

Diabetes-Induced Testicular Injury: Potential Protective Roles of Curcumin, *Garcinia kola*, and *Momordica charantia* in Rats

Ayşenur Kaya Mutlu^{1,2}  | Süleyman Kaplan^{2,3} 

¹Department of Histology and Embryology, Faculty of Medicine, Karamanoğlu Mehmetbey University, Karaman, Türkiye | ²Department of Histology and Embryology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye | ³School of Life Sciences and Bioengineering, Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania

Correspondence: Süleyman Kaplan (skaplan@omu.edu.tr; skaplanomu@yahoo.com)

Received: 5 March 2026 | **Revised:** 13 June 2026 | **Accepted:** 30 June 2026

Keywords: curcumin | diabetes mellitus | *Garcinia kola* | *Momordica charantia* | testis

ABSTRACT

Background: Diabetes mellitus (DM) induces multifaceted cellular and structural alterations in the male reproductive system, thereby predisposing to infertility.

Objectives: This study aimed to evaluate the morphological, stereological, biochemical, histopathological, and ultrastructural changes induced by DM in testicular tissue, and to investigate the potential therapeutic effects of curcumin (Cur), *Garcinia kola* (GK), and *Momordica charantia* (MC) in the diabetic testis.

Materials and Methods: Sixty 12-week-old male Wistar albino rats were randomly divided into six groups: Control, Sham, DM, DM+Cur, DM+GK, and DM+MC. No intervention was applied to the Control group, whereas olive oil was administered by oral gavage to the Sham group. Experimental DM was induced by a single intraperitoneal injection of streptozotocin (50 mg/kg). In the antioxidant-treated groups, rats received Cur (100 mg/kg), GK (100 mg/kg), or MC (100 mg/kg) by oral gavage for four weeks. Stereological analyses were performed using the Cavalieri principle to calculate volume fractions of Sertoli and germ cells. Immunohistochemical analyses were performed for caspase-3, claudin-11, and occludin.

Results: DM was associated with increased oxidative stress, reduced germ cell volume fractions, decreased claudin-11 and occludin expression, and lowered serum testosterone levels. None of the antioxidants significantly lowered fasting blood glucose levels compared to the untreated diabetic group. Cur partially attenuated oxidative stress and improved germ cell volumes. GK modulates apoptotic activity, alters the expression and localization of tight junction proteins, and provides marked morphological protection. Although MC increased tight junction protein expression, its protective effects were limited by persistent disruption of tight junction localization and elevated apoptosis.

Conclusions: Cur, GK, and MC exert varying degrees of partial protection against DM-induced testicular damage. Their effects might be potentially mediated by antioxidant, anti-inflammatory, antidiabetic, and anti-apoptotic mechanisms, highlighting their potential to preserve testicular structure under diabetic conditions; however, further validation is required to determine optimal dosing and the full therapeutic mechanisms.

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1 | Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder that constitutes a major global health burden due to its steadily increasing prevalence and association with multiple systemic complications [1]. Approximately 589 million adults worldwide were affected by DM in 2025, with projections indicating a rise to 853 million by 2050 [2]. Although genetic predisposition plays a role, lifestyle-related factors such as aging, urbanization, unhealthy dietary habits, and physical inactivity are considered the primary drivers of this increase [3].

The increasing tendency of diabetes to occur at younger ages may render infertility problems among men of reproductive age more prevalent [4, 5]. DM leads to impairment of spermatogenesis, a decline in sperm quality, and consequently contributes to male infertility either directly or indirectly, particularly through diabetes-related complications such as diabetic neuropathy [6]. Chronic hyperglycemia disrupts the hypothalamic–pituitary–gonadal (HPG) axis, leading to reduced secretion of gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone, which in turn contributes to testicular and stromal atrophy, seminiferous tubule damage, and loss of spermatogenic cells [7].

The blood–testis barrier (BTB), which preserves the microenvironment required for normal spermatogenesis, is formed by gap and tight junctions between Sertoli cells [8]. Elevated glucose levels have been shown to inhibit the expression of tight junction proteins claudin-11 and occludin [9–11]. Reduced expression of these proteins disrupts BTB integrity, allowing immune cells to infiltrate seminiferous tubules and exert autoimmune activity against spermatids [9].

The development and progression of diabetes involve multiple pathophysiological mechanisms, including hyperglycemia, dyslipidemia, hypertension, oxidative stress, chronic inflammation, mitochondrial dysfunction, and endoplasmic reticulum (ER) stress [5]. Oxidative stress reflects an imbalance between excessive reactive oxygen species (ROS) production and impaired antioxidant defenses [12], leading to ER stress, mitochondrial dysfunction, DNA damage, and NF- κ B-mediated inflammation. Consequently, oxidative stress activates apoptotic signaling pathways and promotes tissue injury [13].

Preserving the morphological integrity of the BTB remains a critical factor in addressing infertility in individuals with diabetes. Although insulin therapy exerts beneficial effects on male fertility, its mechanisms in maintaining homeostasis and the impact of long-term exposure on spermatogenesis remain unclear [14]. Recent studies have increasingly explored natural compounds and dietary supplements as potential therapeutic strategies to attenuate diabetes-related complications and reproductive dysfunction, with growing interest in plant-derived products due to their perceived safety and cost-effectiveness [15].

Curcumin (Cur) is a natural polyphenol with antioxidant, anti-inflammatory, and antidiabetic properties that improve pancreatic β -cell function, reduce insulin resistance, and protect against oxidative damage [16, 17]. It normalizes antioxidant enzyme activities, thereby reducing lipid peroxidation [18, 19], and exerts

anti-inflammatory effects through suppression of NF- κ B signaling and inhibition of pro-inflammatory cytokines, including IL-6, TNF- α , and MCP-1 [20, 21]. Cur attenuates hyperglycemia-induced testicular damage [22], protects against mitochondrial- and ER-dependent apoptosis [23], and improves serum FSH, LH, and testosterone levels [24–26].

Garcinia kola (GK), also known as bitter kola, is traditionally used for managing metabolic and inflammatory disorders, including diabetes [15, 27]. In diabetic animal models, GK treatment improves metabolic parameters and enhances antioxidant and anti-inflammatory biochemical markers [28]. Through multiple mechanisms, including inhibition of lipid peroxidation, enhancement of antioxidant defenses, and preservation of the HPG axis, GK seed administration ameliorates testicular oxidative damage, improves testicular morphology, and restores serum FSH, LH, and testosterone levels in diabetic rats [29, 30].

Momordica charantia (MC), commonly known as bitter melon, is widely used as a complementary or alternative therapy for DM and its complications [31, 32]. In diabetic rats, MC administration increases serum insulin, testosterone, and gonadotropin levels, enhances testicular antioxidant defenses, preserves testicular morphology, and reduces spermatogenic and Sertoli cell apoptosis [33]. However, some studies have reported potential adverse reproductive effects, including antisteroidogenic and antispermato-genic actions, as well as sperm membrane and acrosomal damage [34, 35].

In this study, we comprehensively investigated diabetes-induced testicular damage and the potential therapeutic effects of Cur, GK, and MC using light and electron microscopy, stereological, immunohistochemical, and biochemical analyses. Given the increasing global use of plant-derived therapeutics and dietary supplements, such quantitative and comparative analyses are essential for monitoring their biological effects. We propose that these antioxidants may contribute to the maintenance of testicular function by modulating the expression of BTB-related proteins and exerting potential antidiabetic and antioxidant effects; furthermore, their comparative evaluation may provide a novel and valuable contribution to the field.

2 | Materials and Methods

2.1 | Animals and Ethics

This study was conducted on sixty 12-week-old male Wistar albino rats (250–300 g). Animals were housed under standard laboratory conditions (22 $\pm$ 2°C, 12-h light/dark cycle) with ad libitum access to standard pellet chow and water. All experimental protocols were reviewed and approved by the Ondokuz Mayıs University Animal Experiments Local Ethics Committee (Decision no: 2023–53, Decision date: August 10, 2023). The study was conducted in compliance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines, the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes, and relevant national regulations. All efforts were made to minimize animal suffering and to reduce the number of animals used throughout the experimental procedures.

