and spermiogenesis index (SPI). To determine the tubular differentiation index, the number of seminiferous tubules that have more than three layers of germinal cells derived from type A of spermatogonia was calculated. To find out the repopulation index, the ratio of active spermatogonia to inactive spermatogonia was calculated and to determine the spermiogenesis index, the ratio of the number of seminiferous tubules with spermatozoids to the empty tubules was calculated.[20, 21]

Statistical Analysis. Results were analyzed using the SPSS version 18. All data were reported as mean ± SEM. To evaluation of significant differences, the comparison of means between each two experimental groups was done by computer program for student-*t* test (paired t-test). Differences were considered to be statistically significant if $P < 0.05$.

Results

Effects of diabetes and metformin treatment on blood glucose levels. The mean blood glucose level in the untreated diabetic rats was significantly higher than metformin treated diabetic and control rats ($P < 0.05$) (Table 1). The mean blood glucose level in metformin treated diabetic rats was higher than of its level in control group, but this difference was not significant ($P < 0.05$).

Effects of diabetes and metformin treatment on body weight and physical parameters of gonads. The average body weight of animals in experimental groups is shown in Table 1. The body weight of animals in diabetic group was significantly ($P < 0.05$) decreased in comparison with control rats. Similarly, the mean body weight of metformin treated diabetic rats was significantly ($P < 0.05$) lower than untreated healthy rats. Diabetic animals that were treated with metformin showed more body weight loss in comparison with untreated diabetic rats, but this reduction in body weight was not statistically significant.

As Table 1 indicates, the weight of right and left testes in diabetic groups decreased significantly ($P < 0.05$) in comparison with the control group. The diameter of the right and left testis in diabetic rats decreased in comparison with control group but, this reduction was not statistically significant. Also, untreated diabetic rats had greater mean testis diameter in comparison with metformin treated diabetic rats but this difference also was not significant. The length of the right and left testis in the untreated diabetic rats was more than two other groups but this increase of length was not significant. Moreover, the mean length of the left testis in metformin treated diabetic rats was more than control rats but also this difference was not significant.

Morphometeric analysis of testicular tissue. As Table 2 shows, in the untreated diabetic rats the mean diameter of testicular capsule decreased in comparison with control and metformin treated diabetic rats but, this reduction was only significant between untreated diabetic and control rats ($P < 0.05$). In metformin treated diabetic rats, the diameter of testicular capsule was decreased but this reduction was not significant in comparison with the control group.

Table 1. Effects of streptozotocin induction of diabetes and metformin treatment on blood glucose level, body weight and gonadal physical parameters

	Control group	*Diabetic group*	*Diabetic+Metformin group*
Body weight (g)	257.500±4.902*†	160±8.976*	152.375±4.511†
Right gonad weight (g)	2.360±0.110*†	1.659±0.134*	1.239±0.194†
Left gonad weight (g)	2.275±0.045*†	1.663±0.152*	1.395±0.195†
Right testis diameter (mm)	11.50±0.018	11.25±0.041	10.50±0.053
Left testis diameter (mm)	11.42±0.029	11.14±0.034	10.57±0.048
Right testis length (mm)	17.87±0.044	18.25±0.036	17.00±0.056
Left testis length (mm)	17.42±0.029	18.57±0.042	17.71±0.042
Blood glucose levels (mg/dl)	158.87 ±9.3358*	261.750±8.7234*‡	167.875±4.1766‡

Results are given as mean ± SEM. *Significant difference between untreated control rats and untreated diabetic rats: $P < 0.05$, †Significant difference between untreated control rats and metformin treated diabetic rats: $P < 0.05$, ‡ Significant difference between untreated diabetic rats and metformin treated diabetic rats: $P < 0.05$. Absence of subscript indicates no significant difference.

The histomorphometeric study of seminiferous tubules showed that, the diameter of tubules decreased in diabetic rats during the course of diabetes. This reduction of diameter was significant between STZ-induced untreated diabetic and control rats ($P < 0.05$). These results were satisfied about the germinal epithelium as well, the height of germinal epithelium of STs decreased in both treated and untreated diabetic groups although, this reduction was significant between control and untreated diabetic rats ($P < 0.05$). About the width of lumen of STs, this parameter increased slightly in diabetic rats in comparison to control rats but, was not significant between experimental groups.

