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Original Article

Green Tea Infusion Ameliorates Histological Damages in Testis and Epididymis of Diabetic Rats

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Abstract

Green tea is a popular drink used for therapeutic purposes to mitigate the consequences of diabetes. In this study, we aimed at evaluating the potential of green tea infusion to ameliorate structural and enzymatic damages caused by hyperglycemia in the testis and epididymis of Wistar rats. For that, nondiabetic and streptozotocin-induced diabetic rats (negative control and diabetes control, respectively) received 0.6 mL of water by gavage. Another set of diabetic animals received 100 mg/kg of green tea infusion diluted in 0.6 mL of water/gavage (diabetes + green tea) daily. After 42 days of treatment, the testes and epididymides were removed and processed for histopathological analysis, micromineral determination, and enzymatic assays. The results showed that treatment with green tea infusion preserved the testicular and epididymal histoarchitecture, improving the seminiferous epithelium and the sperm production previously affected by diabetes. Treatment with green tea reduced tissue damages caused by this metabolic condition. Given the severity of hyperglycemia, there was no efficacy of the green tea infusion in maintaining the testosterone levels, antioxidant enzyme activity, and microminerals content. Thus, our findings indicate a protective effect of this infusion on histological parameters, with possible use as a complementary therapy for diabetes.

Key words: antioxidant defense, *Camellia sinensis*, epididymal duct, hyperglycemia, pathology, sperm production

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Introduction

Diabetes mellitus is a chronic metabolic disease in which the individual has persistent hyperglycemia. The patient's clinical condition may be associated with either insufficient insulin production by pancreatic beta cells, characterizing type 1 diabetes, or tissue resistance to insulin, in the case of type 2 diabetes (La Vignera et al., 2012). Excessive glucose, characteristic of the hyperglycemic condition, results in molecular and cellular changes, leading to an increase in oxidative stress and vascular changes that result in significant complications on the metabolic status of patients. In type 1 diabetes, the absence of insulin production results in body mass loss, since insulin stimulates glucose uptake by cells altering their metabolism (Ueda-Wakagi et al., 2019).

Under diabetic conditions, body organs are more vulnerable to the imbalance of endogenous antioxidant defenses that, in turn, cause severe damages to the tissues, intensifying metabolic alterations and disturbing the body homeostasis (Nazem et al., 2015). These alterations also influence male reproduction. For instance, the persistence of the hyperglycemic state has been associated with cases of subfertility or infertility in men (Ding et al., 2015; Maresch et al., 2018). Indeed, diabetes induces reproductive disorders, such as alteration in the hypothalamus–pituitary–gonadal axis, reduction in serum testosterone levels, dysregulation of antioxidant enzymes with the generation of reactive oxygen species (ROS), and damage to testis and epididymis tissues (Murray et al., 1985; Sexton & Jarow, 1997; Bal et al., 2011; Martins et al., 2014). Likewise, experimental studies have shown that streptozotocin (STZ)-induced diabetes results in DNA damage and decreased sperm count in the testis, accompanied by a decrease in epididymal sperm motility (Shrilatha & Muralidhara, 2007; Navarro-Casado et al., 2010; Souza et al., 2019).

Overall, the testis is responsible for spermatogenesis and steroidogenesis, which guarantee the formation of sperm and testosterone, respectively (Alves et al., 2013). Through a series of cellular transformations, supported by Sertoli cells and indirectly by Leydig cells, there is the formation of sperm from spermatogonia (Dias et al., 2017), making these cells fundamental in organ homeostasis. However, the sperm released by the testes are still immobile and unable to fertilize. They will acquire these abilities during their transit in the luminal microenvironment of the epididymis, an organ attached to the testis, and responsible for sperm maturation and storage (Sullivan & Mieusset, 2016).

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In this context, structural or metabolic changes in these organs might result in potential fertility problems.

The use of insulin therapy and lifestyle changes are the most suitable treatments for the control of diabetes (Meneses et al., 2015). However, recently Wagner et al. (2021) clarified that insulin therapy alone did not protect the testis from damage caused by hyperglycemia. Thus, complementary treatments to the use of insulin can be interesting for the patient's well-being. In this context, the use of plant compounds has opened different perspectives in this research. For instance, green tea from *C. sinensis* is rich in catechins, which have great antioxidant potential (Hininger-Favier et al., 2009; Fu et al., 2017). The polyphenols present in tea have potential antidiabetic and anti-obesity (Gennaro et al., 2015). Studies have reported an improvement in testis and epididymis parameters of healthy rats (Abdelrazek et al., 2016; Mahmoudi et al., 2018; Hassan et al., 2019; Opuwari & Monsees, 2020), as well as an amelioration of sperm motility and germ cells epithelium in animals under diabetic conditions after treatment with green tea (Dias et al., 2017).

Although studies have already been carried out on the antidiabetic properties of green tea infusion, the effects of tea on the protection of the male reproductive organs of hyperglycemic rats are not yet fully understood. Thus, the present study aimed to evaluate whether the ingestion of green tea infusion can protect the testis and epididymis from possible damages caused by STZ-induced diabetes. For this, we analyzed the morphology and tissue stereology, the activities of antioxidant enzymes, and the mineral content in both organs, in addition to the hormonal status.

Materials and Methods

Preparation of the C. sinensis *Infusion*

Samples from five lots of Leão® — Food and Beverages (Coca-Cola Company®, lot LO159) were purchased and further homogenized to prepare the infusion. Leaves were mixed in distilled water (1:40 w/v), heated to 80°C, and stirred for 20 min to ensure adequate extraction of phytochemical content from the leaves of *C. sinensis* (Perva-Uzunalić et al., 2006). The mixture was filtered in a 0.45 μm porous paper filter, frozen at −80°C, and lyophilized. The lyophilized infusion powder was dosed and resuspended in distilled water at the time of administration.

Green Tea Infusion Phytochemical Analysis

The analysis of the total phenolic content was performed in triplicate using the Folin-Ciocalteau reagent. The result was expressed as milligrams of gallic acid equivalent per gram (g) of lyophilized samples of green tea infusion [mg GAE/g green tea infusion (gti)]. The antioxidant capacity of ferric-reducing antioxidant power (FRAP) and 2,2′-Azino-bis-[3-ethylbenzothiazoline-6-sulfonic acid] (ABTS) was measured using an ultraviolet (UV)-spectrophotometer (BEL UV-M51, BEL Photonics, Italy). The antioxidant capacity by the ABTS method was expressed in μmol of Trolox equivalent (TE) per g of lyophilized tea samples (μmol TE/g gti), and the antioxidant capacity by the FRAP method expressed as μmol of $FeSO_4$ equivalent per g of lyophilized samples of tea (μmol $FeSO_4$/g gti). The epigallocatechin-3-gallate (EGCG) content was analyzed using high-performance liquid chromatography (HPLC) (Prominence LC-20A, Shimadzu, Kyoto, Japan) and expressed by the %EGCG in the total of green tea infusion powder content.

Animals

Juvenile male Wistar rats ($n = 18$, 30 days old) were provided by the Central Animal Laboratory of the Center of Biosciences and Health of the Universidade Federal de Viçosa (UFV). Two animals were housed in each polypropylene cage and maintained under controlled temperature (22°C) and 12–12 h light/dark. They had free access to rat chow and drinking water. This study was conducted following the recommendations by the National Council for the Control of Animal Experimentation (CONCEA). All the experimental procedures were reviewed and approved by the Committee on the Ethics and Use of Animal Experiments of UFV (CEUA Process No. 53/2018).

Experimental Design

After one week of acclimatization, 12 animals were randomly selected and diabetes was induced by a single intraperitoneal (i.p.) STZ injection (Sigma Chemical Co., St Louis, MO, USA) at 60 mg/kg of body weight (BW) in 0.01 M sodium citrate buffer (pH 4.5) (Shrilatha & Muralidhara, 2007). After 2 days of induction, blood from the tail vein was collected to measure blood glucose levels using a glucometer (Accu-Chek® Performa, Roche LTDA). Animals presenting a blood glucose level higher than 250 mg/dL were included in this study. They were divided into two experimental groups of six animals each. While healthy rats (negative control) and diabetic animals (diabetes control) received 0.6 mL of water per gavage, other diabetic rats received 100 mg/kg BW of green tea infusion in 0.6 mL of water by gavage (Diabetes + green tea group). The dose administered corresponded to the 1,400 mL/day of green tea consumed by humans (Mineharu et al., 2011). Experimental treatments were provided daily and lasted 42 days, between the postnatal day (PND) 40 and PND 82. While rats on PND 40 are peripubertal males, on PND 82 they are considered sexually mature, comprising the final maturation period of the testis and epididymis (Picut & Remick, 2017). Finally, fasting blood glucose levels in blood samples from the tail vein and BW were recorded weekly during the test period.

Euthanasia and Tissue Collection

After 42 days of treatment, the animals were weighed and euthanized with deep anesthesia (sodium thiopental; 60 mg/kg i.p.). During sedation, blood was collected by cardiac puncture for hormonal analysis. The testes and epididymides were removed, dissected, and weighed. The organs on the left side were separated for biometric, histological, and stereological analyses, whereas the organs on the right side were frozen in liquid nitrogen and stored at −80°C for enzymatic and mineral determination. The tunica albuginea of the left testis of each animal was removed and weighed. The value obtained was subtracted from the total testicular weight to calculate the testicular parenchyma weight (Dias et al., 2020).

Serum Testosterone

The blood collected by cardiac puncture during euthanasia was centrifuged at 2,000×*g* for 15 min, and the serum was stored at −80°C for later use in the quantification of serum testosterone (Souza et al., 2016). The testosterone quantification was performed at the Veterinary Laboratory of Tecsa® (Belo Horizonte, Brazil), using the chemiluminescence methodology. The results were expressed in ng/dL.