2.2 | Experimental Design

Animals were randomly assigned into six groups (each $n = 10$): Control, Sham, DM, DM+Cur, DM+GK, and DM+MC. No intervention was applied to the control group, whereas rats in the Sham group received olive oil by oral gavage. Experimental DM was induced by a single intraperitoneal injection of streptozotocin (STZ; 50 mg/kg, Cayman 13104, USA), freshly dissolved in 0.01 M citrate buffer (pH 4.5) [36]. The STZ dose of 50 mg/kg was selected based on a previous study and established laboratory experience to induce a stable, severe model of insulin-deficient diabetes. To prevent acute hypoglycemia, animals were provided with a 5% glucose solution during the first 24 h following STZ administration [37]. DM was confirmed by measuring fasting blood glucose levels, and rats with levels above 250 mg/dL were considered diabetic [26]. No animals were excluded from the final analysis, as all rats met this sub-acute inclusion criterion and survived the experimental period. Following DM induction, rats in the antioxidant-treated groups received 100 mg/kg Cur [21], 100 mg/kg GK [27], or 100 mg/kg MC [38] by oral gavage once daily for 4 weeks. Following a 4-week course of antioxidant treatment, a 1-week interval was allowed to evaluate the cellular effects of the final treatment, and animals were sacrificed at the end of the 5-week experimental period.

Cur (Sigma-Aldrich, St Louis, USA; C1386) was prepared by dissolving in olive oil. GK seeds were grated, homogenized into powder form, and dissolved in distilled water. The olive oil macerate of MC was obtained as a commercial extract from a local pharmacy (Kamer Pharmacy, Samsun, Türkiye) and manufactured by Botafarma Health Products (Samsun, Türkiye). This standardized product was prepared through cold maceration, combining 1 part MC fruit with 5 parts olive oil (1:5 w/w) in sealed amber glass containers for 3 months. The final filtrate was subjected to microbiological and quality testing in accordance with the manufacturer's specifications to ensure consistency. All antioxidant preparations were freshly prepared weekly. Body weights were measured weekly, and oral gavage volumes were adjusted accordingly to ensure accurate dosing; for example, animals weighing 300 g received 1 mL, whereas those weighing 260 g received 0.85 mL of the prepared solutions.

2.3 | Body Weight Measurement

Body weights of the animals were measured at the beginning and at the end of the study. Relative body weight (RBW) changes were calculated using the following formula (1):

$$\text{RBW changes (\%)} = \frac{\text{Final} - \text{Initial}}{\text{Initial}} \times 100 \quad (1)$$

2.4 | Tissue Processing and Fixation

At the end of the experimental period (5 weeks), animals were anesthetized with ketamine (50 mg/kg) / xylazine (10 mg/kg) and subjected to transcardiac perfusion with a fixative solution containing 2% paraformaldehyde and 2% glutaraldehyde. The right testes were used for stereological analyses and fixed in Bouin's solution, while one of the left testes was fixed in 2%

glutaraldehyde for ultrastructural analysis, and the other was fixed in a mixed fixative containing 2% paraformaldehyde and 2% glutaraldehyde for immunohistochemical analysis.

The right testicular tissues fixed in Bouin's solution were immersed in lithium carbonate-containing 70% ethanol, prepared from a 1% lithium carbonate (Li_2CO_3) stock solution, to remove the yellow coloration of the Bouin's solution. The solution was replaced several times until the tissue discoloration was complete. The tissues were then dehydrated through a graded ethanol series (80%, 96%, and 100%), cleared in xylene, and infiltrated with molten paraffin. Subsequently, the tissues were embedded in paraffin blocks, which were coded to allow blind stereological analyses.

2.5 | Stereological Analysis

Systematic uniform random sampling was applied to paraffin blocks, and sections were obtained at a sampling fraction of 1/144. For stereological analyses, 5- μm -thick sections were cut using a rotary microtome (Leica RM2245, Nussloch, Germany), and for immunohistochemical analyses, 4- μm -thick sections were cut using the same microtome. Sections were incubated overnight at 60°C to ensure paraffin removal and proper tissue adhesion. An average of 20 sections per block was obtained. After deparaffinization, sections intended for stereological analysis were stained with hematoxylin and eosin for stereological analysis.

Light microscopy images were acquired using a trinocular research microscope equipped with a digital imaging system, including a color camera (Axioscope 5 microscope and AxioCam 208 camera, Carl Zeiss, Jena, Germany). Considering the section size, an image sampling fraction of 1/5 was applied at $\times 5$ (A-Plan, NA 0.12) objective magnification. Starting from an empty area near the upper right region of each section, five consecutive steps were moved at $\times 5$ magnification, and the seminiferous tubule corresponding to the upper right corner of the field was then imaged at $\times 40$ (A-Plan, NA 0.65) magnification. This procedure was repeated systematically across the section (Figure 1).

The volume fractions of spermatogonia, spermatocytes, spermatids, and Sertoli cells were estimated using a point-counting approach based on the Cavalieri principle with ImageJ software (ImageJ 1.46r, Java 1.6.0–20; National Institutes of Health, MD, USA). A point-counting grid was superimposed on each image, with each point representing an area of 500 μm^2 . The total number of points intersecting each cell type, the seminiferous epithelium, and the tubular lumen was recorded. Volume fractions were calculated as the ratio of points superimposed on each cell type to the total number of points superimposed on the seminiferous epithelium and expressed as percentages (%). The volume fraction represents the relative proportion of each cell type within the seminiferous epithelium rather than a direct measurement of individual cell volume. The reason we chose this as the main parameter is that in diabetic atrophy, not only the numerical loss of cells but also volumetric changes such as cytoplasmic shrinkage, reduction, or edema/vacuolization, and their proportions within the epithelium may directly reflect spermatogenesis capacity.



FIGURE 1 | Acquisition of $\times 40$ magnification images used for the calculation of volume fractions of seminiferous tubule cells. Tubule selection was performed using an unbiased, systematic random sampling method with a standard counting frame to avoid bias.

The total volume of seminiferous tubules was estimated using the Cavalieri principle on $\times 5$ objective (A-Plan, NA 0.12) images obtained with an image sampling fraction of 1/5 from systematically sampled $5\mu\text{m}$ -thick sections. A point-counting grid with an area per point of $20,000\mu\text{m}^2$ was applied using ImageJ. Total volume was calculated using the following formula (2):

$$V = \sum P_i \times (P(a)) \times t \quad (2)$$

In this formula, “ V ” represents the volume, “ $\sum P_i$ ” is the total number of points hitting the structure of interest, “ $(P(a))$ ” is the area represented by each point, and “ t ” is the section thickness. For both stereological analyses, grid spacing was determined by pilot studies to ensure that the coefficient of error (CE) remained below 5%. In addition, coefficients of variation (CV) were calculated to assess intergroup variability for the stereological estimations [39–41].

2.6 | Immunohistochemical Analysis

To determine apoptotic cell density in testicular tissue, a caspase-3 primary antibody (orb500767, Biorbyt, USA; 1:750) was used. To evaluate potential damage to and recovery of tight junctions in the BTB, claudin-11 (ab7474, Abcam, Cambridge, England; 1:750) and occludin (ab216327, Abcam, Cambridge, England; 1:300) primary antibodies were applied. Immunoreactivity was detected using a Mouse and Rabbit Specific HRP/AEC (ABC) Detection IHC Kit containing biotinylated secondary antibodies and HRP-labeled streptavidin (ab93705, Abcam, USA). The immunohistochemical assessment was qualitative and focused on evaluating the presence, intensity, and localization patterns of the proteins relative to negative control sections. To ensure objectivity, sections were evaluated blindly by a researcher (AKM). The entire section was thoroughly scanned under light microscopy, and representative fields showing consistent staining patterns were captured.