Effects of diabetes and metformin treatment on the epithelium of seminiferous tubules. The quantitative analysis of the cells of seminiferous tubules revealed that, in the untreated diabetic rats, the number of Sertoli cells significantly ($P < 0.05$) decreased in comparison with the control group. While, in metformin treated diabetic rats the number of Sertoli cells showed very little change (Table 3). In addition, untreated diabetic rats had smaller number (not significant) of Sertoli cells in their seminiferous tubules in comparison with metformin treated diabetics. In the untreated diabetic rats the average number of spermatogonia decreased in comparison with other groups but, this difference was not significant. As Table 3 shows, the number of primary spermatocytes reduced after development of diabetic condition and this reduction was significant among untreated diabetic rats, metformin treated diabetic and control rats ($P < 0.05$). In the untreated diabetic rats, the number round spermatids decreased in comparison with other groups but this difference was only significant ($P < 0.05$) between untreated diabetic and control rats (Table 3).

Effects of diabetes and metformin treatment on spermatogenesis. Table 4 shows the three indices determined for spermatogenesis. The tubular differentiation index and spermiation index were significantly ($P < 0.05$) reduced in both diabetic groups in comparison with the control group. Metformin treated diabetic rats had higher TDI and SPI in comparison with other diabetic group but these higher levels were not significant. The repopulation index in the untreated diabetic rats significantly ($P < 0.05$) reduced in comparison with other experimental groups. In this connection, this index reduced in metformin treated diabetic rats but this decline was not significant between this group and the control group.

Table 2. Morphometeric analysis of testicular capsule and seminiferous tubules in experimental groups after induction of diabetes and treatment with metformin

	Control group	*Diabetic group*	*Diabetic+Metformin group*
Capsule Diameter (µm)	61.662 ± 2.204*	53.548 ± 2.719*	55.071 ± 7.405
ST Diameter (µm)	467.304 ± 7.852*	394.015 ± 26.319*	426.650 ± 29.598
GE Height (µm)	148.121 ± 4.214*	108.521 ± 8.846*	125.441 ± 8.434
ST Lumen Diameter (µm)	171.061 ± 8.238	176.937 ± 11.803	175.767 ± 14.857

Results are given as mean ± SEM., * significant difference between untreated control rats and untreated diabetic rats: $P < 0.05$, Absence of subscript indicates no significant difference. ST indicates seminiferous tubule; GE indicates germinal epithelium.

Table 3. Effects of streptozotocin induction of diabetes and metformin treatment on the cellular population of germinal epithelium of STs in experimental groups

	Control group	*Diabetic group*	*Diabetic+Metformin group*
Sertoli cells	17.66 ± 1.115*	13.50 ± 1.5220*	17.16 ± 1.5147
Spermatogonia cells	60 ± 2.0816	44.83 ± 5.7062	58.33 ± 1.2292
Primary spermatocytes	60.33 ± 2.3475*	51 ± 2.1291*‡	59.66 ± 1.2560‡
Spermatids	176.66 ± 11.4183*	149.50 ± 11.9798*	162.16 ± 9.7482

Results are given as mean ± SEM. *Significant difference between untreated control rats and untreated diabetic rats: $P < 0.05$, ‡ Significant difference between untreated diabetic rats and metformin treated diabetic rats: $P < 0.05$, Absence of subscript indicates no significant difference.

Table 4. Effects of streptozotocin induction of diabetes and metformin treatment on spermatogenesis indices

	Control group	*Diabetic group*	*Diabetic+Metformin group*
TDI (%)	90.6412 ± 1.9382*†	79.8396 ± 1.9863*	82.4000 ± 1.6309†
SPI (%)	88.6903 ± 0.8404*†	72.6835 ± 4.1050*	79.1228 ± 1.3185†
RI (%)	82.7449 ± 2.9411*	60.5072 ± 3.2252*‡	78.6964 ± 4.0623‡

Results are given as mean ± SEM. *Significant difference between untreated control rats and untreated diabetic rats: $P < 0.05$, †Significant difference between untreated control rats and metformin treated diabetic rats: $P < 0.05$, ‡ Significant difference between untreated diabetic rats and metformin treated diabetic rats: $P < 0.05$, Absence of subscript indicates no significant difference.

