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Research Paper Physiology

Impact of L-Carnitine On Testicular Changesin Diabetic Rats

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ABSTRACT

About 90% of diabetic patients have disturbances in sexual function, including a decrease in libido, impotence and infertility.This investigation was conducted to evaluate the effects of diabetes on the structure and function of testicular tissue and to evaluate the effect of L-carnitine on these diabetic induced testicular changes. Diabetes was induced in male adult rats by a single intraperitoneal injection of streptozotocin. The rats were divided into 5 groups: control, diabetic and 3 treated groups using L-carnitine or insulin or both for four weeks in diabetic rats. The body and testicular weights together with hormonal and antioxidant enzymes parameters as well as histomorphological studies were affected significantly in diabetes. Results showed that L-carnitine had significant effect in improving the diabetic testicular changes and biochemical parameters. Administration of L-carnitine revealed a synergistic effect with insulin as a potentially beneficial agent to reduce testicular damage in adult diabetic rats, probably by decreasing oxidative stress and increasing the levels of gonadotropins which affect the spermatogenesis process.

KEYWORDS : Diabetes, gonadotropins, insulin, L-carnitine, spermatogenesis, Testis

Introduction

Diabetes mellitus is a serious metabolic disorder with numerous complications. 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(22). It is well known that diabetes mellitus is a degenerative disease that has deleterious effects on male reproductive function, possibly through an increase in oxidative stress (26). About 90% of diabetic patients have disturbances in sexual function, including a decrease in libido, impotence and infertility (22). 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 (26). The effects of diabetes were reported to the lack of insulin and subsequently the impairment of regulatory action of this hormone on both Leydig and Sertoli cells (26).

In fact, it has been observed that the function of the hypothalamic–pituitary–gonadal axis is highly sensitive to the adverse metabolic conditions prevailing in diabetes mellitus (17). Thepituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are the hormones that regulate gonadal function; their coordinated secretion and action allow for an extremely precise control of testicular function. The main target tissues for LH are testicular Leydig cells, where this gonadotropin regulates the local and systemic concentrations of sex steroids, mainly androgens (24). Reduction of insulin secretion in STZ-induced diabetic rats alters the spermatogenesis through the change in serum FSH levels (6). Moreover, the relationship between insulin and LH has been demonstrated and according to these findings insulin regulates serum LH levels (22).In this regard, it was well known that gonadal dysfunction and decrease in testosterone production lead to insufficient production of spermatozoids (17).

Attention has been paid to the search of effective drugs in the field of traditional medicine. Levocarnitine is a vitamin-like compound which is abundantly present in mammalian plasma and tissues (13). It plays an important role as a cofactor in cellular energy production in the mitochondrial matrix. L-carnitine aids in the transport of activated acyl groups across the mitochondrial inner membrane, and it is needed for the oxidation of long-chain fatty acids in the mitochondria of all cells (12). L-carnitine exhibits a wide range of biological activities including anti-inflammatory (16), cardioprotective (11), gastroprotective (9), antiapoptotic and neuroprotective properties (25). In several experimental and clinical studies on groups of patients with idiopathic oligoasthenospermia, the total carnitine level in their seminal plasma was found to be low (13). Mamoulakis*et al.* (18) proved that L-carnitine deficiency is often contributed in diabetes mellitus and it has been thought that it may play important role in development of its complications.

So, the aim of the present study was to investigate the effect of STZ-induced diabetes on the structure and function of testicular tissue and to evaluate the possible role of L-carnitine on the testicular function in diabetic rats.

Materialsand Methods

A. Experimental Animals

A total of fifty apparently healthy adult male wistar rats (12-16 weeks old) that initially weighed approximately 150-200 g were used in this study. The animals were purchased from and were housed under standard condition for 2 weeks before experiments were conducted at the Medical Experimental Research Centre (MERC) in Mansoura, Egypt. The rats were acclimatized for one week. They were kept in cages with five rats per cage in a controlled environment that was maintained under a 12 hour light/dark cycle, a temperature of 24°C (±3°C) and 50-70% humidity. The rats were supplied with a standard diet and water ad-libitum. Experiments were performed according to the *Guide for the Care and Use of Laboratory Animals* (Institute for Laboratory Animal Research, National Research Council, Washington, DC: National Academy Press, no. 85-23, revised 1996). This research was approved by the Medical Research Ethics Committee of Mansoura University.