Histological Processing

Left testes and epididymides were immersed in Karnovsky's fixative solution (2.5% glutaraldehyde, 4% paraformaldehyde in 0.1 M, pH 7.2 sodium phosphate buffer) for 24 h (Karnovsky, 1965). After the fixation period, the epididymides were segmented into four regions (initial segment, caput, corpus, and cauda). The fragments of these regions and fragments of the testes were dehydrated in crescent series of ethanol (70, 80, 90, and 100%) and embedded in 2-hydroxyethyl methacrylate (Historesin®, Leica Microsystems, Nussloch, Germany). Semi-serial sections of 3 μm thickness were obtained using a rotating microtome (RM 2255, Leica, Nussloch, Germany), stained with toluidine blue/sodium borate (1%), and mounted with Entellan (Merck, Germany).

Histopathology

Testis and epididymis tissues ($n = 6$/group) were evaluated qualitatively under light microscopy. In the former, 200 seminiferous tubules per animal were analyzed in non-serial sections considering the components of seminiferous tubules (lumen and seminiferous epithelium containing Sertoli and germ cells) as well as the components of the interstitium (Leydig cells, connective tissue, and blood and lymphatic vessels). Tubules that exhibited any change in the structure, such as cellular vacuolization, desquamation, displacement, and presence of cellular debris in the lumen, were classified as abnormal (Leite et al., 2017). Epididymal regions, in turn, were analyzed qualitatively according to the integrity of the duct structure, including epithelial morphology and luminal content. Hence, the presence of germ cells in the lumen, vacuoles in the epididymal epithelium, and inflammatory foci in the interstitium were considered histological alterations (Kempinas & Klinefelter, 2014).

Testicular Morphometry and Stereology

Morphometric and stereological analyses of the testis ($n = 6$/group) were performed using the Image-Pro Plus 4.5 software (Media Cybernetics, Silver Spring, MD, USA). To estimate the proportion of testicular parenchyma components, we used a grid with 266 intersections over 10 random images of the testicular parenchyma of each animal, obtained using a bright-field microscope (Olympus BX-53, Tokyo, Japan) equipped with a digital camera (Olympus DP73, Tokyo, Japan), totalizing 2.660 points per animal. Matching points were recorded in the tubular compartment (tunica propria, seminiferous epithelium, and lumen) and intertubule. The percentage of points in each component was calculated using the formula: volumetric proportion (%) = (number of points in the interest structure/2,660 total points) × 100. The volume (mL) of each testicular component was calculated through a formula: % of the interest structure × volume of the testicular parenchyma/100. Once the mammalian testis density is around 1, the weight was considered the same as the volume (Tae et al., 2005). The mean tubular diameter, epithelium height, and luminal diameter (μm) were obtained in 30 cross-sections of seminiferous tubules per animal, being used the tubules with the most circular contour (Johnson & Neaves, 1981).

The volumetric proportion of the intertubular components was obtained by counting 1,000 points in images on the intertubular compartment per animal. Points were counted on the nucleus and cytoplasm of the Leydig cell, lymphatic space, blood vessels, macrophages, and connective tissue. The percentage of points in each component was calculated using the formula: the volumetric proportion of intertubular compartment (%) = (number of points in intertubular component/1,000 total points) × 100. The volume of each intertubular component per testes (mL) was calculated by the formula: the proportion of the element on testis/(100 × parenchymal mass of one testis) (Russell et al., 1990).

The nuclear diameter of 30 Leydig cells was measured in 400× magnification, allowing the calculation of the volume occupied by its nucleus and cytoplasm, as well as the total volume of cells. The volume of the Leydig nucleus was obtained using the average nuclear diameter and the formula: $4/3\pi R^3$, in which R = nuclear diameter/2. The volume of Leydig's cytoplasm was estimated using the formula: percentage of cytoplasm × nuclear volume obtained/nuclear percentage. Cell volume was estimated by adding nuclear and cytoplasmic volumes (Russell et al., 1990; Dias et al., 2020). These values were expressed in μm^3. From the calculations presented by Dias et al. (2020), it was possible to obtain the total number of Leydig cells in the testis by dividing the total volume occupied by Leydig cells in the testicular parenchyma by the individual volume of a Leydig cell.

Germ Cell Counting

The germ cell and Sertoli cell counts were performed in sections of seminiferous tubules in stage 1 of the spermatogenesis cycle (Amann & Schanbacher, 1983). In 20 sections of seminiferous tubules, the numbers of type A spermatogonia, primary spermatocytes at preleptotene/leptotene, primary spermatocytes at pachytene, the number of round spermatids, and the number of Sertoli cells were counted. To obtain the corrected number of these cells, the diameter of the nucleus, or nucleolus in the case of Sertoli cells, was measured in 30 cells of each type and using the formula described by Amann & Almquist (1962), we obtained the corrected number of cells of the germinal epithelium. Afterward, it was possible to calculate the number of germ cells in the epithelium, mitotic index, meiotic index, Sertoli cell index, the number of Sertoli cells per testis, the total support capacity of the Sertoli cell, and spermatogenic yield. All formulas were described by Dias et al. (2019).

Daily sperm production was calculated using the formula: daily sperm production = total volume of the seminiferous tubule (μm^3) × corrected number of round spermatids/12.9 (duration of the seminiferous epithelium cycle in Wistar rats) × area cross-section of the seminiferous tubule at stage 1 of the seminiferous epithelium cycle (μm^2) × histological section thickness (3 μm) (Amann, 1961).

Epididymal Morphometry and Stereology

For epididymal morphometry ($n = 6$/group), digital images of the four epididymal regions, initial segment, caput, corpus, and cauda, were obtained using an optical microscope (Olympus BX-53, Tokyo, Japan) equipped with a digital camera (Olympus DP73, Tokyo, Japan). We measured the ductal diameter, epithelium height, and luminal diameter (μm) through 30 cross-sections per region. The proportion of epididymal components was performed using a grid with 266 intersections in 10 digital images, totaling 2,660 points per animal in each epididymal region. Coincident points were registered in the epithelium, lumen with and without sperm, lamina propria, smooth muscle, connective tissue, and blood vessels, whereas their proportion was calculated according to Souza et al. (2016).

Activity of Antioxidant Enzymes

The right testis was used to obtain 100 mg of tissue per animal (n = 5/group) to perform the enzymatic analysis. In the case of the epididymis, this organ was divided into two portions, proximal (initial segment + caput regions) and distal portions (corpus + cauda regions), to guarantee 100 mg each. The samples were homogenized in PBS (pH 7.4) and centrifuged at 3,500 × g for 10 min (5°C). The supernatant was used to measure the activities of catalase (CAT) (Aebi, 1984), superoxide dismutase (SOD) (Dieterich et al., 2000), and glutathione-S-transferase (GST) (Habig et al., 1974).

Mineral Microanalysis

The proportion of chemical elements in the testis and the proximal and distal regions of the epididymis (n = 5/group) was estimated in fragments of frozen organs (Ladeira et al., 2020). Briefly, the fragments were dried in an oven at 60°C for 96 h, coated with carbon (Quorum Q150 T, East Grinstead, West Sussex, UK), and analyzed in a scanning electron microscope (JEOL, JSM-6010LA) with a detector bypass silicon with X-ray radiation. The proportion of manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), and selenium (Se) was obtained, and the results were expressed as the average percentage value of the proportions between the elements present in the samples.

Statistical Analysis

The normality was analyzed using the Shapiro–Wilk test. Subsequently, the results were submitted to two separate Student's t-test according to previous studies (Souza et al., 2019; Ladeira et al., 2021). The first analysis evaluated the effectiveness of type 1 diabetes induction comparing healthy animals (negative controls) with STZ-induced diabetic animals (diabetes control). Another test was performed to compare the effect of green tea infusion in diabetic animals (diabetes + green tea) to their controls (diabetes control). Differences were considered significant when $p \leq 0.05$. The analyzes were performed using the GraphPad Prism 7.0 software (GraphPad Software Inc., San Diego, CA, USA). Results were expressed as mean ± standard deviation (mean ± SD).

Results

Validation of Male Reproductive Injuries Caused by STZ-Induced Diabetes

Serum glucose levels were higher in diabetic animals when compared to nondiabetic rats ($p \leq 0.05$), confirming the effectiveness of STZ in inducing diabetes (Table 1). As expected, diabetic rats showed lower final BW, testis and epididymis weights, and serum testosterone levels than nondiabetic rats ($p \leq 0.05$; Table 1). Rats under diabetes conditions showed other expected alterations in the testis, including morphometry and volumetry, germ cell count, DSP, and Leydig cells stereology, beside the impairment of antioxidant enzyme activity (Tables 2 and 3; Fig. 3).

Green Tea Infusion Phytochemical Analysis

The total amount of phenolic components in the green tea infusion powder was 3.88 ± 2.49 mg GAE/g gti. From this, 19.38% was detected as the EGCG content after analysis using HPLC methodology. The green tea infusion presented an antioxidant capacity of 3.26 ± 0.06 μmol TE/g gti by the ABTS assay and 46.38 ± 4.10 μmol $FeSO_4$/g gti by the FRAP assay.

Serum Glucose Levels, Biometric Parameters, and Serum Testosterone Levels in Diabetic Groups

Diabetic animals treated with green tea showed higher glucose levels than non-treated diabetic animals ($p \leq 0.05$; Table 1). Additionally, diabetic animals receiving green tea presented higher albuginea weight than their controls ($p \leq 0.05$; Table 1). The other biometric parameters did not differ between groups ($p > 0.05$; Table 1). The serum testosterone of diabetic animals treated with green tea did not change compared to untreated diabetic animals ($p > 0.05$; Table 1).