Histological observations of testicular tissue. The histological investigations of testicular tissue in different groups demonstrated that in the untreated diabetic rats the STs were irregular in shape, the normal organization of germinal epithelium was reduced and the cells of germinal epithelium had abnormal cellular attachment as well, some extent depletion in spermatogenic cells was seen and in some severely affected tubules, the spermatogonia cells were the major cell type that seen (Fig. 2 and 3). While in the testicular tissue of the control rats the seminiferous tubules had compacted and organized germinal cells and all types of cells had normal cellular attachment also, five or more cell layers were seen in the epithelium of seminiferous tubules (Fig. 1). Moreover, in the untreated diabetic rats

the interstitial connective tissue had the amorphous material (Fig. 3). The multinucleated cells with two or three nucleus were seen in some of the seminiferous tubules in the untreated diabetic cases, while in other two experimental groups this type of cells were not observed (Fig. 4). The histological investigation of testicular tissue in metformin treated diabetic rats revealed that, the degree of histological changes of testis reduced in this group in comparison with untreated diabetic group. The comparison of the histology of testicular tissue between this group and the control rats showed no any remarkable differences in the architecture of testicular tissue (Fig. 5).

Histochemical study of testicular tissue. In the control group, the histochemical study of the samples stained with Oil-Red-O technique showed that, the positive reaction for lipid droplets existed mainly in developing spermatozoids and round spermatids. Positive reaction was also seen in Sertoli and spermatogonia cells but, the degree of reactivity was weaker than spermatozoids and round spermatids. The positive reaction sites were manifested as small granules in the cytoplasm of these cells (Fig. 6). In contrast, in the untreated diabetic rats, the histochemical analysis demonstrated remarkable positive reaction in spermatids and developing spermatozoids. Moreover, the lipid accumulation increased in the cytoplasm of Sertoli and spermatogonia cells and the large lipid droplets were seen in the cytoplasm of these cells (Fig. 7). These positive reactivity were also seen in the metformin treated diabetic rats, but this group showed improved condition since the amount of reactivity and the size of lipid droplets were decreased in comparison with untreated diabetic rats (Fig. 8). To confirm the results of Oil-Red-O method, the Sudan Black B technique was done. The results from this staining method (black spots) were similar and confirmed previous histochemical method (Figures not shown).

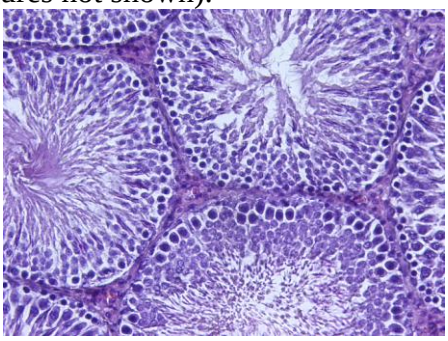

Fig 1. Light micrograph of testicular tissue of a rat from the control group. The seminiferous tubules have ordinary shape, their epithelium is structurally intact and shows normal association of germ cells. H&E (200×)

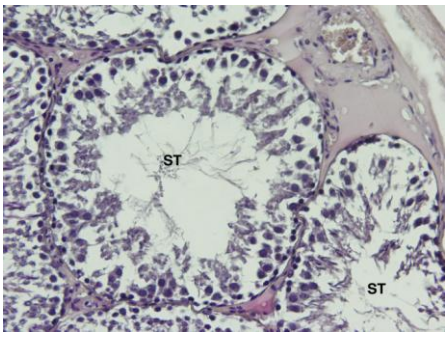

Fig 2. Cross section showing part of testicular tissue form untreated diabetic rat. The seminiferous tubules (ST) have irregular shape and the germinal epithelium is disorganized. Depletion of germ cells is seen. H&E (200×)

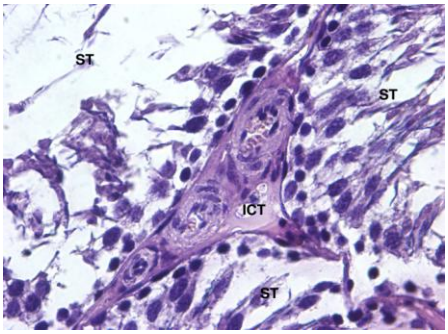

Fig 3. Cross section showing part of three seminiferous tubules (ST) of a rat from untreated diabetic group. The edema was attenuated in interstitial connective tissue (ICT). H&E (400×)