Chemicals

L-carnitine was purchased from MEPACO-MEDIFOOD in the form of syrup (300 mg/ml L-carnitine). The L-carnitine syrup was diluted in a physiological saline solution and was subcutaneously administrated each day at a dose of 40 mg/kg body weight for 4 weeks (20). All rats were weighed once per week to calculate the average weight for each treatment; these weights were used to determine the required amount of L-carnitine and the volume of saline solution to obtain the calculated dose. Bovine insulin was injected subcutaneously (8-10 IU/day) for 4 weeks of treatment (36).

Experimental Design

A total of fifty rats were designated into five groups (10 rats each). : (1) control group (C), (2) diabetic group (D), (3) L-carnitine treated diabetic group (L) (4) Insulin treated diabetic group (I) and (5) Combined L-carnitine and insulin treated group (L&I).

In this study, the streptozotocin (Sigma, ST. Louis, MO, USA) was used for induction of diabetes in rats. Freshly prepared STZ (dissolved in citrate buffer of pH 4.5) in a dose of 45 mg/kg was administered intraperitone-

ally to the overnight fasting rats (8). An equal volume of vehicle (citrate buffer solution) was injected alone to each of the non diabetic control group. The rats were allowed to drink a 5% glucose solution overnight to overcome the drug-induced hypoglycemia. Plasma glucose levels were measured with an Accu-Check Go strip test in a glucometer. Blood sugar levels were measured before and after 48 h of STZ induction. After 48 h of STZ induction, the rats whose blood glucose levels were ≥300 mg/dl were considered as diabetic and were used in this study (35). From all rats used in this study, only 3 rats from group (D) and 2 rats from group (L) were deteriorated and died after induction of diabetes. The survived rats were sacrificed at the end of the treatment period.

Blood Sampling

Immediately after sacrificing, the blood from each animal was collected into clean centrifuge tubes. The blood was left to coagulate and was then centrifuged at 3000 rpm for 30 minutes to separate the serum. The separated serum was stored at -20°C for subsequent biochemical and hormonal analyses.Glucose concentrations were determined on a Hitachi 912 Chemistry Analyzer that used a hexokinase (Boehringer Mannheim, Indianapolis, IN) reaction whose reaction product was measured by spectrophotometric absorption at 340 nm (5).

ELIZA of Hormones

Serum insulin levels were determined by an enzyme-linked immunosorbent assay (ELISA) (DRG Instruments GmbH, Germany) using a commercial (Rat) kit (Crystal Chem, Chicago, Ill) and determined by a spectrophotometeric absorption at 450 nm(5). Serum testosterone levels were measured by another ELISA assay (DRG Instruments, Marburg, Germany) which is based on the principle of competitive binding between Testosterone in the test specimen and Testosterone-HRP conjugate for a constant amount of rabbit anti-Testosterone and determined by a spectrophotometeric absorption at 450 nm. Normal serum testosterone range is 0.1-18 ng/ml and its analytic sensitivity is 0.1 ng/ml. Serum FSH and LH levels were also determined by ELISA assays using a specific commercial kit supplied by Lab Service Co. (Egypt) according to the method of (19) and determined by a spectrophotometeric absorption at 450 nm. Detection range of serum FSH :2.47-200 ng/mL.The minimal detectable concentration of Rat Luteinizing hormone by this assay is estimated to be about 0.5ng/ml and its detection range is 0.5-20 ng/ml

Tissue Samplingfor Biochemical Analysis

The testes were weighed and homogenized for measuring dismutase, reduced glutathione and catalase activities. Superoxide dismutase activity was determined according to Nishikimi and collaborators (23), the reduced glutathione level was determined according to the method of Beutler and collaborators (7) and catalase activity was determined according to Aebi (4).

HistologicalExaminations

For histology, testes were fixed in Bouin's solution overnight. Histological sections (5µm) were prepared and stained with hematoxylin–eosin before being observed under a microscope (BH-2; Olympus, Tokyo, Japan).

Statistical Analysis

The data was analysed on SPSS version 17.0 (SAS 2004).[1] The mean and standard error of mean (SE) values were calculated for quantitative variables. Data between the groups were analyzed by using one-way analysis of variance (ANOVA), followed by post-hoc Tukey HSD. $P< 0.05$ was considered statistically significant.