Table 1. Serum Glucose and Testosterone Levels and Biometric Data of Nondiabetic Animals (Negative Control Group) and STZ-Induced Diabetic Rats Treated or Not with Green Tea.

Parameters	Negative Control	Diabetes Control	Diabetes + Green Tea
Serum glucose levels (mg/dL)	85.38 ± 7.83	475.00 ± 33.14*	542.80 ± 42.20#
Initial BW (g)	84.26 ± 14.97	81.27 ± 9.46	81.75 ± 7.57
Final BW (g)	288.10 ± 44.16	93.08 ± 23.42*	99.75 ± 13.04
Testis (g)	1.51 ± 0.12	0.72 ± 0.30*	0.73 ± 0.17
Testis (g/100 g)	0.51 ± 0.03	0.72 ± 0.22	0.72 ± 0.07
Albuginea weight (g)	0.05 ± 0.01	0.02 ± 0.01*	0.04 ± 0.004#
Parenchyma weight (g)	1.46 ± 0.12	0.69 ± 0.29*	0.69 ± 0.17
Epididymis (g)	0.43 ± 0.04	0.13 ± 0.07*	0.13 ± 0.05
Epididymis (g/100 g)	0.14 ± 0.01	0.13 ± 0.06	0.12 ± 0.04
Testosterone (ng/dL)	77.08 ± 10.30	18.07 ± 5.24*	19.75 ± 3.24

Mean ± SD.
*Significant difference ($p \leq 0.05$) between negative control and diabetes control groups by Student's t-test.
#significant difference ($p \leq 0.05$) between diabetes control and diabetes + green tea groups by Student's t-test (n = 6 animals/group).

Table 2. Testicular Morphometry and Volumetric Proportion (%) of Testicular Parenchyma Components and Morphometry of Seminiferous Tubules in Stage 1 of Nondiabetic Animals (Negative Control Group) and STZ-Induced Diabetic Rats Treated or Not with Green Tea.

Parameters	Negative Control	Diabetes Control	Diabetes + Green Tea
Testicular morphometry			
Tubular diameter (μm)	275.00 ± 28.12	230.13 ± 7.65*	244.60 ± 8.61#
Luminal diameter (μm)	113.80 ± 7.92	97.84 ± 2.67*	104.64 ± 10.65
Epithelium height (μm)	80.57 ± 5.31	66.14 ± 3.71*	71.85 ± 1.31#
Volumetric proportion			
Seminiferous tubules (%)	87.84 ± 1.97	92.54 ± 0.99*	89.16 ± 0.99#
Seminiferous epithelium (%)	47.99 ± 1.24	45.58 ± 1.20*	48.99 ± 1.27#
Tunica propria (%)	7.61 ± 1.40	7.22 ± 1.58	5.59 ± 1.07
Lumen (%)	32.24 ± 1.48	39.74 ± 0.56*	34.58 ± 1.67#
Intertubule (%)	12.16 ± 1.97	7.46 ± 0.99*	10.84 ± 1.73#
Morphometry of tubular section in stage I			
Type A spermatogonia	5.34 ± 1.14	3.73 ± 0.27*	3.90 ± 0.32
Primary spermatocyte at preleptotene/leptotene	23.47 ± 1.73	20.99 ± 2.6	24. 96 ± 0.84#
Primary spermatocyte at pachytene	27.50 ± 1.90	23.30 ± 4.29	22.08 ± 3.76
Round spermatid	76.12 ± 2.07	42.88 ± 9.60*	57.15 ± 8.10#
Number of germinative cell	132.50 ± 3.27	90.91 ± 11.49*	108.10 ± 11.01#
Sertoli cell	7.93 ± 0.88	8.67 ± 1.07	8.32 ± 0.92
Mitotic index	4.59 ± 1.24	5.66 ± 0.98	6.41 ± 0.36
Meiotic index	2.78 ± 0.25	1.86 ± 0.41*	2.63 ± 0.47#
Sertoli cell index	9.68 ± 1.17	5.06 ± 1.52*	6.96 ± 1.49
Number of Sertoli cells (×10^6)	122.68 ± 19.26	136.40 ± 51.98	116.01 ± 30.92
Support capacity of Sertoli cells	16.87 ± 1.98	10.66 ± 2.12*	13.05 ± 2.12
Spermatogenic yield	14.76 ± 3.00	11.63 ± 3.20	14.71 ± 2.38
Daily sperm production (×10^6)	96.65 ± 4.32	21.64 ± 17.46*	54.48 ± 8.70#

Mean ± SD.
*Significant difference ($p \leq 0.05$) between negative control and diabetes control groups by Student's *t*-test.
#Significant difference ($p \leq 0.05$) between diabetes control and diabetes + green tea groups by Student's *t*-test ($n = 6$ animals/group).

Histopathology of Testis and Epididymis

The testis of nondiabetic animals showed seminiferous tubules exhibiting seminiferous epithelium with regular layers and distribution of germ cells in its structure, including Sertoli cells at the base of the epithelium, and no presence of epithelial vacuolization (Figs. 1a, 1b). The intertubule, in turn, was formed by Leydig cells with concentric nuclei as well as other tissue components, such as macrophages, lymphatic, and blood vessels (Fig. 1c). On the contrary, diabetic animals presented vacuolization at the base of the germinal epithelium, cell detachment, and a large number of cells in the lumen of the seminiferous tubules (Figs. 1d–1f). The testis of diabetic animals treated with green tea showed an attenuation of the alterations observed in testes rats from the diabetes control group, especially regarding the number of nucleated cells in the lumen of the tubule (Figs. 1g–1i).

Moreover, the epididymis of nondiabetic animals showed regular tissue architecture, with epithelium, lumen, and interstitium with no alterations in all duct regions (Figs. 2a, 2d, 2g, 2j). By contrast, diabetic rats presented vacuolization in most parts of the epididymal epithelium and a massive presence of germ cells in the lumen along the entire duct (Figs. 2b, 2b′, 2e, 2e′, 2h, 2h′, 2k, 2k′). This vacuolation remained present in the initial segment of the epididymis from diabetic animals treated with green tea. In these animals, we also observed a diminished presence of germ cells in the lumen of the four regions compared to rats from the diabetes control group (Figs. 2c, 2c′, 2f, 2f′, 2i, 2i′, 2l, 2l′).

Testicular Morphometry and Stereology, and Germ Cell Counting

Testis from diabetic animals treated with green tea infusion showed higher tubular diameter, epithelium height, and proportion of seminiferous epithelium and interstitium than non-treated diabetic rats ($p \leq 0.05$; Table 2). Moreover, diabetic animals receiving green tea showed a lower percentage of seminiferous tubules and lumen than non-treated diabetic animals, with an increase of intertubule proportion ($p \leq 0.05$; Table 2). The morphometry of luminal diameter and the proportion of tunica propria did not alter among animals from both diabetic groups.

With respect to the cell population in the seminiferous epithelium, animals from diabetes + green tea group showed a higher number of primary spermatocytes at preleptotene/leptotene,

Table 3. Volumetric Proportion (%) and Volume (mL) of the Intertubular Elements and Morphometry of Leydig Cells of Nondiabetic Animals (Negative Control Group) and STZ-Induced Diabetic Rats Treated or Not with Green Tea.

Parameters	Negative Control	Diabetes Control	Diabetes + Green Tea
Volumetric proportion			
Connective tissue (%)	0.50 ± 0.19	0.53 ± 0.27	0.62 ± 0.32
Lymphatic space (%)	4.39 ± 1.67	1.25 ± 0.35*	4.05 ± 1.08#
Blood vessel (%)	1.24 ± 0.68	2.53 ± 0.26*	1.20 ± 0.25#
Macrophages (%)	0.15 ± 0.07	0.18 ± 0.09	0.24 ± 0.08
Leydig cell (%)	6.10 ± 1.67	2.81 ± 0.40*	3.49 ± 0.51#
Volume			
Connective tissue (mL)	0.014 ± 0.004	0.006 ± 0.0009*	0.008 ± 0.006
Lymphatic space (mL)	0.12 ± 0.05	0.03 ± 0.01*	0.05 ± 0.04
Blood vessel (mL)	0.03 ± 0.01	0.01 ± 0.006	0.01 ± 0.006
Macrophages (mL)	0.004 ± 0.001	0.002 ± 0.001*	0.003 ± 0.001
Leydig cell (mL)	0.17 ± 0.04	0.04 ± 0.01*	0.04 ± 0.01
Morphometry of Leydig cells			
Nuclear diameter (μm)	6.81 ± 0.26	6.05 ± 0.32*	6.53 ± 0.29#
Nuclear percentage (%)	0.91 ± 0.22	0.66 ± 0.12*	0.83 ± 0.11#
Cytoplasm percentage (%)	5.19 ± 1.37	2.14 ± 0.39*	2.66 ± 0.53
Nuclear volume (μm^3)	166.08 ± 19.28	116.81 ± 18.41*	146.63 ± 19.34#
Cytoplasmatic volume (μm^3)	963.30 ± 122.20	381.40 ± 88.63*	527.32 ± 87.45#
Cell volume (μm^3)	1135.38 ± 121.49	498.21 ± 92.37*	674.97 ± 99.65#
Number of cells/testis ($\times 10^6$)	157.61 ± 48.31	78.51 ± 29.84*	78.12 ± 20.84

Mean ± SD.
*Significant difference ($p \leq 0.05$) between negative control and diabetes control groups by Student's *t*-test.
#Significant difference ($p \leq 0.05$) between diabetes control and diabetes + green tea groups by Student's *t*-test ($n = 6$ animals/group).

round spermatic, and germinative cells than rats from the diabetes control group ($p \leq 0.05$; Table 2). Rats from diabetes + green tea group also exhibited increased meiotic index and daily sperm production ($p \leq 0.05$; Table 2). The number and support capacity of Sertoli cells and the mitotic index did not differ between animals in both diabetes groups ($p > 0.05$; Table 2).