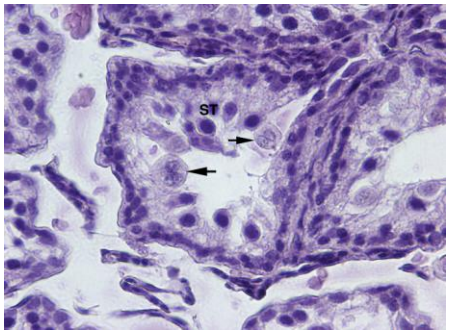

Fig 4. Light micrograph showing part of testicular tissue from an animal rendered diabetic. The giant cell formation with two or three nucleus (arrows) is seen in the lumen of irregular shaped seminiferous tubule (ST). H&E (400×)

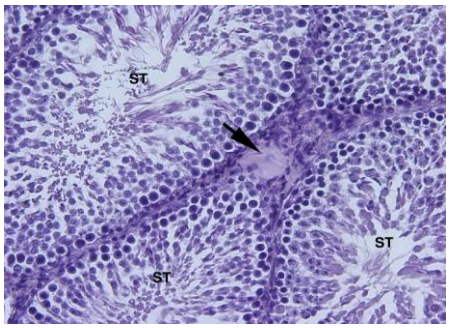

Fig 5. Cross section showing part of testicular tissue of a rat from metformin treated diabetic group. The histological changes are reduced and the seminiferous tubules (ST) have normal structure. The interstitial edema (arrow) was seen with less intensity. H&E (200×)

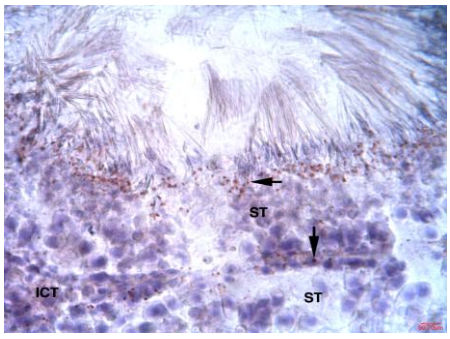

Fig 6. Cross section showing part of two seminiferous tubules (ST) and interstitial connective tissue (ICT) in testis from control rat. Red spots (arrows) are the positive reaction sites for lipid droplets. Oil-Red-O staining (400×)

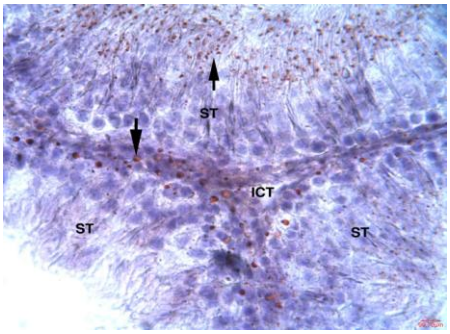

Fig 7. Cross section showing part of three seminiferous tubules (ST) and interstitial connective tissue (ICT) in testis from untreated diabetic rat. Red spots (arrows) are the positive reaction sites for lipid droplets. The number and the size of positive reaction sites increased after induction of diabetes. Oil-Red-O staining (400×)

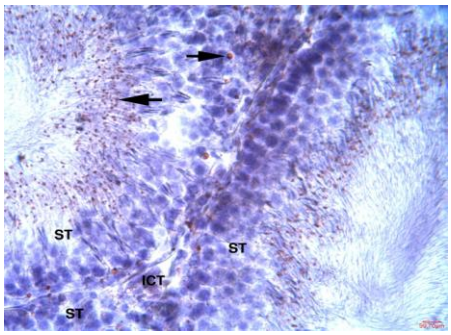

Fig 8. Cross section showing part of three seminiferous tubules (ST) and interstitial connective tissue (ICT) in testis from metformin treated diabetic rat. Red spots (arrows) are the positive reaction sites for lipid droplets. The number and the size of positive reaction sites decreased after treatment with metformin in comparison to untreated diabetic rats. Oil-Red-O staining (400×)