Results

Table 1

Parameter (g)	(C)	(D)	(L)	(I)	(L&I)
Body weight	257.5±4.9	160±8.9*	190.3±6.7*#	220.4±5.5*#∞	235.8±3.8#∞ €
Rt . gonad	2.47±0.163	1.68±0.061*	1.94±0.09*	2.06±0.123*#	2.27±0.193#∞
Lt. gonad	2.34±0.045	1.66±0.154*	1.85±0.095*	2.18±0.066*#	2.21±0.072#∞

Data were expressed as mean ±SE, significance means ***P***<o.o5. *: significant as compared with control group, #: significant as compared with diabetic group, ∞: significant as compared with L-carnitine treated group and €: significant as compared with insulin treated group. Control group(C), Diabetic group (D), L-Carnitine treated group (L), Insulin treated group (I) and Insulin + L-Carnitine treated group (L&I)

Body Weight and Gonadal Weights

Changes in body and gonadal weight are shown in Table (1). All diabetic groups experienced a significant ($P< 0.001$) loss in body weight as well as gonadal weight as a result of STZ administration. This decrement was reduced significantly with administration of L-carnitine or insulin, and it was nearly diminished with (L&I) group as compared to control group.

Table 2

Parameter	(C)	(D)	(L)	(I)	(L&I)
Plasma glucose (mg/dl)	72.5±6.8	433.2±10.1*	288.4±15.8*#	150.6±14.9*#∞	106.4±8.9#∞ €
Serum insulin (µU/l)	50.03±18.63	20.63±7.61*	22.25 ±8.94*	30.89±6.87*#	35.16±12.05*#∞

Data were expressed as mean ±SE, significance means ***P***<o.o5. *: significant as compared with control group, #: significant as compared with diabetic group, ∞: significant as compared with L-carnitine treated group and €: significant as compared with insulin treated group. Control group(C), Diabetic group (D), L-Carnitine treated group (L), Insulin treated group (I) and Insulin + L-Carnitine treated group (L&I)

Levels of Plasma Glucose and Serum Insulinin Experimental Groups

The levels of plasma glucose were significantly increased ($P< 0.001$) in STZ-induced diabetic rats in comparison with Control group (from72.5±6.8 to 433.2±10.1) (Table 2). There was also, a significant ($P< 0.001$) reduction in the mean of insulin level in the STZ-induced diabetic group in comparison to the untreated control rats (from 50.03±18.6 to 20.63±7.61). There was significant reduction in glucose level in treated groups especially (L&I) group showed nearly normal glucose level giving insignificant changes ($P>0.05$) as compared with the control group (from 106.4±8.9 to72.5±6.8). The level of insulin did not show any improvement in L-carnitine treated group as compared with diabetic rats. Although, there was significantly increased in the level of insulin ($P< 0.05$) with treatment especially insulin treated group and combined treated group which gave significant increment as compared with diabetic group (from 20.63±7.61to 30.89±6.87 and 35.16±12.05) (Table 2).

Table 3

Parameter (ng/ml)	(C)	(D)	(L)	(I)	(L&I)
FSH	155.4 ± 15.28	75.7±8.93*	93.4 ± 5.12*#	112.4 ± 6.25#∞	130.3±9.9#∞ €
LH	4.5 ± 0. 5	1.5±0.13*	2.6 ± 0. 45*#	3.4 ± 0.26 *#∞	4.08 ± 0. 2#∞
Testosterone	2.36 ± 0.4	0.8±0.1*	1.5 ± 0.3*#	2.02 ± 0.3#∞	2.69 ± 0.2#∞€

Data were expressed as mean ±SE, significance means P<o.o5. *: significant as compared with control group, #: significant as compared with diabetic group, ∞: significant as compared with L-carnitine treated group and €: significant as compared with insulin treated group. Control group(C), Diabetic group (D), L-Carnitine treated group (L), Insulin treated group (I) and Insulin + L-Carnitine treated group (L&I)

SerumGonadotropins and Testosterone Levels in Experimental Groups

Table (3) showed that the levels of FSH reduced significantly ($P< 0.05$) in STZ-induced diabetic rats in comparison with Control group (from 155.4 ± 15.28 to 75.7±8.93). There was also, a significant ($P< 0.05$) reduction in the mean of LH level in the STZ-induced diabetic group in comparison to the untreated control rats (from 4.5 ± 0. 5 to 1.5±0.13). Together with significant reduction in blood testosterone level in diabetic group compared with control group (from 2.36 ± 0.4 to 0.8±0.1). As regards treated groups, there were significant elevation of all parameters as compared with diabetic group especially combined treated group results which approached control levels.