The volumetric proportion and the volume of most intertubular components did not change among diabetic rats after treatment with green tea, only the percentage of Leydig cells increased ($p \leq 0.05$; Table 3). Likewise, Leydig cells from testis of rats that received an infusion of green tea had a higher nuclear diameter, nuclear proportion, cytoplasmic and nuclear volume, and cell volume than Leydig cells from testes of non-treated diabetic animals ($p \leq 0.05$; Table 3). The percentage of cytoplasm and the number of cells/testis did not change between groups with diabetes ($p > 0.05$; Table 3).

Epididymal Morphometry and Stereology

Morphometric analyses of the initial segment epididymis revealed a decrease in ductal diameter, luminal diameter, and epithelium height in diabetic animals when compared to the same region in nondiabetic animals ($p \leq 0.05$; Table 4). Likewise, diabetic animals showed a reduction in the proportion of lumen with sperm and connective tissue, as well as an increase in the percentage of lumen without sperm, compared to their control rats. After green tea intake, in turn, the initial segment epididymis of diabetic animals showed higher luminal diameter and percentage of lumen with sperm than the initial segment of non-treated diabetic rats ($p \leq 0.05$; Table 4).

In the caput region, the ductal diameter and epithelium height decreased in the epididymis of diabetic animals compared to their controls. Additionally, diabetes caused a reduction in the proportion of lumen with sperm and an increase in the percentage of lumen without sperm and connective tissue ($p \leq 0.05$; Table 4). Diabetic animals treated with green tea showed an increase in the epithelium height and proportion of lumen with sperm, with a consequent reduction in the percentage of lumen without sperm ($p \leq 0.05$; Table 4).

The morphometry performed in the corpus region showed a decreased ductal diameter, epithelium height, and luminal diameter in the epididymis of diabetic animals compared to nondiabetic animals. The infusion of green tea restored all these parameters in the diabetes + green tea group ($p \leq 0.05$; Table 4). When analyzing the proportions of the epididymis components, only the proportion of lumen without sperm was increased in diabetes control animals compared to controls ($p \leq 0.05$). Green tea ingestion did not protect the organ against this loss ($p \leq 0.05$; Table 4).

In the cauda region, the ductal and luminal diameters and epithelium height were also lower in the epididymis of diabetic animals when compared to their controls ($p \leq 0.05$; Table 4). These parameters were ameliorated in diabetic animals treated with green tea ($p \leq 0.05$; Table 4). The proportion of lumen with sperm was lower in diabetic control animals, while the

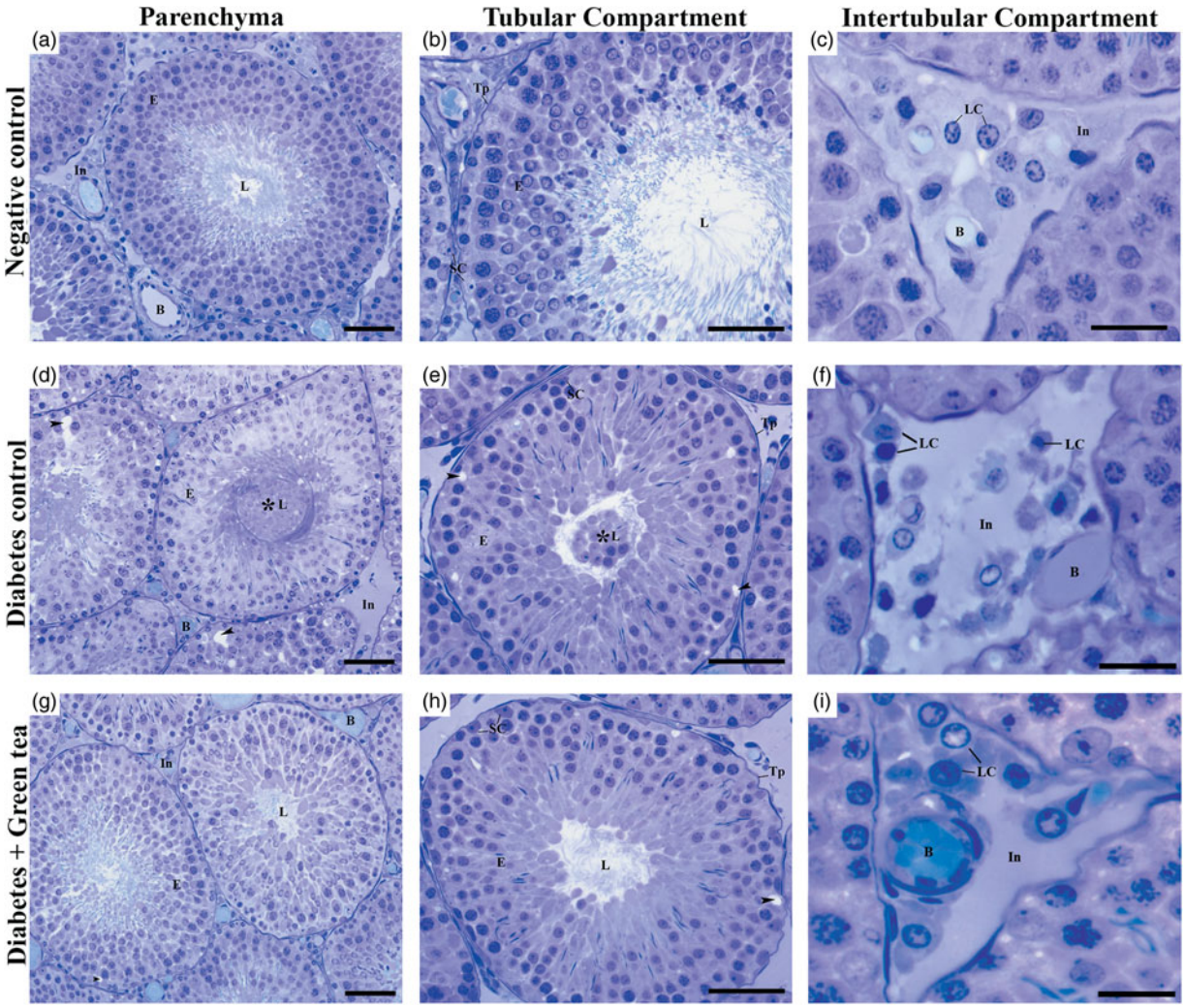

Fig. 1. Histological sections of the testis of nondiabetic animals (negative control) and STZ-induced diabetic Wistar rats treated with green tea infusion (diabetes + green tea) or not (diabetes control). (**a,d,g**) Negative control; (**b,e,h**) diabetes control; (**c,f,i**) diabetes + green tea. In, intertubular compartment; E, seminiferous epithelium; L, lumen; B, blood vessels; Tp, tunica propria; SC, Sertoli cell; Spg, spermatogonia; Sp, spermatocyte; Rs, round spermatid; LC, Leydig cell; arrowhead, vacuole; *, germ cell detachment. Toluidine blue. Scale bars: 50 μm.

proportion of lumen without sperm increased in these animals compared to their control rats. The infusion of green tea protected the region against these changes ($p \leq 0.05$; Table 4). Other results from stereological analysis that did no exhibit significant differences among groups are shown in Supplementary Table 1.

Activity of Antioxidant Enzymes in Testis and Epididymis Tissues

SOD activity was not altered in the testicular and epididymal portions of diabetic animals after ingestion of green tea ($p > 0.05$; Fig. 3a). Likewise, CAT activity did not change in these organs of rats in both groups with diabetes ($p > 0.05$; Fig. 3b). By contrast, diabetic animals treated with green tea showed lower activity of GST in the testis compared to diabetic animals ($p \leq 0.05$; Fig. 3c), exhibiting no alteration in the epididymis ($p > 0.05$; Fig. 3c).

Proportion of Trace Elements in Testis and Epididymis Tissues

Regarding the testis, only the proportion of Se decreased in the diabetic rats compared to nondiabetic animals ($p \leq 0.05$), with no differences between groups for the proportion of the other trace elements ($p > 0.05$; Fig. 4a). Moreover, diabetic animals presented lower proportion of Zn in the proximal epididymis of diabetic animals compared to their controls ($p \leq 0.05$; Fig. 4b). The ingestion of green tea did not alter the proportion of chemical elements in the testis and proximal epididymis of diabetic rats ($p > 0.05$; Figs. 4a, 4b).

In the distal portion of the epididymis, there were no significant differences between the nondiabetic rats and diabetic animals only for the chemical elements analyzed ($p > 0.05$; Fig. 4c). In diabetes + green tea group, in turn, green tea intake increased the proportion of Cu and reduced Fe compared to non-treated diabetic animals ($p \leq 0.05$; Fig. 4c). No changes were observed between diabetes groups for the other trace elements contained in the distal portion of the epididymis ($p > 0.05$; Fig. 4c).