Discussion

Increase of blood glucose level leads to structural and functional changes in target organs of diabetic patients.[2] In our study, the mean blood glucose level of STZ-induced untreated diabetic rats was significantly increased above the normal level, and this elevation in blood glucose levels was approximately constant through the course of diabetes. According to Rossetti et al., reduction in pancreatic β-cell mass is associated with development of diabetes. In this study, streptozotocin was used for induction of diabetes. This

alkylating agent causes pancreatic β-cell death reducing the population of these cells. The overall effect of streptozotocin on β-cells leads to development of insufficient production of insulin and consequently, the elevation of blood glucose level occurs. In this study, the mean blood glucose level in the untreated diabetic rats was significantly higher than untreated healthy (control) and metformin treated diabetic groups. The metformin can reduce blood glucose levels in the presence of insulin; therefore, this drug can be used in treatment of patients with non insulin dependent diabetes mellitus.[14] This drug also reduces gluconeogenesis and increases insulin mediated glucose disposal.[1] Insulin resistance is the most important factor in development of diabetes mellitus, and metformin can reduce the resistance of target cells to insulin.[14] A mechanism by which metformin improves the insulin sensitivity in diabetic patients, may be through enhancement in binding of insulin to its receptors in several cell types.[1] According to Rossetti et al., metformin treatment of diabetic rats for six weeks leads to a significant improvement in glucose tolerance without any increase in insulin secretion. Metformin decreases blood glucose, but does not lower glucose levels below normal; thus, does not affect the blood glucose levels in nondiabetics.[22] In this study, the mean blood glucose level of metformin treated diabetic rats was reduced markedly in comparison with untreated diabetic rats, meanwhile the mean blood glucose level in this group was higher than untreated control rats. Our results indicate that, metformin treatment can reduce blood glucose levels in diabetic rats. These results are in accordance with the previous similar studies.[1, 13, 22] The blood glucose lowering effect of metformin is dependent to presence of insulin, therefore the results of our study indicate that, the STZ with a dose of 45 mg kg^{-1} of body weight of rats could not able to degenerate all pancreatic β cells and so did not lead to a complete lack of β-cell population.

Our experiment revealed that, the body weight of untreated diabetic rats significantly reduced during the course of diabetes. This reduction of body weight was also seen in metformin treated diabetics. The reduction of body weight can be due to breakdown of tissue proteins in diabetic rats.[1, 23] In this study, analysis of results from the measurement of body weight indicated that, treatment of diabetic rats by metformin with a dose of 100 mg kg^{-1} of body weight daily for 56 days has no effect on increase of body weight in diabetic rats. Some previous studies reported that, treatment of diabetic rats by metformin with a dose of 25 mg kg^{-1} of body weight for 28 days has no effect on increase of body weight. According to Yanardag et al., treatment of diabetic rats by metformin with a dose of 25 mg kg^{-1} of body weight caused more body weight loss in comparison with untreated diabetic rats. In our study, diabetic rats that treated with metformin showed more body weight loss in comparison to untreated diabetic group. Therefore, our results are in accordance with the results of similar previous studies.[1, 13, 22] Metformin can reduce the adipose tissue mass, therefore does not cause weight gain.[14, 24] In our study, a reduction of the weight of gonads was seen in both diabetic groups meanwhile, our results showed no significant differences in diameter and length of testes between experimental groups. This reduction of gonadal weight was accompanied with general body weight loss. The most gonadal weight loss was seen in metformin treated group which had more body weight loss in comparison with other groups. The ratio of gonadal weight to body weight in control group was 1.8 %, this ratio in metformin treated diabetic rats was 1.72 % and in the untreated diabetic rats was 2.07 %. The comparison of gonad/body weight ratio between experimental groups indicated that, the loss of gonadal weight in diabetic groups was dependant to body

weight changes. The elevation of this ratio in the untreated diabetic group seemed to be connected to accumulation of amorphous material in the interstitial connective tissue of testis. Therefore, this reduction of gonadal weight may be happened due to general body weight loss and/or reduction of the weight of epididymides (was not measured) in STZ-induced untreated and metformin treated diabetic rats.