2.7 | Light and Electron Microscopic Analysis

Left testis tissue fixed in 2% glutaraldehyde was dissected under a stereomicroscope and processed according to standard electron microscopy tissue processing procedures. Semi-thin

sections ($0.5\mu\text{m}$) were cut from resin-embedded testicular tissues using an ultramicrotome (Leica EM UC7, Nussloch, Germany) and stained with toluidine blue for light microscopic evaluation. Histopathological images were acquired using a camera-equipped light microscope (Olympus BX43 microscope and Olympus SC50 camera, Olympus, Center Valley, PA, USA). Subsequently, ultrathin sections (90 nm) were obtained from the resin blocks using the same ultramicrotome, mounted on 200-mesh copper grids, and contrasted with uranyl acetate and lead citrate using an automated staining system (Leica EM AC20, Nussloch, Germany). Electron micrographs were obtained using an electron microscope (JEOL JSM-7001F, JEOL Ltd., Tokyo, Japan) at the Ondokuz Mayıs University Advanced Research and Application Center.

2.8 | Biochemical Analysis

During perfusion, blood samples from each animal were collected into anticoagulant-free tubes, centrifuged at 3000 rpm for 10 min at 4°C , and the separated serum was stored at -80°C until biochemical analyses were performed.

All biochemical analyses were performed at the Department of Medical Biochemistry, Faculty of Medicine, Ondokuz Mayıs University. Serum levels of follicle-stimulating hormone (FSH, mIU/mL), luteinizing hormone (LH, mIU/mL), and testosterone (ng/mL) were measured using Rat FSH (Elabscience, EA0015Ra, China), Rat LH (Elabscience, EA0013Ra, China), and Rat Testosterone (Elabscience, EA0023Ra, China) ELISA kits, respectively. Optical density (OD) values were measured at 450 nm using a microplate reader (BioTek Synergy-4, USA).

Metabolic analyses, including total antioxidant status (TAS) and total oxidant status (TOS), were performed using commercially available assay kits (TOS Metabolism Assay Kit, Elabscience, E-BC-K802-M; TAS Metabolism Assay Kit, Elabscience, E-BC-K801-M) based on a colorimetric method. Absorbance measurements were obtained using a microplate reader set at 660 nm (BioTek Synergy-4 microplate reader, USA). TAS values were calculated based on a Trolox reference standard and expressed as mmol Trolox equivalents per liter (mmol Trolox Eq./L), whereas TOS values were expressed as $\mu\text{mol H}_2\text{O}_2$

equivalents per liter ($\mu\text{mol H}_2\text{O}_2 \text{ Eq./L}$). The oxidative stress index (OSI) was calculated to assess overall oxidative stress using the formula: $\text{OSI} = [(\text{TOS} / 1000) / \text{TAS}] \times 100$ [42].

2.9 | Statistical Analysis

Statistical analyses were performed using GraphPad Prism software (version 8.0, San Diego, USA). Data distribution normality was assessed using the Shapiro–Wilk test. For normally distributed data, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied, whereas non-normally distributed data were analyzed using the Kruskal–Wallis test followed by Dunn's post hoc test. Data were expressed as mean $\pm$ standard deviation, and a p -value ≤ 0.05 was considered statistically significant.

3 | Results

3.1 | Body Weights

At the end of the experiment, the mean body weight of the DM group was significantly lower than that of the Control group ($p \leq 0.0001$). The Sham group exhibited a significantly higher mean body weight than the DM group ($p \leq 0.01$). There were no significant differences in mean body weight among the DM group and the DM+Cur, DM+GK, and DM+MC groups ($p > 0.05$). The mean body weight of the Control group was significantly higher than that of the DM+Cur and DM+GK groups ($p \leq 0.001$ and $p \leq 0.01$, respectively). Similarly, the mean body weight of the Sham group was significantly higher than that of the DM+Cur group ($p \leq 0.05$). No significant difference was observed between the DM+MC group and the Control or Sham groups ($p > 0.05$) (Figure 2a).

3.2 | Relative Body Weight Changes (%)

Evaluation of relative body weight changes revealed weight gain in the Control and Sham groups, whereas weight loss was observed in all diabetic groups. The Control group showed a significantly greater increase in body weight compared with the DM group ($p \leq 0.0001$). Likewise, the Sham group exhibited a significantly higher relative weight change than the DM group ($p \leq 0.01$). Although weight loss in the DM+Cur, DM+GK, and DM+MC groups was less pronounced than in the DM group, these differences were not statistically significant ($p > 0.05$). The relative body weight change in the Control group was significantly higher than that in the DM+Cur and DM+GK groups ($p \leq 0.01$). In addition, the Sham group showed a significantly higher relative weight change compared with the DM+GK group ($p \leq 0.05$) (Figure 2b).

3.3 | Fasting Blood Glucose Levels at the fourth Week

At the fourth week of antioxidant administration, fasting blood glucose levels in the DM group were significantly higher than those in the Control and Sham groups ($p \leq 0.0001$). Fasting blood

glucose levels in the DM+Cur, DM+GK, and DM+MC groups were compared with the DM group; there was no difference ($p > 0.05$). However, fasting blood glucose levels in the DM+Cur group were significantly higher than those in both the Control and Sham groups ($p \leq 0.001$) (Figure 2c).

3.4 | Fasting Blood Glucose Levels at the Perfusion (Fifth Week)

At the end of the experiment (fifth week), fasting blood glucose levels were significantly higher in the DM group than in the Control group ($p \leq 0.001$) and the Sham group ($p \leq 0.0001$). Fasting blood glucose levels in the DM+Cur, DM+GK, and DM+MC groups were not different compared with the DM group ($p > 0.05$). On the other hand, fasting blood glucose levels were significantly higher in the DM+Cur and DM+GK groups than in the Control group ($p \leq 0.05$). Likewise, fasting blood glucose levels in the DM+Cur and DM+GK groups were significantly higher than those in the Sham group ($p \leq 0.01$). In addition, the DM+MC group showed a significantly higher fasting blood glucose level compared with the Sham group ($p \leq 0.05$) (Figure 2d).

3.5 | Stereological Findings

In seminiferous tubule images obtained at $40\times$ magnification, the volume fractions (%) were calculated by dividing the number of points hitting spermatogonia, spermatocytes, spermatids, and Sertoli cells by the total number of points hitting the seminiferous epithelium. In addition, images were acquired at $5\times$ magnification, and seminiferous tubule volume (mm^3) was calculated by counting the number of points that hit the seminiferous tubules. Using data obtained from all groups, the coefficient of error (CE) and coefficient of variation (CV) values were calculated and are presented in Table 1. Based on the grid spacing used for estimation, many points covered the entire seminiferous tubule area, while fewer points covered cell populations within a smaller reference area. This distribution is methodologically expected and acceptable in the point-counting method of stereology, as the sampling error (i.e., CE) remains well below the biological variation (i.e., CV).

Comparison of the spermatogonium volume fraction (%) among groups revealed a significant decrease in the DM group compared with the Sham group ($p \leq 0.01$). Similarly, the DM+MC group showed a significantly lower spermatogonium volume fraction (%) than the Sham group ($p \leq 0.05$). In contrast, the DM+GK group exhibited a significant increase in spermatogonium volume fraction (%) compared with the DM group ($p \leq 0.05$), with no significant difference relative to the Sham group ($p > 0.05$). No significant differences were observed between the DM group and the DM+Cur or DM+MC groups ($p > 0.05$) (Figure 3a).

Analysis of Sertoli cell volume fraction (%) demonstrated a significant increase in the DM+Cur group compared with the DM+MC group ($p \leq 0.01$). However, no statistically significant differences were observed between the DM+Cur, DM+GK, or DM+MC groups and the Control or Sham groups ($p > 0.05$) (Figure 3b).