Discussion

The results provide pioneer information concerning the protective effects of green tea on testis and epididymis of STZ-induced diabetic rats. We found that treatment with green tea infusion

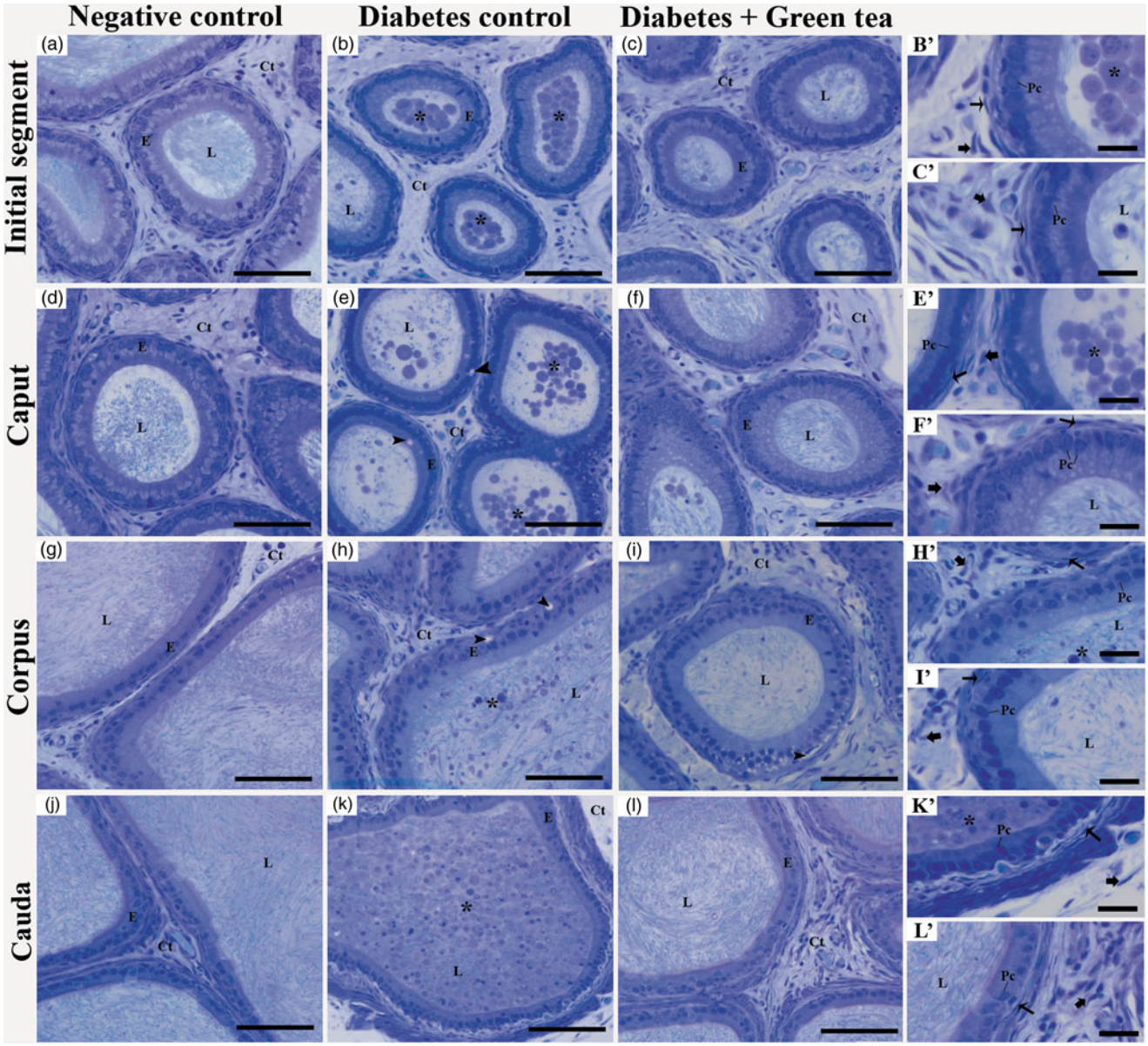

Fig. 2. Histological sections of the epididymis of nondiabetic animals (negative control) and STZ-induced diabetic Wistar rats treated with green tea infusion (diabetes + green tea) or not (diabetes control). (**a,d,g,j**) Negative control; (**b,b′,e,e′,h,h′,k,k′**) diabetes control, (**c,c′,f,f′,i,i′,l,l′**) diabetes + green tea. Ct, connective tissue; E, epithelium; L, lumen; Pc, principal cells; *, germ cells in lumen; arrowhead, vacuoles; thin arrow, smooth muscle cells; thick arrow, fibroblast. Toluidine blue. Scale bars: (**a–l**): 50 μm; (**b′,c′,e′,f′,h′,i′,k′,l′**): 10 μm.

attenuated the alterations in testis and epididymis tissues caused by the hyperglycemic state, reducing the occurrence of epithelial damages and ameliorating aspects related to spermatogenesis. However, this treatment showed no effective antidiabetic and antioxidant potential and did not combat low testosterone levels and the imbalance in the mineral content caused by the metabolic disease. For instance, the maintenance of the hyperglycemic state is related to the expression of glucose transporters 1 and 2 in STZ-induced animals, which elevated the glucose uptake and, consequently, its levels in the blood (Boyer et al., 1996; Wong et al., 2009).

As expected, hyperglycemia influenced the loss of BW. This metabolic condition alters cell metabolism, interfering with ATP synthesis, cell function, and hormone production (Anderson & Thliveris, 1986; Seethalakshmi et al., 1987). The latter, for example, was altered in STZ-induced diabetic rats from both groups. Those animals presented serum testosterone at low levels. This fact resulted in the loss of organs' weight and the disorganization of seminiferous epithelium, epithelial vacuolization, and cell detachment. Notably, testis and epididymis are well known as androgen-dependent organs (Mäkelä et al., 2019). Moreover, in STZ-induced diabetic animals, studies indicated that low testosterone level is a relevant factor acting on the morphometrical alterations observed here, such as reduction in the tubular diameter, the epithelial height, and the number of Sertoli and germ cells that, in the end, influenced the diminished DSP (Kianifard et al., 2012; Korejo et al., 2016; Das et al., 2017; Corrêa et al., 2019; Souza et al., 2019). Altogether, these results confirmed the adverse effects of diabetes on the testis and epididymis of Wistar rats.

In the current study, green tea infusion did not recover serum testosterone levels previously affected by diabetes. Studies

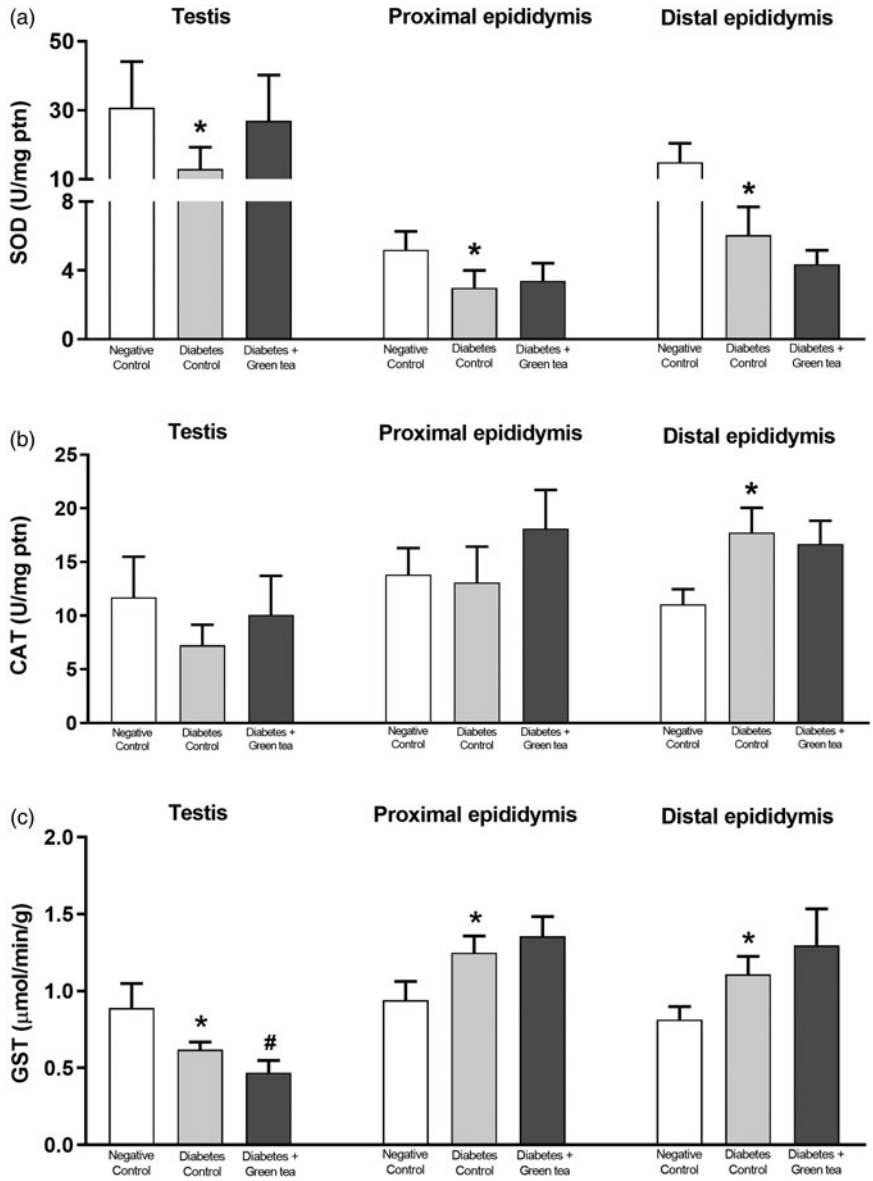

Fig. 3. Activity of antioxidant enzymes in the testis and epididymis of nondiabetic animals (negative control) and STZ-induced diabetic Wistar rats treated with green tea extract (diabetes + green tea) or not (diabetes control). (**a**) SOD, superoxide dismutase; (**b**) CAT, catalase; (**c**) GST, glutathione S-transferase. Mean ± SD. *Significant difference ($p \leq 0.05$) between negative control and diabetes control groups by Student's *t*-test. #Significant difference ($p \leq 0.05$) between diabetes control and diabetes + green tea groups by Student's *t*-test ($n = 5$ animals/group).

reported that the EGCG from green tea might exert a negative role in testosterone production. Catechin seems to act negatively on Leydig's cell activity, using cell-signaling pathways to inhibit hormone production. Chandra et al. (2011) and Das & Karmakar (2015) reported a decrease in its concentration in healthy animals treated with green tea extract. Figueroa et al. (2009), in turn, found a direct reduction in the production of this hormone by Leydig cells by inhibition of either the protein kinase A/protein kinase C signaling pathways or the steroidogenic enzymes 3β- and 17β-hydroxysteroid dehydrogenases.