This study shows the effects of diabetes on the histological structure of testis. The abnormal spermatogenesis in diabetic conditions was reported in several studies.[25, 26] Our results revealed that, in the untreated diabetic rats the spermatogenesis was disrupted in comparison with the control and metformin treated diabetic rats. Decrease of the diameter of seminiferous tubules was reported in previous studies.[2, 27] In the untreated diabetic rats, decrease in the diameter of STs was accompanied with depletion in the height of germinal epithelium; this finding indicates that, the STs became atrophied during the course of diabetes. These histological observations in STs, illustrated depressed cellular activity of spermatogenic cells. In our study, the number of spermatogonia cells decreased in diabetic testes, but this reduction was not significant. In the untreated diabetic rats, the number of primary spermatocytes decreased significantly in comparison with the control and metformin treated diabetic rats. This finding indicates that, the conversion of spermatogonia to primary spermatocytes is reduced in diabetic conditions. Diminished tubular differentiation and spermiation indices in diabetic rats confirm this change in cellular activities of seminiferous tubules. The reduction of repopulation index in the untreated diabetic rats shows that, the number of inactive spermatogonia increased after induction of diabetes and consequently, the number of primary spermatocytes derived from spermatogonia cells reduced. These alterations in cellular conversion and/or activity lead to reduction in spermatozoid production. Some previous studies reported the increase in interstitial area in testicular tissue of diabetic rats.[28] In this study, the increase in interstitial connective tissue and amorphous material was seen in testes of diabetic rats. This increase of interstitial connective tissue is associated with microvascular angiopathy in diabetic conditions.[29] Our results of histological changes of testicular tissue were similar and in accordance with the previous studies which reported the degeneration and necrosis of STs, giant cell formation and interstitial changes.[10, 11, 30] In diabetic rats treated with metformin, the all histologic observations seemed to be improved in comparison with untreated diabetic rats. These findings specify that, metformin can be able to diminish the side effects of diabetes on the function and structure of reproductive system through the lowering of the elevated blood glucose levels.

Histochemical results between experimental groups indicated that, untreated diabetic rats showed a considerable lipid content in their STs in comparison with other groups. Increase of lipid contents in the cells of testicular tissue may be due to a reduction in steroid biosynthesis because after induction of diabetes, the blood plasma levels of testicular steroids reduced in comparison with control rats (data was not shown). Another reason for the lipid accumulation in testicular tissue may be due to an interruption in glucose metabolism.[31] Because the glucose is the main source of the energy in germinal cells therefore, the reduction of glucose metabolism in diabetic conditions could result in lipid accumulation in affected testicular tissue.

It is concluded that, the blood glucose has an important and key role in the complications of diabetes mellitus.

Acknowledgments

We would like to thank all staff members of Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University for their help.