Table 4

Parameter	(C)	(D)	(L)	(I)	(L&I)
Catalase (U/mg)	3.69± 0.6	0.4±0.1*	1.6± 0.2*#	2.8± 0.4#	3.19±0.5#∞
GSH(mg/mg)	54.9 ± 2.1	20.5±3.2*	29.6±1.5*#	38.1 ± 3.2*#∞	50.6 ± 4.7#∞ €
SOD(U/mg)	83.6± 6.7	20.8±2.4*	37.4± 3.4*#	61.8 ± 4.6*#∞	75.2± 5.7#∞ €

Data were expressed as mean ±SE, significance means *P*<o.o5. *: significant as compared with control group, #: significant as compared with diabetic group, ∞: significant as compared with L-carnitine treated group and €: significant as compared with insulin treated group. Control group(C), Diabetic group (D), L-Carnitine treated group (L), Insulin treated group (I) and Insulin + L-Carnitine treated group (L&I)

Levels of Antioxidant Enzymes Activities in Experimental Groups

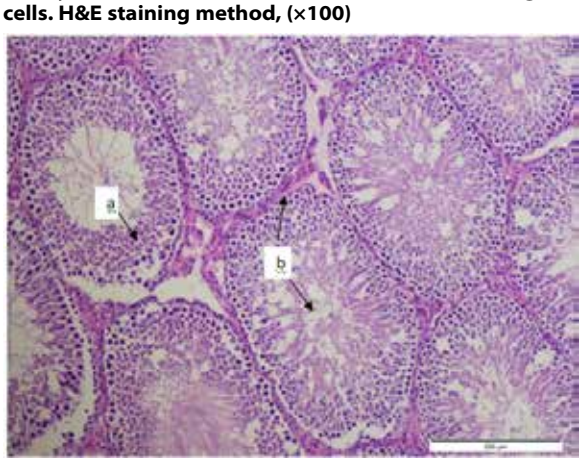
Table (4) showed significant decrease (*P*<0.001) in all antioxidant enzymes in diabetic group compared with control group. This decrease was significantly reduced with L-carnitine treatment. The levels of antioxidant enzymes increased significantly with insulin and approached normal with combined treatment.

Figures will be here

Evaluation of Testicular Changes in Hematoxylin-Eosin Stained Sections

It was observed that the testis tissue in the control group was covered with an albugineous layer and the complete seminiferous tubule cell series was present (Fig.1).

Fig.1. Light micrograph of testicular tissue of a rat belongs to the control group. The seminiferous tubules (ST) had compacted and organized germinal cells and all types of cells had normal cellular attachment with normal size and layering; their epithelium is structurally intact and shows normal association of germ cells. H&E staining method, (×100)



Moreover, 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). The histological investigations of testicular tissue demonstrated that in the untreated diabetic rats the seminiferous tubules were irregular in shape, the normal organization of germinal epithelium was reduced as well, some extent depletion in spermatogenic cells was seen and the interstitial connective tissue showed amorphous material (Fig.2). L-Carnitine significantly attenuated the diabetes-induced morphological changes and germ cell apoptosis in the diabetic rat testis (Fig.3). The testicular tissue in L- carnitine or insulin treated diabetic rats revealed that, the degree of histological changes of testis was reduced in these groups in comparison with untreated diabetic group (Fig.3 and 4). The comparison of the histology of testicular tissue between the combined treated group and the control rats showed no any remarkable differences in the architecture of testicular tissue (Fig. 5).

Discussion

Injecting a single dose of cytotoxic streptozocin reduced the β cell mass in islets of Langerhans and resulted in frank hyperglycaemia and insulin resistance (8). In our study, the mean blood glucose level of diabetic rats significantly increased with significant decrease in insulin level (Table 2). In treated groups the glucose levels were improved significantly and agreed with Molfino*et al.* (21) who has been documented that levocarnitine supplementation significantly improves insulin sensitivity and stimulates glucose oxidation in type 2 diabetic patients, thereby improving glycaemic control in T2DM. Also, Hadadinezhad group(15)have shown that L-carnitine increases the activity of the pyruvate dehydrogenase complex, which increases glucose catabolism.