In addition to the above, the treatment with green tea did not improve the oxidative environment elicited by diabetes in testis and epididymis, since it did not alter the activity of antioxidant enzymes, except testicular GST. This result may indicate a possible accumulation of superoxide ions and hydrogen peroxide in those tissues. In particular to hydrogen peroxide, the elevation of its concentration acts in the activity of CAT and GST, which work to neutralize this non-radical oxidant and avoid the oxidation of cell components, such as lipids, proteins, and nucleic acids. Thus, the reduced activity of GST in the testis of diabetic animals treated with green tea might reveal an ongoing ROS production that leads the GST enzyme to its exhaustion (Adewole et al., 2007), culminating with its reduction. The decreased proportion of Se in the testis may also contribute to this result by altering the activity of glutathione-related enzymes (Rotruck et al., 1973). While it is well characterized that diabetes disturbs the adaptative mechanism of the testis to act against the ROS (Souza et al., 2019), the antioxidant role of green tea is still controversial. Das et al. (2017) reported an increase in the activity of SOD and CAT in the testis of hyperglycemic rats treated with

Table 4. Morphometry of the Epididymal Regions of Nondiabetic Animals (Negative Control Group) and STZ-Induced Diabetic Rats Treated or Not with Green Tea.

Parameters	Negative Control	Diabetes Control	Diabetes + Green Tea
Ductal diameter (μm)			
Initial segment	158.34 ± 6.22	120.98 ± 9.70*	123.71 ± 3.82
Caput	258.44 ± 4.30	232.52 ± 24.34*	246.03 ± 15.55
Corpus	262.11 ± 21.07	184.72 ± 11.46*	206.93 ± 16.06#
Cauda	379.95 ± 33.41	243.54 ± 24.73*	282.32 ± 16.06#
Luminal diameter (μm)			
Initial segment	79.89 ± 10.64	67.12 ± 5.54*	58.79 ± 4.72#
Caput	197.40 ± 6.26	179.44 ± 25.80	185.00 ± 16.80
Corpus	201.91 ± 20.69	138.56 ± 7.74*	149.92 ± 15.65#
Cauda	324.91 ± 32.12	198.94 ± 21.72*	231.29 ± 16.18#
Epithelium height (μm)			
Initial segment	39.22 ± 2.96	26.93 ± 4.20*	32.45 ± 3.39
Caput	30.52 ± 2.26	26.53 ± 1.26*	30.51 ± 1.81#
Corpus	30.10 ± 0.58	23.07 ± 1.97*	28.50 ± 0.77#
Cauda	27.52 ± 1.08	22.29 ± 1.55*	25.51 ± 0.53#
Lumen with sperm (%)			
Initial segment	19.16 ± 0.99	8.00 ± 4.14*	16.77 ± 1.32#
Caput	53.82 ± 5.00	42.51 ± 1.41*	47.74 ± 1.72#
Corpus	46.37 ± 4.71	41.88 ± 9.12	43.60 ± 2.74
Cauda	57.12 ± 1.04	39.93 ± 3.73*	50.53 ± 2.83#
Lumen without sperm (%)			
Initial segment	3.72 ± 1.27	18.00 ± 6.61*	10.33 ± 7.97
Caput	3.84 ± 1.56	9.16 ± 0.78*	6.49 ± 1.24#
Corpus	0.40 ± 0.89	5.19 ± 2.56*	3.76 ± 0.56
Cauda	0.20 ± 0.44	12.43 ± 10.04*	2.13 ± 2.03#

Mean ± SD.
*Significant difference ($p \leq 0.05$) between negative control and diabetes control groups by Student's *t*-test.
#Significant difference ($p \leq 0.05$) between diabetes control and diabetes + green tea groups by Student's *t-test* ($n = 6$ animals/group).

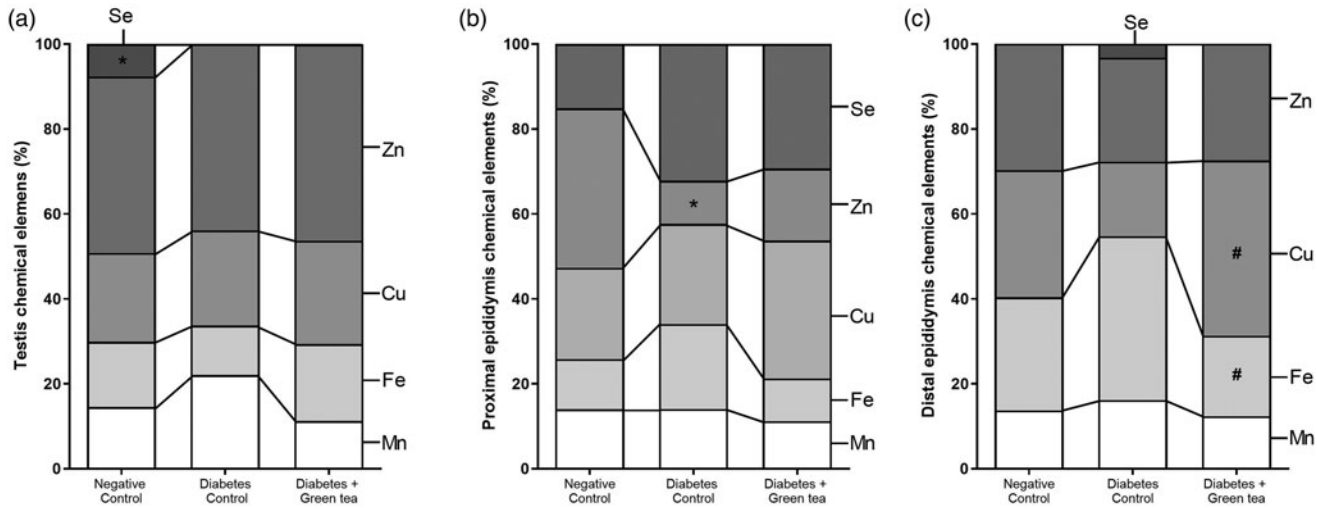

Fig. 4. Proportion of chemical elements in testis and epididymis of nondiabetic animals negative control) and STZ-induced diabetic Wistar rats treated with green tea extract (diabetes + green tea) or not (diabetes control). (**a**) Testis chemical elements, (**b**) proximal epididymis chemical elements, and (**c**) distal epididymis chemical elements. Manganese (Mn), iron (Fe), copper (Cu), selenium (Se), and zinc (Zn). Mean ± SEM. *Significant difference ($p \leq 0.05$) between negative control and diabetes control groups by Student's *t*-test. #Significant difference ($p \leq 0.05$) between diabetes control and diabetes + green tea groups by Student's *t*-test ($n = 5$ animals/group).

green tea, whereas Roychoudhury et al. (2017) described the pro-oxidant activity of green tea catechins in reproductive organs. Therefore, the maintenance of the oxidant environment in the testis of animals treated with green tea was due to the severe hyperglycemic state caused by STZ-induced diabetes, the drastic reduction in serum testosterone, and the oxidative potential (anti or pro) of catechins found in green tea.

Although the adverse antioxidant and hormonal environment, our findings revealed that green tea intake ameliorated testicular morphometric parameters primarily affected by diabetes. Herein, the increase in the tubular diameter, epithelial height, and the number of cell types of the seminiferous epithelium may reflect the spermatogenic status. Such changes can directly infer the production of male gametes (França & Russell, 1998), resulting in the improvement in the DSP observed in this study. Another fact that indicated a positive effect of green tea on the quality of the seminiferous tubules concerns the Sertoli cells. Although the ratio of the Sertoli cells was similar between diabetic animals treated or not with green tea, their seminiferous tubules and epididymal duct presented fewer germ cells inside their lumen. The hyperglycemic condition can affect the performance of the blood-testicular barrier and bring complications to the metabolism of Sertoli cells (Alves et al., 2013). For instance, the presence of vacuolization at the base of the seminiferous epithelium and the detachment of these cells with the consequent presence of nucleated cells in the lumen of testis and epididymis suggest a possible loss of Sertoli cells function in diabetic animals, impairing the adhesion between this cell type with germ cells (Lanning et al., 2002). Collectively, our findings may evidence an improvement in the Sertoli-Sertoli barrier by green tea, as reported by Kaplanoglu et al. (2013) and Mahmoudi et al. (2018). Dias et al. (2017) documented an increase in the glucose uptake by Sertoli cells *in vitro* promoted by the EGCG. The greater uptake by Sertoli cells may favor its perfect functioning despite the severe metabolic state. Thus, our results suggest that the green tea treatment may protect the tubular compartment and improve sperm production.

Furthermore, diabetic animals treated with green tea presented alterations in the proportion of intertubular components. The increase in blood vessels is one of the mechanisms associated with reduced fertility due to the hyperglycemic state, which causes an increase in the testis temperature with a consequent impairment in the spermatogenesis and Sertoli cell's functioning (Anderson & Thliveris, 1986). The decrease in the proportion of vessels by green tea ingestion may be explained by the antiangiogenic role of EGCG that inhibits the activity of members of the vascular endothelial growth factor family (Rashidi et al., 2017). In addition, the decrease in the volume and proportion of the lymphatic space of diabetic animals may characterize a problem in maintaining the circulation of fluids produced in the organ (Hirai et al., 2012). In the end, the improvement in the proportion of intertubular components in treated rats can guarantee a better functioning of the organ, maintaining homeostasis and providing an environment conducive to spermatogenesis.