References

1. Yanardag R, Ozsoy-Sacan O, Bolkent S, et al. Protective effects of metformin treatment on the liver injury of streptozotocin-diabetic rats. Hum Exp Toxicol 2005; 24: 129-135.
2. Cai L, Chen S, Evans T, et al. Apoptotic germ-cell death and testicular damage in experimental diabetes: prevention by endothelin antagonism. Urol Res 2000; 28: 342-347.
3. Orth JM, Murray FT, Bardin CW. Ultrastructural Changes in Leydig Cells of Streptozotocin-induced Diabetic Rats. Anat Rec 1979; 195: 415-430.
4. Kuhn-Velten N, Waldenburger D, Staib W. Evaluation of Steroid Biosynthetic Lesions in Isolated Leydig Cells from the Testes of Streptozotocin-Diabetic Rats. Diabetol 1982; 23: 529-533.
5. Morimoto S, Mendoza-Rodriguez CA, Hiriat M, et al. Protective effect of testosterone on early apoptotic damage induced by streptozotocin in rat pancreas. J Endocrinol 2005; 187: 217-224.
6. Ballester J, Carmen Munoz M, Dominguez J, et al. Insulin-Dependent Diabetes Affect Testicular Function by FSH- and LH-linked Mechanisms. J Androl 2004; 25: 706-719.
7. Cameron DF, Orth J, Murray FT. Morphological alteration in the testes from diabetic man and rat. Diabetes 1982; 31: 11A.
8. Steger RW, Rabe M. The effect of diabetes mellitus on endocrine and reproductive function. Proc Soc Exp Biol Med 1997; 214: 1-11.
9. Ozdemir O, Akalin PP, Baspinar N, et al. Pathological changes in the acute phase of streptozotocin-induced diabetic rats. Bull Vet Inst Pulawy 2009; 53: 783-790.
10. Sanguinetti RE, Ogawa K, Kurohmaru M, et al. Ultrastructural changes in mouse Leydig cells after streptozotocin administration. Exp Anim 1995; 44: 71-73.
11. Ozturk F, Gul M, Agkadir M, et al. Histological alterations of rat testes in experimental diabetes. T Kin J Med Sci 2002; 22: 173-178.
12. Navarro-Cassado L, Juncos-Tobarra MA, Chafer-Rudilla M, et al. Effect of experimental diabetes and STZ on male fertility capacity. Study in rats. J Androl 2010; 108: 007260.
13. Rossetti L, De Fronzo RA, Gharezi R, et al. Effect of Metformin Treatment on Insulin Action in Diabetic Rats: In Vivo and In Vitro Correlations. Metabol 1990; 39: 425-435.
14. Bailey CJ, Turner RC. Metformin. New Eng J Med 1996; 334: 574-579.
15. Malder H, Ahren B, Sundler F. Islet amyloid polypeptide (amylin) and insulin are differentially expressed in chronic diabetes induced by streptozotocin in rats. Diabetologia 1996; 39: 649-657.
16. Yanardag R, Ozsoy-Sacan O, Bolkent S, et al. Protective effects of metformin treatment on the liver injury of streptozotocin-diabetic rats. Hum Exp Toxicol 2005; 24: 129-135.
17. Tanaka Y, Uchino H, Shimizu T, et al. Effect of metformin on advanced glycation endproduct formation and peripheral nerve function in streptozotocin-induced diabetic rats. Eur J Pharmacol 1999; 376: 17-22.
18. Venkateswaran S, Pari L. Antioxidant effect of Phaseolus vulgaris in streptozotocin-induced diabetic rats. Asia Pacific J Clin Nutr 2002; 11(3): 206-209.
19. Ballester J, Dominguez J, Carmen M, et al. Tungstate treatment improves Leydig cell function in streptozotocin-diabetic rats. J Androl 2005; 26(6): 706-715.

20. Shetty G, Wilson G, Huhtaniemi I, et al. Gonadotropin releasing hormone analogs and testosterone inhibits the recovery of spermatogenesis in irradiated rats. Endocrinology 2000; 141: 1735-1745.
21. Meistrich M, Wilson G, Porter K. Restoration of spermatogenesis in DBCP-treated rats by hormone suppression. Toxicol Sci 2003; 76(2): 418-426.
22. Ewis SA, Abdel-Rahman MS. Effect of metformin on glutathione and magnesium in normal and streptozotocin-induced diabetic rats. J Appl Toxicol 1995; 15: 387-390.
23. Andulla B, Varadacharyulu NCh. Antioxidant role of mulberry leaves in streptozotocin-diabetic rats. Clin Chim Acta 2003; 338: 3-10.
24. Stumvoll M, Nurjhan N, Perriello G, et al. Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. New Eng J Med 1995; 333: 550-554.
25. Cameron DF, Murray FT, Drylie DD. Interstitial compartment pathology and spermatogenic disruption in testes from impotent diabetic men. Anat Rec 1985; 213: 53-62.
26. Rossi GI, Aeschlimann M. Morphometric studies of pituitary gland and testes in rats with streptozotocin-induced diabetes. Andrologia 1982; 14: 532-542.
27. Guneli E, Tugyan K, Ozturk H, et al. Effect of melatonin on testicular damage in streptozotocin-induced diabetes rats. Eur Surg Res 2008; 40: 354-360.
28. Hassan G, Abdel Moneium T. Structural changes in the testes of streptozotocin-induced diabetic rats. Suez Canal Univ Med J 2001; 4(1): 17-25.
29. Williamson JR, Kilo C. Capillary basement membranes. Diabetes 1983; 32: 96-100.
30. Anderson JE, Thliveris JA. Testicular histology in streptozotocin-induced diabetes. Anat Rec 1986; 214: 378-382.
31. Farooqui SM, Al-Begdadi F, O´Donnel JM. et al. Degenerative changes in spermatogonia are associated with loss of glucose transporter (Glut 3) in abdominal testes of surgically induced unilateral cryptorchidism in rats. Biochem Biophys Res Commun 1997; 23