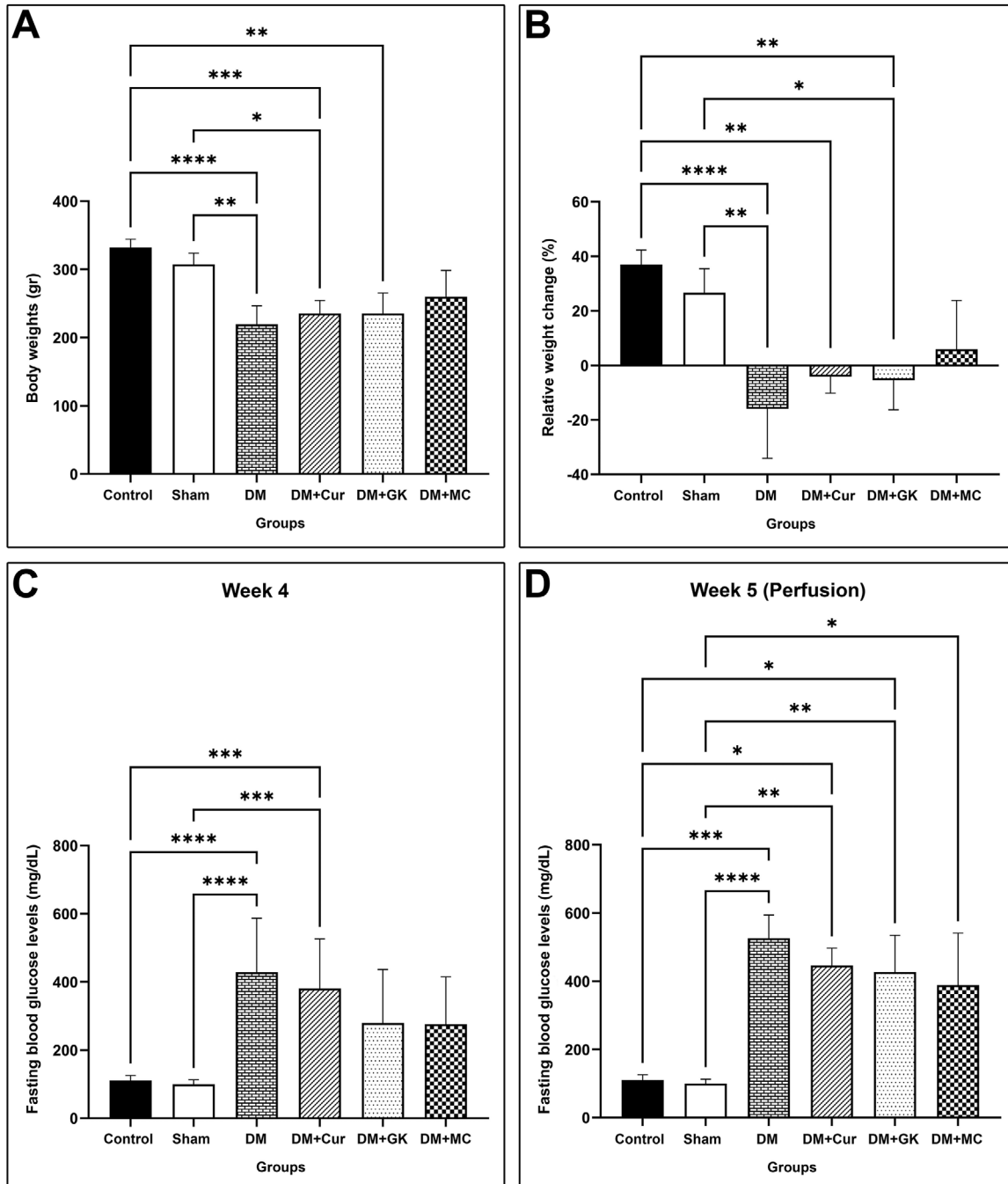


FIGURE 2 | Graphs showing the comparison among groups of mean body weights (g) (A), relative weight changes (%) (B), fasting blood glucose levels measured immediately after discontinuation of antioxidant treatments (fourth week) (C), and fasting blood glucose levels measured at the time of perfusion at the end of the experimental period (fifth week) (D). Values are expressed as mean $\pm$ standard deviation. Statistically significant differences are indicated as $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***), and $p \leq 0.0001$ (****).

The spermatocyte and spermatid volume fractions (%) were significantly higher in the DM+Cur group compared with the DM group ($p \leq 0.05$). No significant differences were observed between the Control and Sham groups ($p > 0.05$). Similarly, no statistically significant differences were found between the DM+GK or DM+MC groups and the Control or Sham groups ($p > 0.05$) (Figure 3c,d).

Evaluation of seminiferous tubule volume (mm^3) revealed no significant differences among the groups ($p > 0.05$) (Figure 3e).

3.6 | Biochemical Findings

3.6.1 | Follicle-stimulating hormone (FSH)

FSH levels did not differ significantly among the Control, Sham, and DM groups ($p > 0.05$). Similarly, no significant differences were observed between the Sham and any experimental groups ($p > 0.05$). In contrast, FSH levels were significantly increased in the DM+MC group compared with the Control and DM groups ($p \leq 0.05$), and in the DM+Cur group compared with the Control and

TABLE 1 | Coefficient of error (CE) and coefficient of variation (CV) values of volume fractions (%) for spermatogonia, spermatocytes, spermatids, and Sertoli cells, as well as the volume measurements of seminiferous tubules.

	Spermatogonium	Sertoli	Spermatocyte	Spermatid	Seminiferous tubule
Control					
CE	0.07	0.10	0.03	0.07	0.02
CV	0.09	0.14	0.11	0.34	0.10
Sham					
CE	0.06	0.09	0.03	0.07	0.02
CV	0.22	0.13	0.11	0.33	0.13
DM					
CE	0.08	0.09	0.03	0.07	0.02
CV	0.18	0.30	0.19	0.67	0.36
DM+Cur					
CE	0.07	0.09	0.03	0.07	0.02
CV	0.11	0.16	0.11	0.22	0.14
DM+GK					
CE	0.08	0.10	0.04	0.10	0.02
CV	0.12	0.24	0.15	0.54	0.30
DM+MC					
CE	0.08	0.12	0.03	0.09	0.02
CV	0.14	0.22	0.12	0.23	0.15

DM groups ($p \leq 0.01$). No significant differences were detected between the DM+GK group and the Control, Sham, or DM groups ($p > 0.05$) (Figure 4a).

3.6.2 | Luteinizing hormone (LH)

Analysis of LH activity revealed no significant differences among any of the experimental groups ($p > 0.05$) (Figure 4b).

3.6.3 | Testosterone

Testosterone levels were significantly lower in the DM group than in the Control group ($p \leq 0.05$). However, no statistically significant differences were observed in the DM+Cur, DM+GK, or DM+MC groups when compared with the Control, Sham, or DM groups ($p > 0.05$) (Figure 4c).

3.6.4 | Total antioxidant status (TAS)

No significant differences in TAS levels were observed among the Control, Sham, and DM groups ($p > 0.05$). In contrast, TAS levels were significantly higher in the DM+Cur and DM+MC groups compared with the Sham group ($p \leq 0.05$). Compared with the Control group, TAS levels were significantly increased in the DM+Cur ($p \leq 0.001$), DM+MC ($p \leq 0.01$), and DM+GK ($p \leq 0.05$) groups (Figure 4d).

3.6.5 | Total oxidant status (TOS)

Comparison of TOS levels among all experimental groups revealed no statistically significant differences ($p > 0.05$) (Figure 4e).

3.6.6 | Oxidative stress index (OSI)

Evaluation of OSI demonstrated a significant decrease in the DM+Cur group compared to the Control group ($p \leq 0.0001$). Similarly, OSI levels were significantly reduced in the DM+GK and DM+MC groups compared with the Control group ($p \leq 0.05$ and $p \leq 0.01$, respectively). In addition, OSI levels were significantly lower in the DM+Cur group compared with the DM group ($p \leq 0.05$) (Figure 4f).