Finally, green tea intake was able to improve stereological parameters of Leydig cells in diabetic animals. This cell type is stimulated by the luteinizing hormone (LH) produced by the pituitary gland. While studies have associated the hyperglycemic state with a decrease in serum LH levels (Seethalakshmi et al., 1987; Mohamed et al., 2018), other studies reported an increase in this hormone level after green tea intake (Chandra et al., 2011; Das & Karmakar, 2015). Hence, green tea may stimulate the LH releasing that, in turn, reached the Leydig cells activating their metabolism (Zirkin & Papadopoulos, 2018). This activation was sufficient to ameliorate the morphological and morphometrical parameters of those cells. However, this benefit was not enough to restore the serum testosterone levels and testis weight. Notably, the intratesticular testosterone levels are 10-fold higher than their serum levels (Turner et al., 1984). Possibly, the positive effect of green tea in testosterone production might be evident in a long-term period of treatment.

In the epididymis, the treatment with green tea was able to mitigate most of the morphometrical changes caused by diabetes. Morphometrical alterations are usually associated with sperm production and testosterone levels (Robaire & Viger, 1995; Hermo & Robaire, 2002; Souza et al., 2019). Despite the low testosterone, the improved DSP by green tea intake influenced the recovery of epididymal parameters, such as luminal diameter, epithelium height, and the percentage of the lumen with sperm in the epididymal regions. Notably, changes in these tissue structures can have consequences on the state of sperm fertilization ability (Hermo & Robaire, 2002). Likewise, the improvement of epididymal and sperm parameters in rats treated with green tea was also reported by Mahmoudi et al. (2018) and Azizi & Mehranjani (2019).

Conclusion

In conclusion, our results showed that the treatment with green tea infusion was effective in protecting testicular and epididymal histoarchitecture from damages caused by hyperglycemia, preserving tissue organization, and reducing histopathologies. However, green tea did not exhibit potential antidiabetics and antioxidants effects. The severe hyperglycemia along with low testosterone levels were the major factors for the maintenance of the altered hormonal and oxidative environment in diabetic rats, even with green tea intake. Nevertheless, our results shed light on new perspectives regarding the treatment of damages caused by hyperglycemia in male reproductive parameters. Further studies should be performed evaluating the potential of green tea treatment in such hyperglycemic conditions in combination with other antidiabetic substances to better understand whether the treatment becomes more effective.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S1431927621012071.

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References

Abdelrazek HMA, Helmy SA, Elsayed DH, Ebaid HM & Mohamed RM (2016). Ameliorating effects of green tea extract on cadmium induced reproductive injury in male Wistar rats with respect to androgen receptors and caspase-3. *Reprod Biol* **16**, 300–308.

Adewole SO, Caxton-Martins EA & Ojewole AO (2007). Protective effect of quercetin on the morphology of pancreatic beta-cells of streptozotocin-treated diabetic rats. *Afr J Trad* **4**(1), 64–74.

Aebi H (1984). [13] catalase in vitro. *Methods Enzymol* **105**, 121–126.

Alves MG, Martins AD, Cavaco JE, Socorro S & Oliveira PF (2013). Diabetes, insulin-mediated glucose metabolism and Sertoli/blood-testis barrier function. *Tissue Barriers* **1**, e23992.

Amann RP (1961). Reproductive capacity of dairy bulls. IV. Spermatogenesis and testicular germ cell degeneration. *Am J Anat* **110**, 49–67.

Amann RP & Almquist JO (1962). Reproductive capacity of dairy bulls. VIII. Direct and indirect measurement of testicular sperm production. *J Dairy Sci* **45**, 774–781.

Amann RP & Schanbacher BD (1983). Physiology of male reproduction. *J Anim Sci* **57**, 380–403.

Anderson JE & Thliveris JA (1986). Testicular histology in streptozotocin-induced diabetes. *Anat Rec* **214**, 378–382.

Azizi P & Mehranjani MS (2019). The effect of green tea extract on the sperm parameters and histological changes of testis in rats exposed to para-nonylphenol. *Int J Reprod BioMed* **17**, 717–726.

Bal R, Türk G, Tuzcu M, Yilmaz O, Ozercan I, Kuloglu T, Gür S, Nedzvetsky VS, Tykhomyrov AA, Andrievsky GV, Baydas G & Naziroglu M (2011). Protective effects of nanostructures of hydrated C60 fullerene on reproductive function in streptozotocin-diabetic male rats. *Toxicology* **282**, 69–81.

Boyer S, Sharp PA, Debnam ES, Baldwin SA & Srai SKS (1996). Streptozotocin diabetes and the expression of GLUT1 at the brush border and basolateral membranes of intestinal enterocytes. *FEBS Lett* **396**, 218–222.

Chandra AK, Choudhury SR, De N & Sarkar M (2011). Effect of green tea (*Camellia sinensis* L.) extract on morphological and functional changes in adult male gonads of albino rats. *Indian J Exp Biol* **49**(9), 689–697.

Corrêa LBNS, da Costa CAS, Ribas JAS, Boaventura GT & Chagas MA (2019). Antioxidant action of alpha lipoic acid on the testis and epididymis of diabetic rats: Morphological, sperm and immunohistochemical evaluation. *Int Braz J Urol* **45**, 815–824.

Das B, Biswas B, Ghosh A, Pakhira BP & Ghosh D (2017). Ameliorative role of ethyl-acetate fraction of methanolic leaf extract of *Camellia sinensis* (green tea) on streptozotocin-induced diabetes linked testicular hypofunction in albino rat: A dose-dependent biochemical and genomic transection study. *J Complement Integr Med* **14**, 1–13.

Das SK & Karmakar SN (2015). Effect of green tea (*Camellia sinensis* l.) leaf extract on reproductive system of adult male albino rats. *Int J Physiol, Pathophysiol Pharmacol* **7**, 178–184.

Dias FCR, Gomes MDLM, de Melo FCSA, Menezes TP, Martins AL, do Carmo Cupertino M, Otoni WC & da Matta SLP (2020). Pfaffia glomerata hydroalcoholic extract stimulates penile tissue in adult Swiss mice. *J Ethnopharmacol* **261**, 113182.

Dias FCR, Martins ALP, de Melo FCSA, do Carmo Cupertino M, Gomes MDLM, de Oliveira JM, Damasceno EM, Silva J, Otoni WC & da Matta SLP (2019). Hydroalcoholic extract of Pfaffia glomerata alters the organization of the seminiferous tubules by modulating the oxidative state and the microstructural reorganization of the mice testes. *J Ethnopharmacol* **233**, 179–189.

Dias TR, Alves MG, Silva J, Barros A, Sousa M, Casal S, Silva BM & Oliveira PF (2017). Implications of epigallocatechin-3-gallate in cultured human Sertoli cells glycolytic and oxidative profile. *Toxicol in Vitro* **41**, 214–222.

Dieterich S, Bieligk U, Beulich K, Hasenfuss G & Prestle J (2000). Gene expression of antioxidative enzymes in the human heart: Increased expression of catalase in the end-stage failing heart. *Circulation* **101**(1), 33–39.

Ding GL, Liu Y, Liu ME, Pan JX, Guo MX, Sheng JZ & Huang HF (2015). The effects of diabetes on male fertility and epigenetic regulation during spermatogenesis. *Asian J Androl* **17**, 948–953.

Figueroa MS, Vieira JSBC, Leite DS, Filho RCOA, Ferreira F, Gouveia PS, Udrisar DP & Wanderley MI (2009). Green tea polyphenols inhibit testosterone production in rat Leydig cells. *Asian J Androl* **11**(3), 362–370.

França LR & Russell LD (1998). The testis of domestic mammals. In *Male Reproduction: A Multidisciplinary Overview*, Martinez-Garcia F & Regadera J (Eds.), pp. 198–219. Madrid: Churchill Communications.

Fu QY, Li QS, Lin XM, Qiao RY, Yang R, Li XM, Dong ZB, Xiang LP, Zheng XQ, Lu JL, Yuan CB, Ye JH & Liang YR (2017). Antidiabetic effects of tea. *Molecules* **22**, 1–19.

Gennaro G, Claudino M, Cestari TM, Ceolin D, Germino P, Garlet GP, De Assis GF & Gronthos S (2015). Green tea modulates cytokine expression in the periodontium and attenuates alveolar bone resorption in type 1 diabetic rats. *PLoS One* **10**, 1–16.

Habig WH, Pabst MJ & Jakoby WB (1974). Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. *J Biol Chem* **249**, 7130–7139.

Hassan E, Kahilo K, Kamal T, Hassan M & Saleh Elgawish M (2019). The protective effect of epigallocatechin-3-gallate on testicular oxidative stress in lead-induced toxicity mediated by Cyp19 gene/estradiol level. *Toxicology* **422**, 76–83.

Hermo L & Robaire B (2002). Epididymal cell types and their functions. In *The Epididymis: From Molecules to Clinical Practice*, Robaire B & Hinton BT (Eds.), pp. 81–102. New York: Springer.

Hininger-Favier I, Benaraba R, Coves S, Anderson RA & Roussel AM (2009). Green tea extract decreases oxidative stress and improves insulin sensitivity in an animal model of insulin resistance, the fructose-fed rat. *J Am Coll Nutr* **28**, 355–361.

Hirai S, Naito M, Terayama H, Qu N, Kuerban M, Musha M, Ikeda A, Miura M & Itoh M (2012). The origin of lymphatic capillaries in murine testes. *J Androl* **33**, 745–751.