Our experiment revealed that, the mean body weight of diabetic rats significantly reduced during the course of diabetes. The reduction of body weight can be due to the breakdown of tissue proteins in diabetic rats (26). In our study, a reduction of the weight of gonads was seen in diabetic group, this reduction of gonadal weight may happen due to testicular atrophy and/or reduction of the weight of epididymides (not measured) in diabetic rats.

The results of this study showed that, the mean blood level of FSH in diabetic rats experiencing a significant reduction in comparison to control rats. FSH acts synergistically with LH in stimulation of androgen synthesis therefore, the reduction of this gonadotropin can play an important role in decrement of testosterone output in diabetic animals as shown in Ballester*et al*. (6). In addition, high circulating glucose and its metabolites in diabetes increases the resistance of the testis towards these hormones, leading to low testosterone levels (30). In our study, the blood LH levels significantly lowered in diabetic rats, which is responsible for normal function of Leydigcells. The effect of insulin on Leydig cells is related to control of the cell proliferation and metabolism (28).

In our study, the mean blood testosterone level in diabetic rats decreased in comparison with untreated healthy rats. This reduction of testicular androgen synthesis agreed with previous studies (22, 31). It is quite logical that a decline in testosterone brings about a constitutional delay in the spermatogenetic processes such as germinal cells differentiation, meiosis and spermiogenesis. The histology data of the present study supported the changes occurring in the testes following the onset of diabetes. These parameters were significantly improved in treated groups and were consistent with reports by (33,32) who found that administration of a dietary supplement containing acetyl-L-carnitine has been shown to cause an increase in testosterone levels by increasing nitric oxide (NO) , that activates the release of luteinising hormone-releasing hormone (LHRH), and cyclic guanosine monophosphate (cGMP) levels via enhanced acetylcholine levels. In our study,the most important finding was that the combined treated group showed the most significant results that revealed a synergistic effect of insulin and L-carnitine on these hormones.

Enhanced oxidative stress and changes in antioxidant capacity are considered to play an important role in the pathogenesis of chronic diabetes mellitus. In hyperglycemia, the production of several reducing sugars (through glycolysis and polyol pathways) is enhanced. These reducing sugars can easily react with lipids and proteins (non enzymaticglycation reaction) (14), increasing the production of reactive oxygen species (ROS) (27). It can be concluded that the induction of free radicals by the cytotoxic effect of STZ caused a dysfunction of pancreatic β cells and resulted in the reduction of insulin secretion (10).

Abd-Allah *et al.* (1) and Yari*et al.* (35) studies also reported that L-carnitine affected the testicular function mainly by increasing the activity of antioxidant enzymes, which is reflected in the increased levels of catalase, superoxide dismutase, reduced glutathione and TAC that lead to reduced levels of free radicals. In our study, the administration of L-carnitine, insulin and a combination of both substances increased the levels of the antioxidant enzymes (i.e., catalase, reduced glutathione and superoxide dismutase), as shown in table (4). The testicular damage observed by the histological examination in diabetic rats was reversed by the administration of L-carnitine alone or with insulin owing to its antioxidant effect. The antioxidant effect of L- carnitine may have been due to the role of L-carnitine in the chelation of free Fe+2 ions with a subsequent reduction in free radical generation (29, 34) or its ability to enhance ATP produc-

tion, which improves the overall level and activity of antioxidant enzymes in the cell (3). The increased level of testicular antioxidant enzymes in the combination group may revealed a synergistic effect of insulin and L-carnitine on these enzymes.

In Conclusion,the results of the present study proved that, the testicular changes in diabetes which may be due to alterations in pituitary gonadotropins and subsequently testosterone. Enhanced oxidative stress is considered to play an important role in the pathogenesis of chronic diabetes mellitus. Administration of L-carnitine is a potentially beneficial agent in reducingthe testicular damage in adult diabetic rats, probably by decreasing oxidative stress and increasing the levels of gonadotropins which affect the spermatogenesis process.