3.7 | Immunohistochemical Findings

3.7.1 | Claudin-11 immunoreactivity

No immunoreactivity was observed in negative controls. In the Control group, claudin-11 showed preserved localization with predominantly membranous positivity in the seminiferous tubules. The Sham group exhibited discontinuous, moderate immunoreactivity mainly near the basal membrane. In the DM group, claudin-11 staining was weak and irregular, with focal loss of immunoreactivity. In the DM+Cur group, widespread immunoreactivity was observed in both basal and apical regions,

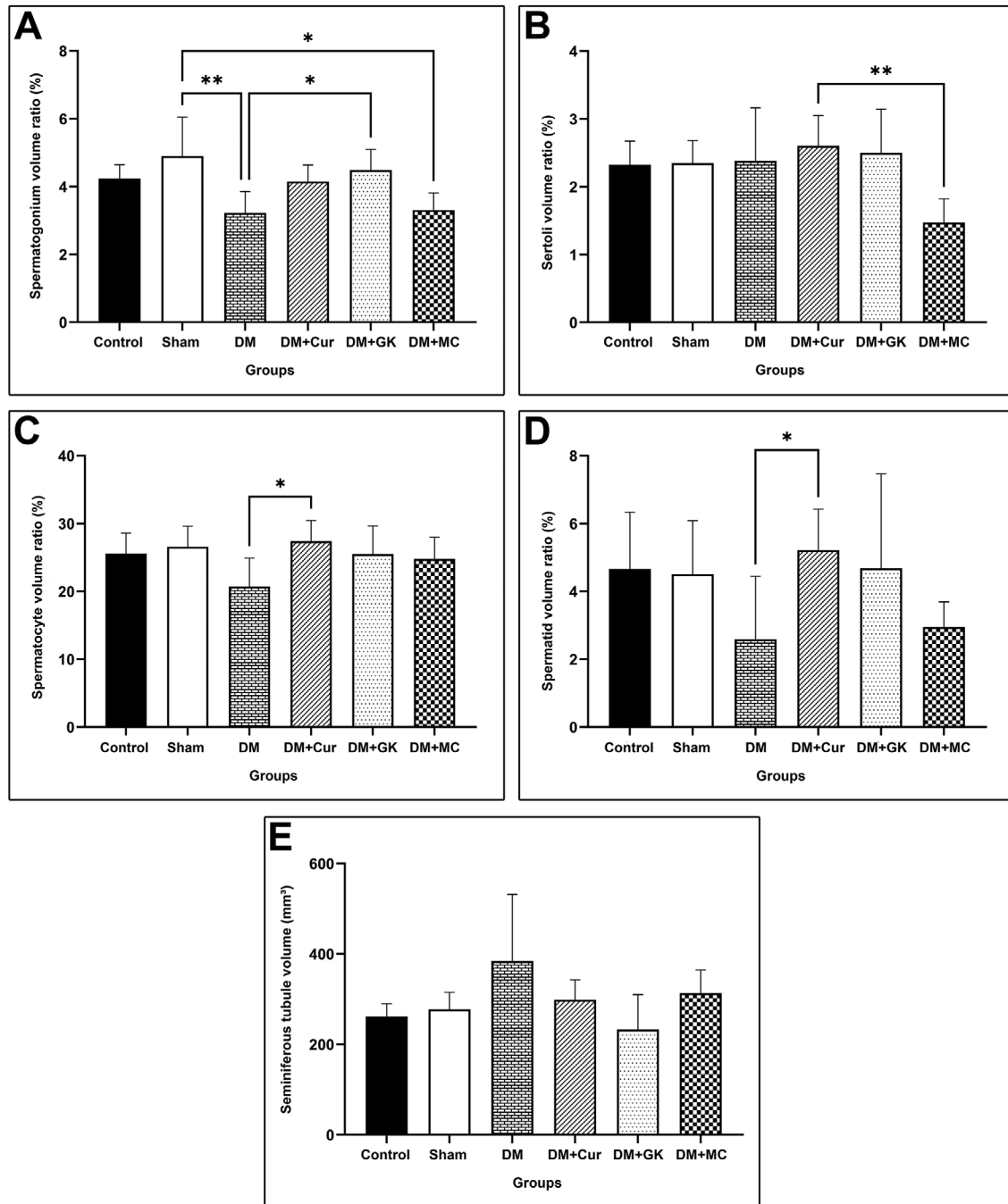


FIGURE 3 | (A–D) Graphs showing the comparison of the volume fractions (%) of spermatogonia, spermatocytes, spermatids, and Sertoli cells within the seminiferous epithelium. (E) Graph showing the comparison among groups of seminiferous tubule volume (mm³). Data are expressed as mean ± standard deviation. Statistically significant differences are indicated as $p \leq 0.05$ (*) and $p \leq 0.01$ (**).

predominantly cytoplasmic. The DM+GK group showed intense immunoreactivity mainly in the basal region, with partial extension toward the apical compartment. In the DM+MC group, marked claudin-11 positivity was observed predominantly in the basal region, with focal apical distribution (Figure 5).

3.7.2 | Occludin immunoreactivity

Negative control sections showed no immunoreactivity. In the Control group, strong, continuous occludin immunoreactivity

was observed at the basal membrane. The Sham group exhibited moderate but discontinuous basal staining. In the DM group, occludin immunoreactivity was weak and detected only in limited areas. The DM+Cur group showed widespread and intense immunoreactivity throughout the epithelium, predominantly in the basal region. In the DM+GK group, membranous occludin positivity was observed mainly around cells near the basal membrane. In the DM+MC group, strong and extensive occludin immunoreactivity was detected throughout the epithelium, particularly in the basal region (Figure 6).

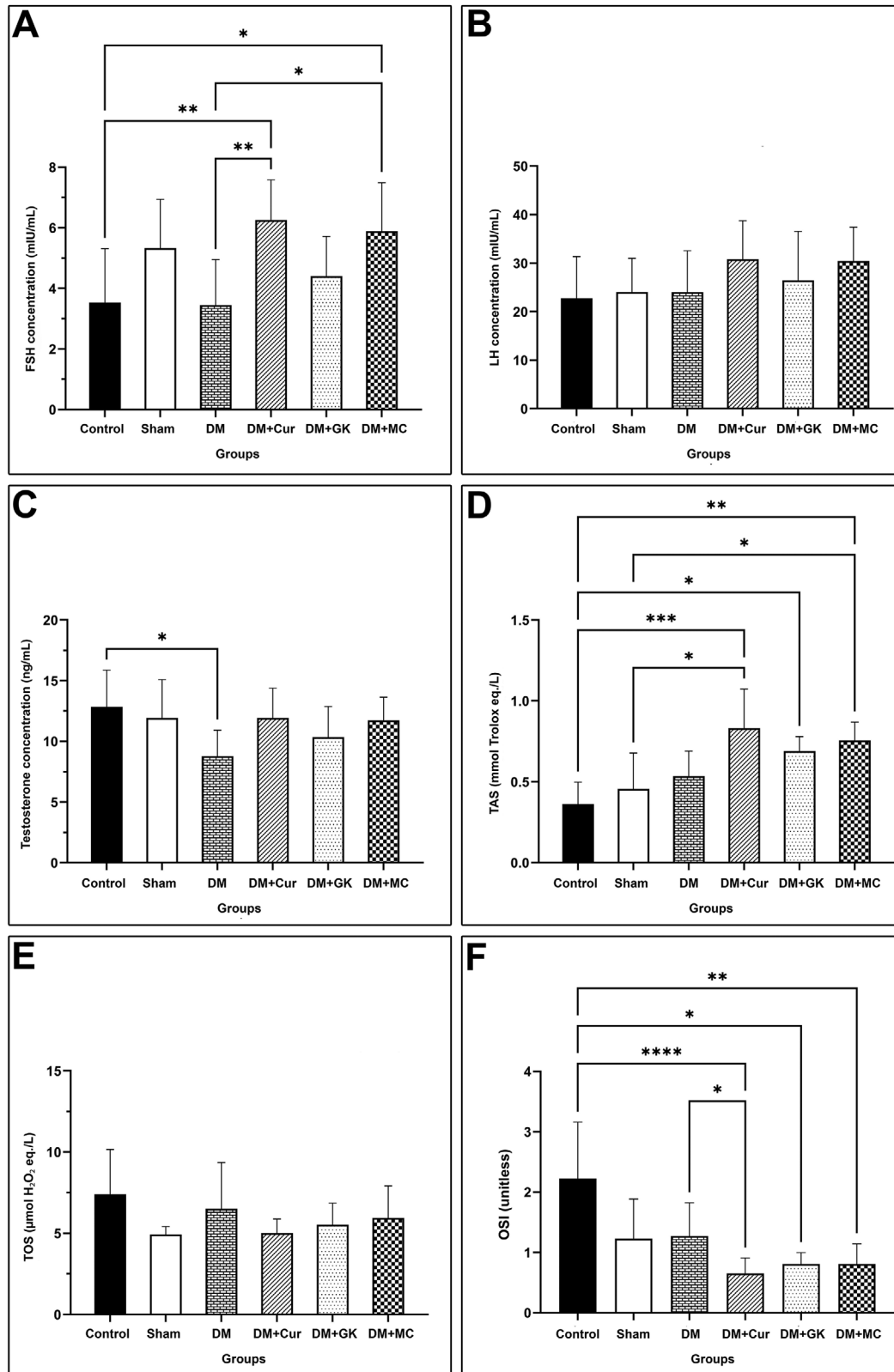


FIGURE 4 | (a–f) Graph showing the comparison among groups of mean FSH (mIU/mL), LH (mIU/mL), testosterone (ng/mL), TAS (mmol Trolox Eq./L), TOS ($\mu\text{mol H}_2\text{O}_2$ Eq./L) levels, and OSI. Values are expressed as mean $\pm$ standard deviation. Statistically significant differences are indicated as $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***), and $p \leq 0.0001$ (****).