Johnson L & Neaves WB (1981). Age-related changes in the Leydig cell population, seminiferous tubules, and sperm production in stallions. *Biol Reprod* **24**, 703–712.

Kaplanoglu GT, Bahcelioglu M, Gozil R, Helvacioglu F, Buru E, Tekindal MA, Erdogan D & Calguner E (2013). Effects of green tea and vitamin E in the testicular tissue of streptozotocin-induced diabetic rats. *Saudi Med J* **34**, 734–743.

Karnovsky MJ (1965). A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J Cell Biol* **15**, 127–137.

Kempinas WG & Klinefelter GR (2014). Interpreting histopathology in the epididymis. *Spermatogenesis* **4**, e979114.

Kianifard D, Sadrkhanlou RA & Hasanzedh S (2012). The ultrastructural changes of the Sertoli and Leydig cells following streptozotocin induced diabetes. *Iran J Basic Med Sci* **15**(1), 623–635.

Korejo NA, Wei QW, Shah AH & Shi FX (2016). Effects of concomitant diabetes mellitus and hyperthyroidism on testicular and epididymal histoarchitecture and steroidogenesis in male animals. *J Zhejiang Univ Sci B* **17**, 850–863.

Ladeira LCM, dos Santos EC, Santos TA, Da Silva J, Lima GDA, Machado-Neves M, Silva RC, Freitas MB & Maldonado IRSC (2021). Green tea infusion prevents diabetic nephropathy aggravation in recent-onset type 1 diabetes regardless of glycemic control. *J Ethnopharmacol* **274**, 114032.

Ladeira LCM, dos Santos EC, Valente GE, da Silva J, Santos TA & Maldonado IRSC (2020). Could biological tissue preservation methods change chemical elements proportion measured by energy dispersive X-ray spectroscopy? *Biol Trace Elem Res* **196**, 168–172.

Lanning LL, Creasy DM, Chapin RE, Mann PC, Barlow NJ, Regan KS & Goodman DG (2002). Recommended approaches for the evaluation of testicular and epididymal toxicity. *Toxicol Pathol* **30**, 507–520.

La Vignera S, Condorelli R, Vicari E, D'agata R & Calogero AE (2012). Diabetes mellitus and minireview sperm parameters. *J Androl* **33**, 145–153.

Leite GAA, Figueiredo TM, Sanabria M, Dias AFMG, Silva PVE, Martins Junior ADC, Barbosa Junior F & Kempinas WDG (2017). Ascorbic acid supplementation partially prevents the delayed reproductive development in juvenile male rats exposed to rosuvastatin since prepuberty. *Reprod Toxicol* **73**, 328–338.

Mahmoudi R, Azizi A, Abedini S, Hemayatkhah Jahromi V, Abidi H & Jafari Barmak M (2018). Green tea improves rat sperm quality and reduced cadmium chloride damage effect in spermatogenesis cycle. *J Med Life* **11**, 371–380.

Mäkelä JA, Koskenniemi JJ, Virtanen HE & Toppari J (2019). Testis development. *Endocr Rev* **40**, 857–905.

Maresch CC, Stute DC, Alves MG, Oliveira PF, de Kretser DM & Linn T (2018). Diabetes-induced hyperglycemia impairs male reproductive function: A systematic review. *Hum Reprod Update* **24**, 86–105.

Martins AD, Alves MG, Bernardino RL, Dias TR, Silva BM & Oliveira PF (2014). Effect of white tea (*Camellia sinensis* (L.)) extract in the glycolytic profile of Sertoli cell. *Eur J Nutr* **53**, 1383–1391.

Meneses M, Silva B, Sousa M, Sá R, Oliveira P & Alves M (2015). Antidiabetic drugs: Mechanisms of action and potential outcomes on cellular metabolism. *Curr Pharm Des* **21**, 3606–3620.

Mineharu Y, Koizumi A, Wada Y, Iso H, Watanabe Y, Date C, Yamamoto A, Kikuchi S, Inaba Y, Toyoshima H, Kondo T & Tamakoshi A (2011). Coffee, Green tea, black tea and oolong tea consumption and risk of mortality from cardiovascular disease in Japanese men and women. *J Epidemiol Community Health* **65**, 230–240.

Mohamed NA, Ahmed OM, Hozayen WG & Ahmed MA (2018). Ameliorative effects of bee pollen and date palm pollen on the glycemic state and male sexual dysfunctions in streptozotocin-induced diabetic wistar rats. *Biomed Pharmacother* **97**, 9–18.

Murray FT, Cameron DF, Orth JM & Katovich MJ (1985). Gonadal dysfunction in the spontaneously diabetic BB rat: Alterations of testes morphology, serum testosterone and LH. *Horm Metab Res* **17**, 495–501.

Navarro-Casado L, Juncos-Tobarra MA, Cháfer-Rudilla M, De Onzoño LÍ, Blázquez-Cabrera JA & Miralles-García JM (2010). Effect of experimental diabetes and STZ on male fertility capacity. Study in rats. *J Androl* **31**, 584–592.

Nazem F, Farhangi N & Neshat-Gharamaleki M (2015). Beneficial effects of endurance exercise with Rosmarinus officinalis labiatae leaves extract on blood antioxidant enzyme activities and lipid peroxidation in streptozotocin-induced diabetic rats. *Can J Diabetes* **39**, 229–234.

Opuwari C & Monsees T (2020). Green tea consumption increases sperm concentration and viability in male rats and is safe for reproductive, liver and kidney health. *Sci Rep* **10**(1), 15269.

Perva-Uzunalić A, Škerget M, Knez Ž, Weinreich B, Otto F & Grüner S (2006). Extraction of active ingredients from Green tea (*Camellia sinensis*): Extraction efficiency of major catechins and caffeine. *Food Chem* **96**, 597–605.

Picut CA & Remick AK (2017). Impact of age on the male reproductive system from the pathologist's perspective. *Toxicol Pathol* **45**, 195–205.

Rashidi B, Malekzadeh M, Goodarzi M, Masoudifar A & Mirzaei H (2017). Green tea and its anti-angiogenesis effects. *Biomed Pharmacother* **89**, 949–956.

Robaire B & Viger RS (1995). Regulation of epididymal epithelial cell functions. *Biol Reprod* **52**, 226–236.

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG & Hoekstra WG (1973). Selenium: Biochemical role as a component of glutathione peroxidase. *Science* **179**, 588–590.

Roychoudhury S, Agarwal A, Virk G & Cho CL (2017). Potential role of green tea catechins in the management of oxidative stress-associated infertility. *Reprod BioMed Online* **34**, 487–498.

Russell L, Ettlin R, Sinhahikim A & Clegg E (1990). Mammalian spermatogenesis. In *Histological and Histopathological Evaluation of the Testis*, Russel L, Ettlin R, Sinha Hikim A & Clegg E (Eds.), pp. 1–40a. Florida: Cache River Press.

Seethalakshmi L, Menon M & Diamond D (1987). The effect of streptozotocin-induced diabetes on the neuroendocrine-male reproductive tract axis of the adult rat. *J Urol* **138**, 190–194.

Sexton WJ & Jarow JP (1997). Effect of diabetes mellitus upon male reproductive function. *Urology* **49**, 508–513.

Shrilatha B & Muralidhara (2007). Occurrence of oxidative impairments, response of antioxidant defences and associated biochemical perturbations in male reproductive milieu in the streptozotocin-diabetic rat. *Int J Androl* **30**, 508–518.

Souza ACF, Bastos DSS, Sertorio MN, Santos FC, Ervilha LOG, de Oliveira LL & Machado-Neves M (2019). Combined effects of arsenic exposure and diabetes on male reproductive functions. *Andrology* **7**, 730–740.

Souza ACF, Marchesi SC, Ferraz RP, De Almeida Lima GD, De Oliveira JA & Machado-Neves M (2016). Effects of sodium arsenate and arsenite on male reproductive functions in Wistar rats. *J Toxicol Environ Health A* **79**, 274–286.

Sullivan R & Mieusset R (2016). The human epididymis: Its function in sperm maturation. *Hum Reprod Update* **22**, 574–587.

Tae HJ, Jang BG, Ahn DC, Choi EY, Kang HS, Kim NS, Lee JH, Park SY, Yang HH & Kim IS (2005). Morphometric studies on the testis of Korean ring-necked pheasant (*Phasianus colchicus karpowi*) during the breeding and non-breeding seasons. *Vet Res Commun* **29**, 629–643.

Turner TT, Jones CE, Howards SS, Ewing LL, Zegeye B & Gunsalus GL (1984). On the androgen microenvironment of maturing spermatozoa. *Endocrinology* **115**, 1925–1932.

Ueda-Wakagi M, Nagayasu H, Yamashita Y & Ashida H (2019). Green tea ameliorates hyperglycemia by promoting the translocation of glucose transporter 4 in the skeletal muscle of diabetic rodents. *Int J Mol Sci* **20**, 24–36.

Wagner IV, Klöting N, Savchuk I, Eifler L, Kulle A, Kralisch-Jäcklein S, Dötsch J, Hiort O, Svechnikov K & Söder O (2021). Diabetes type 1 negatively influences Leydig cell function in rats, which is partially reversible by insulin treatment. *Endocrinology* **162**, 4.

Wong TP, Debnam ES & Leung PS (2009). Diabetes mellitus and expression of the enterocyte renin-angiotensin system: Implications for control of glucose transport across the brush border membrane. *Am J Physiol Cell Physiol* **297**, 601–610.

Zirkin BR & Papadopoulos V (2018). Leydig cells: Formation, function and regulation. *Biol Reprod* **99**(1), 101–111.