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Fig.2. Cross section showing part of three seminiferous tubules (ST) of a rat from diabetic group. the seminiferous tubules were irregular in shape, the normal organization of germinal epithelium was reducedwith depletion in spermatogenic cells. H&E staining method (×200)

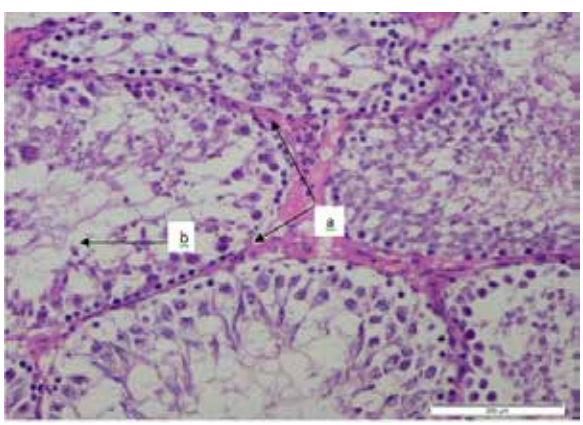


Fig.3. Light micrograph of testicular tissue of a rat belongs to the L-carnitine treated group. The irregularity of seminiferous tubules (ST) was reduced and there were some regeneration of the germinal epithelium and germ cells. H&E staining method (×100)

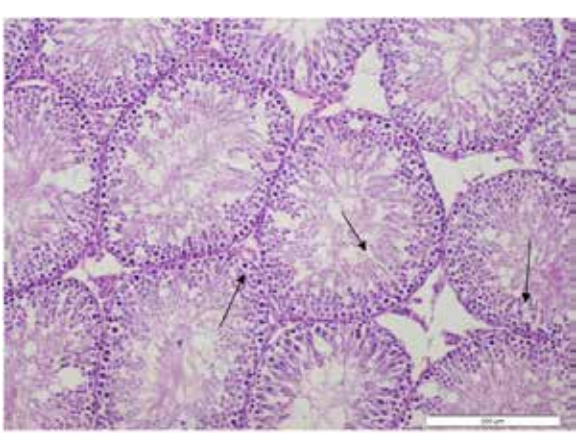


Fig.4. Cross section showing part of testicular tissue of a rat from Insulin treated diabetic group. The histological changes were reduced and the seminiferous tubules (ST) were increased in size with normal layering and regeneration of sertoli cells and spermatids formation. H&E (×100)

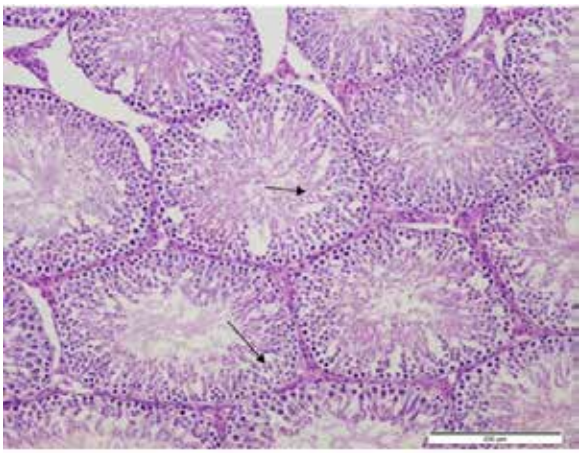
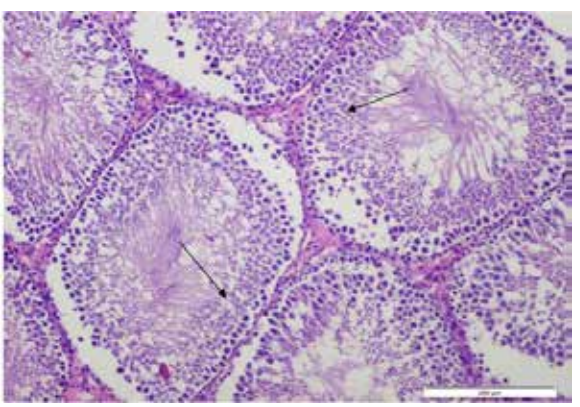


Fig.5. Light micrograph of testicular tissue of a rat belongs to the combined treatment group. The seminiferous tubules (ST) had ordinary shape with normal size and layering; their epithelium was structurally intactand showed normal association of germ cells. H&E staining method, (×100)



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