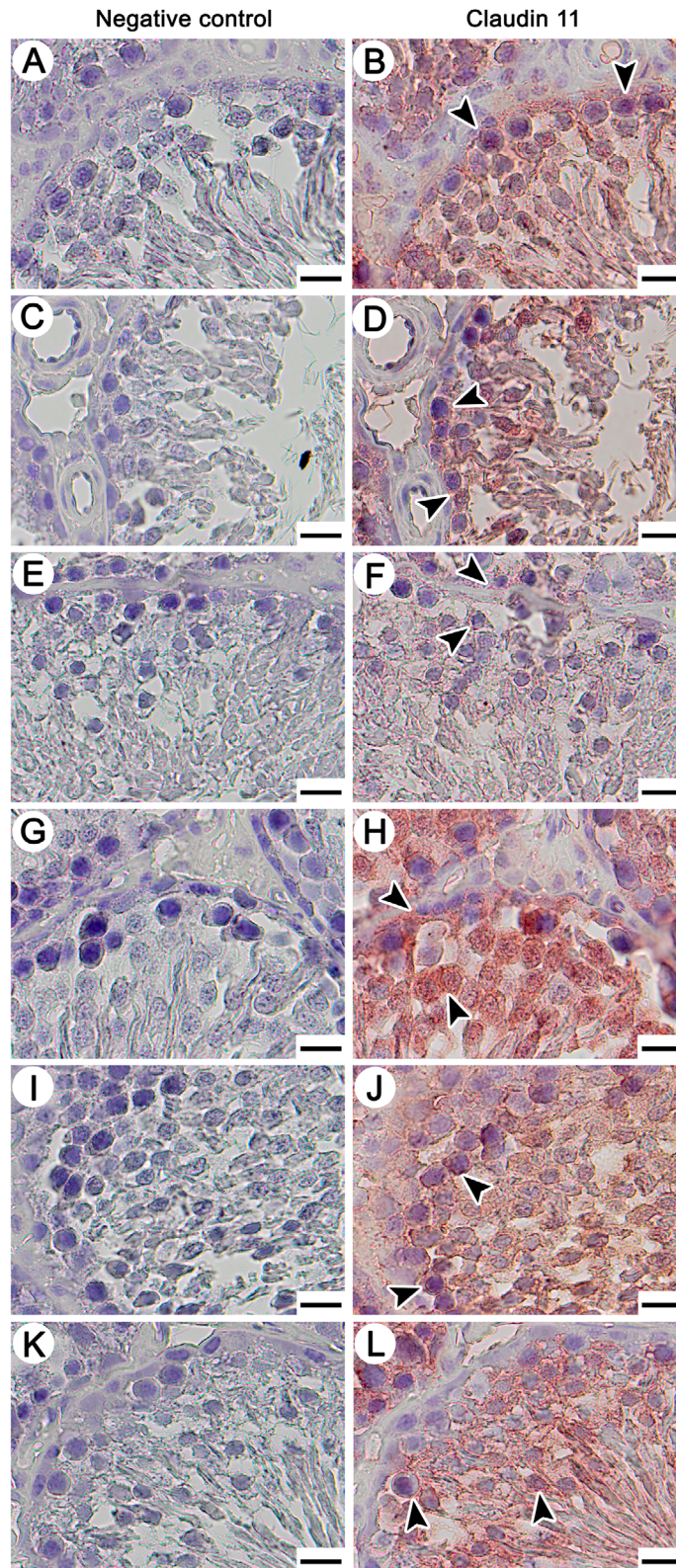


FIGURE 5 | (A–L) Representative images showing claudin-11 immunostaining and negative controls. (B) In the control group, claudin-11 staining is observed along the basement membrane of the seminiferous tubule epithelium, predominantly in a membranous pattern (arrowhead). (D) In the sham group, moderate claudin-11 staining is observed in the basal region of the seminiferous tubule, with locally discontinuous distribution near the basement membrane (arrowhead). (F) In the DM group, claudin-11 staining appears weak and irregular in the seminiferous tubule epithelium, with complete loss of staining in some areas (arrowhead). (H) In the DM+Cur group, claudin-11 staining is distributed in both basal and apical regions, showing widespread cytoplasmic localization (arrowhead). (J) In the DM+GK group, intense claudin-11 staining is predominantly localized in the basal region, with focal extension toward the apical region (arrowhead). (L) In the DM+MC group, prominent claudin-11 staining is observed in the seminiferous tubule, mainly localized in the basal region with extension toward the apical region (arrowhead). Bars: 15 μ m.

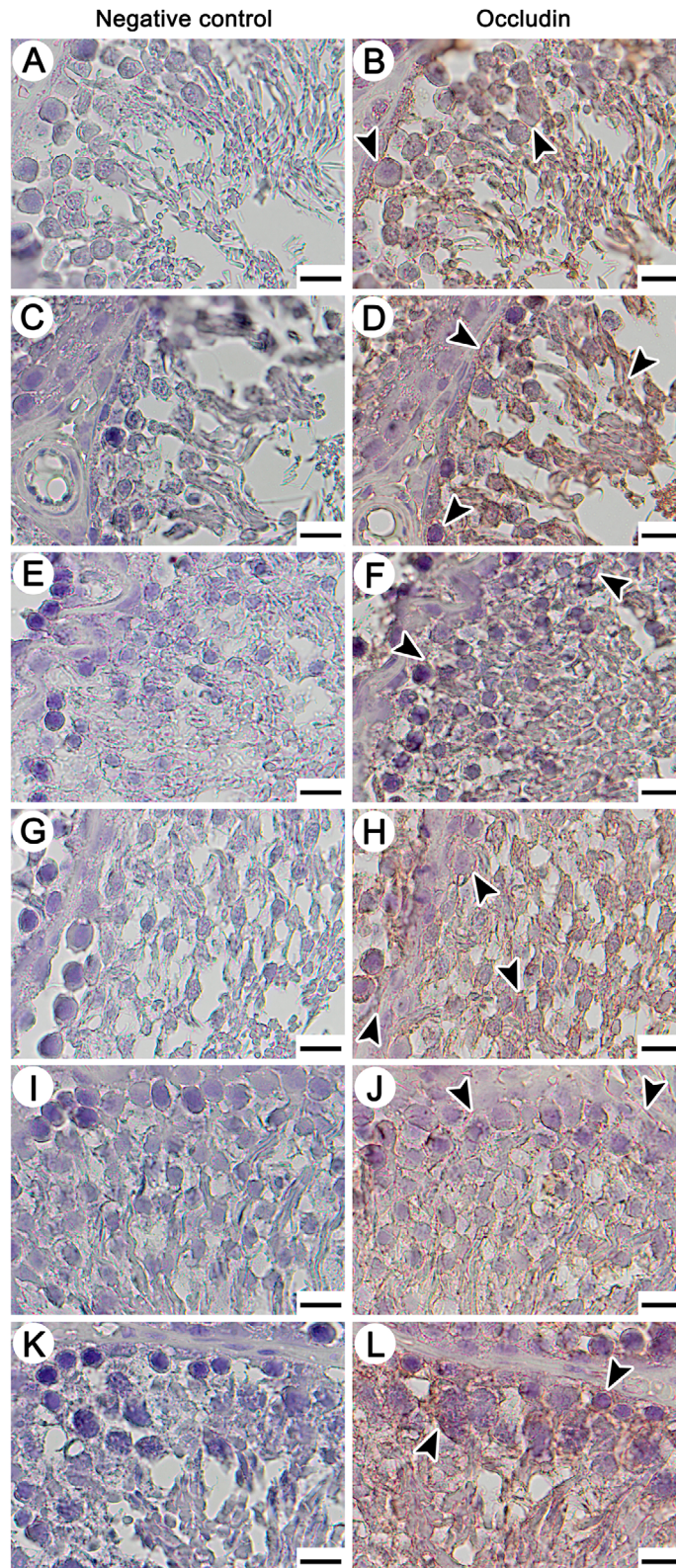


FIGURE 6 | (A–L) Representative images showing occludin immunostaining and negative controls. (B) In the control group, occludin staining is observed as a thin band near the basement membrane of the seminiferous tubule (arrowhead). (D) In the sham group, moderate occludin staining is observed parallel to the basement membrane, with a non-diffuse and locally discontinuous pattern (arrowhead). (F) In the DM group, occludin staining appears weak and localized in some areas of the seminiferous tubule epithelium (arrowhead), with minimal staining observed overall. (H) In the DM+Cur group, occludin staining shows a widespread distribution within the seminiferous tubules (arrowhead), predominantly localized in the basal region with diffuse extension throughout the epithelium. (J) In the DM+GK group, occludin staining is prominent near the basement membrane of the seminiferous tubule, exhibiting a thin linear pattern around the cells (arrowhead). (L) In the DM+MC group, distinct occludin staining is observed, mainly parallel to the basement membrane (arrowhead), with widespread staining throughout the epithelium. Bars: 15 μ m.

3.7.3 | Caspase-3 immunoreactivity

No caspase-3 immunoreactivity was observed in negative controls. In the Control and Sham groups, caspase-3 expression was low and limited to a few germ and Sertoli cells. In the DM group, caspase-3 immunoreactivity markedly increased in germ cell lineages and Sertoli cells, predominantly nuclear. In the DM+Cur group, caspase-3 positivity was observed in some spermatogonia and Sertoli cells, while spermatocytes and spermatids exhibited strong nuclear staining. In the DM+GK group, caspase-3 immunoreactivity was weak and restricted to a few germ cells. In contrast, the DM+MC group showed pronounced caspase-3 immunoreactivity with both cytoplasmic and nuclear localization across the seminiferous epithelium (Figure 7).

3.8 | Light Microscopic Findings

In the control group, the seminiferous tubules and interstitial tissue exhibited a preserved morphology, with intact epithelial organization, continuous basal membranes, and a normal layering of spermatogenic cells. Numerous mature spermatozoa were observed within the tubular lumen, and Leydig cells showed normal morphology.

The Sham group largely maintained normal seminiferous tubule architecture; however, focal disorganization of germ cell arrangement, increased intercellular spaces, and the presence of degenerative germ cells were occasionally observed.

In the DM group, marked structural deterioration was evident, including disruption of seminiferous epithelium, loss of germ cell organization, thickening and discontinuity of the basal membrane, extensive cellular degeneration, and accumulation of cellular debris within the lumen. Interstitial fibrosis, reduced vascularization, and altered Leydig cell morphology were also observed.

In the DM+Cur group, partial preservation of seminiferous tubule architecture was noted. Although epithelial thickness was reduced in some areas and apoptotic cells were present, the overall spermatogenic cell sequence was largely maintained. Interstitial vascularization was preserved, accompanied by increased macrophage infiltration.

The DM+GK group exhibited a relatively well-preserved seminiferous tubular structure, with maintained epithelial integrity and a largely normal germ cell hierarchy. Despite occasional degenerative cells and residual bodies, basal membrane continuity and interstitial organization were generally preserved.

In the DM+MC group, seminiferous tubules showed preserved epithelial organization with regular germ cell layering and intact basal membranes. Interstitial tissue demonstrated normal morphology and high vascularization, although macrophage accumulation and focal interstitial edema were observed (Figure 8).

3.9 | Electron Microscopic Findings

In the control group, seminiferous epithelium and interstitial tissue displayed preserved ultrastructural organization. Spermatogenic cells at different developmental stages, Sertoli cells, basal membrane, and myoid cells showed normal morphology, consistent with active and intact spermatogenesis.

The Sham group largely maintained normal ultrastructural features, including preserved basal membrane continuity and well-organized Sertoli and Leydig cells. Minor ultrastructural alterations were observed in some spermatogonia, while overall cellular organization remained intact.

In the DM group, severe ultrastructural damage was evident, characterized by disrupted cell boundaries, degeneration of Sertoli and germ cells, discontinuity and thickening of the basal membrane, cytoplasmic vacuolization, and accumulation of cellular debris within the tubular lumen. Alterations in sperm ultrastructure, including abnormalities in dense fibers and fibrous sheath thickening, were also observed.

In the DM+Cur group, ultrastructural organization was partially preserved. Basal membrane continuity and germ cell nuclear integrity were largely maintained, although focal mitochondrial damage, lipid droplets, apoptotic features, and the presence of macrophages were observed.

The DM+GK group exhibited relatively preserved ultrastructure of seminiferous epithelium and interstitial tissue. Sertoli and germ cells showed mostly intact morphology, with limited degenerative changes. Leydig cells displayed well-preserved ultrastructure, while sperm flagella exhibited focal alterations in fibrous and dense fiber components.

In the DM+MC group, seminiferous epithelium showed preserved ultrastructural organization, including intact basal membrane, well-defined Sertoli and germ cell morphology, and normal acrosomal structures. Although residual bodies, vacuolization, and focal alterations in axonemal microtubules were observed, overall ultrastructural integrity was largely maintained (Figure 9).

4 | Discussion

In the present study, STZ-induced diabetes resulted in marked systemic and testicular alterations, including persistent hyperglycemia, body weight loss, hormonal imbalance, germ cell depletion, increased apoptotic activity, and disruption of BTB integrity. Administration of Cur, GK, and MC attenuated these changes to different extents, revealing distinct patterns of biochemical, cellular, and structural protection.

Consistent with the catabolic nature of uncontrolled diabetes, the DM group exhibited significant body weight loss compared with the Control and Sham groups. Weight loss is a well-recognized consequence of hyperglycemia and insulin deficiency [43–45] and is commonly attributed to increased lipid and muscle breakdown,

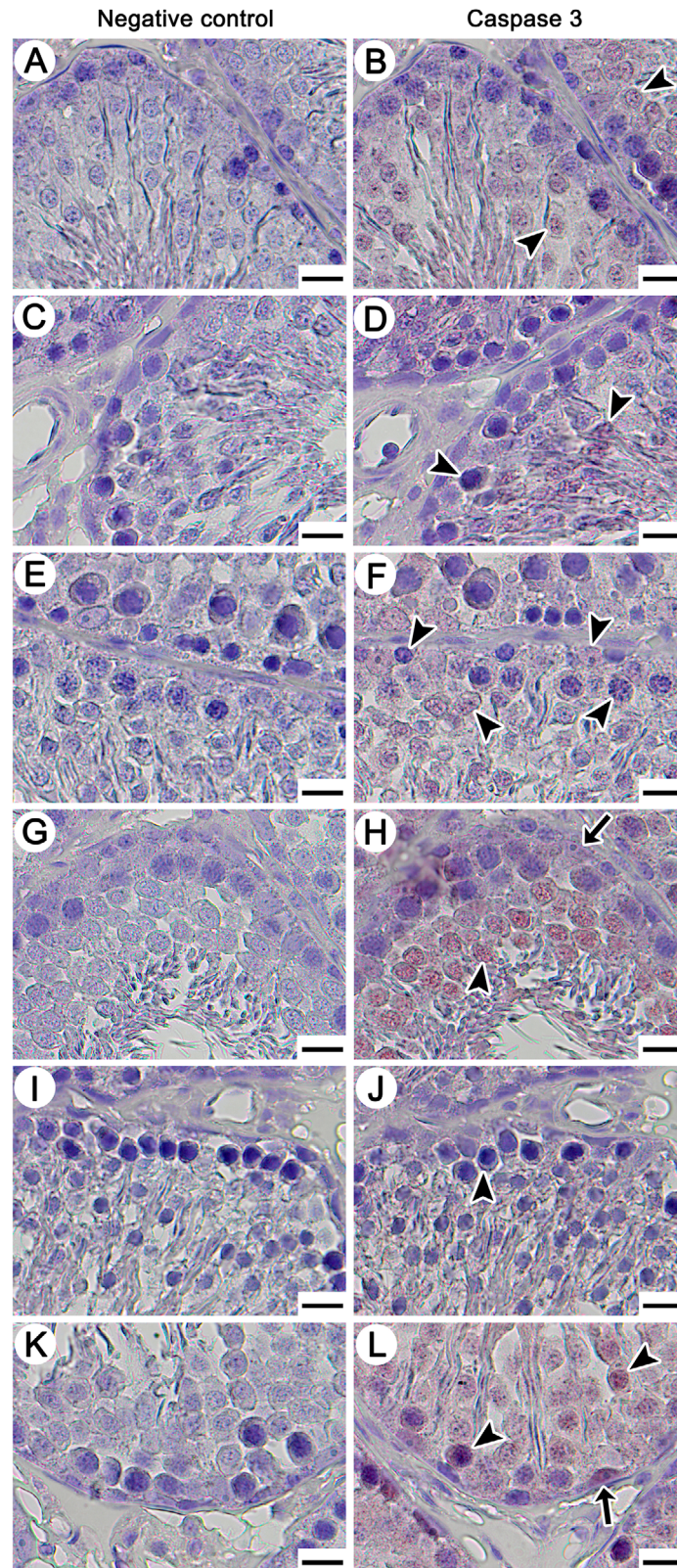


FIGURE 7 | (A–L) Representative images showing caspase-3 immunostaining and negative controls. (B) In the control group, low-intensity cytoplasmic caspase-3 staining is observed in some Sertoli and germ cells of the seminiferous tubule epithelium (arrowhead). (D) In the sham group, focal caspase-3 staining is detected in spermatocytes and spermatids in some seminiferous tubules. (F) In the DM group, caspase-3 staining is prominently observed in the seminiferous tubules, with predominant nuclear localization observed in spermatogenic and Sertoli cells (arrowhead). (H) In the DM+Cur group, pronounced caspase-3 staining is observed in cells of the spermatogenic lineage in most seminiferous tubules (arrowhead). Low-frequency staining is present in spermatogonia and Sertoli cells (arrow), whereas spermatocytes and spermatids show strong nuclear staining. (J) In the DM+GK group, weak cytoplasmic caspase-3 staining is observed in some germ cells (arrowhead), with minimal overall staining. (L) In the DM+MC group, caspase-3 staining is prominent throughout the seminiferous tubule epithelium. Distinct staining is observed in spermatogonia (arrowhead) and

excessive protein catabolism, and dehydration [46, 47]. Although antioxidant-treated groups tended to show less weight loss, no significant differences in final body weight or relative weight change were observed compared with the DM group. These findings indicate that, under the applied dose and duration, Cur, GK, and MC did not exert a systemic effect sufficient to reverse diabetes-associated weight loss. This suggests that insulin-deficiency-driven catabolic processes remain dominant in severe STZ-induced diabetes, while antioxidant treatment may provide only partial systemic benefit.

Fasting blood glucose levels were markedly elevated in the DM group at both the fourth week (end of treatment) and the fifth week (1 week after treatment withdrawal) compared with the Control and Sham groups. This finding is consistent with the cytotoxic action of STZ on pancreatic β cells, leading to impaired insulin secretion and sustained hyperglycemia [48, 49]. At the fourth week, fasting glucose levels in the antioxidant-treated groups tended to be lower than those in the DM group, but did not reach statistical significance; however, the DM+Cur group remained significantly higher than the Control and Sham groups. After treatment cessation, fasting glucose levels tended to increase in all antioxidant-treated groups. DM+Cur and DM+GK differed significantly from both the Control and Sham groups, whereas DM+MC differed only from the Sham group and showed relatively more stable glucose values. These results indicate that none of the antioxidants alone was sufficient to control severe hyperglycemia at the applied dose and duration. The severity of the STZ-induced diabetic model, characterized by substantial β -cell destruction and insulin deficiency, may have limited the hypoglycemic effects of the antioxidants at the applied dose and duration. The partial improvements observed during treatment and the increase in blood glucose levels after treatment withdrawal suggest that sustained administration may be necessary to achieve glycemic control, with MC showing a relatively more persistent effect under the present conditions.

When endocrine findings were evaluated, serum testosterone levels were significantly lower in the DM group than in the Control group. This may be an indicator of diabetes-related testicular damage and Leydig cell dysfunction. Previous studies have demonstrated that insulin deficiency and hyperglycemia adversely affect Leydig cell steroidogenesis and reduce testosterone production [50, 51]. Contrary to expectations, no significant difference was observed between the Control and DM groups in terms of FSH and LH levels. Under normal circumstances, an increase in LH secretion from the pituitary gland would be expected in response to a decrease in testosterone; the absence of this response suggests a diabetes-induced suppression at the pituitary level. FSH levels were significantly increased in the DM+Cur and DM+MC groups compared with both the Control and DM groups. The fact that testosterone levels in these groups approached normal levels, while FSH increased, suggests that the antioxidant and antidiabetic phytochemical components of Cur and MC stimulate pituitary-gonadal axis activity [33, 52]. How-

ever, serum LH levels remained statistically unchanged across all experimental groups. This suggests that the observed therapeutic benefits, especially the prominent antioxidant effects in the Cur group, may be mediated by local cytoprotection of testicular tissue rather than a systemic restoration of the hormonal axis. Taken together, these findings indicate that Cur, GK, and MC may partially and variably improve diabetes-associated hormonal alterations.

Systemic oxidative stress analysis showed a significant increase in TAS levels in all antioxidant-treated groups, indicating enhanced antioxidant capacity in the Cur, GK, and MC groups. In addition, the reduction in oxidative stress levels observed in the DM+Cur group compared with the DM group, as well as the lower levels detected in all antioxidant-treated groups, suggests a suppression of oxidative load. Cur produced the most pronounced reduction in oxidative stress indices, whereas MC and GK showed more modest effects.

Stereological analysis demonstrated substantial diabetes-related alterations in germ cell populations. The volume ratio of spermatogonia was markedly reduced in the DM group compared with the Sham group. This finding suggests that olive oil may have prevented diabetes-induced apoptosis in spermatogonial cells, consistent with previous reports [34, 53]. In the DM+MC group, spermatogonial cells were compared with those in the Sham group. Depending on the dose and duration, MC may have exerted cytotoxic effects on spermatogonia, leading to cell loss. This finding is supported by the significant anti-caspase 3 activity observed in spermatogonia, particularly in the DM+MC group. Like existing studies, no significant difference was found in spermatogonium volume ratio between the DM+MC and Control groups in our study [34, 53]. The data obtained suggest that olive oil may have a mild protective and supportive effect in the Sham group. However, the increase in the spermatogonial volume ratio observed in the DM+GK group compared with the DM group may indicate a cytoprotective effect of GK. The low intensity of anti-caspase 3 activity observed in the DM+GK group in our immunohistochemical analyses confirms the cytoprotective effect of GK.

The Sertoli cell volume ratio did not differ significantly between the DM and Control groups, suggesting relative resistance to volumetric loss. However, preserved volume ratio does not imply preserved function. In diabetic models, Sertoli cells exhibit reduced lactate production, mitochondrial dysfunction, and increased BTB permeability [54–56]. In agreement, our immunohistochemical findings demonstrated reduced or absent claudin-11 and occludin expression in the DM group, indicating disruption of BTB integrity. These results suggest that DM primarily impairs Sertoli cell function and barrier organization rather than causing overt Sertoli cell loss.

Among antioxidant-treated groups, the Sertoli cell volume ratio was significantly higher in DM+Cur than in DM+MC; this difference was mainly driven by the pronounced reduction observed in

some Sertoli cells (arrow), while moderate staining is present in other spermatogenic cells. Staining is observed at both the cytoplasmic and nuclear levels. Bars: 15 μ m.

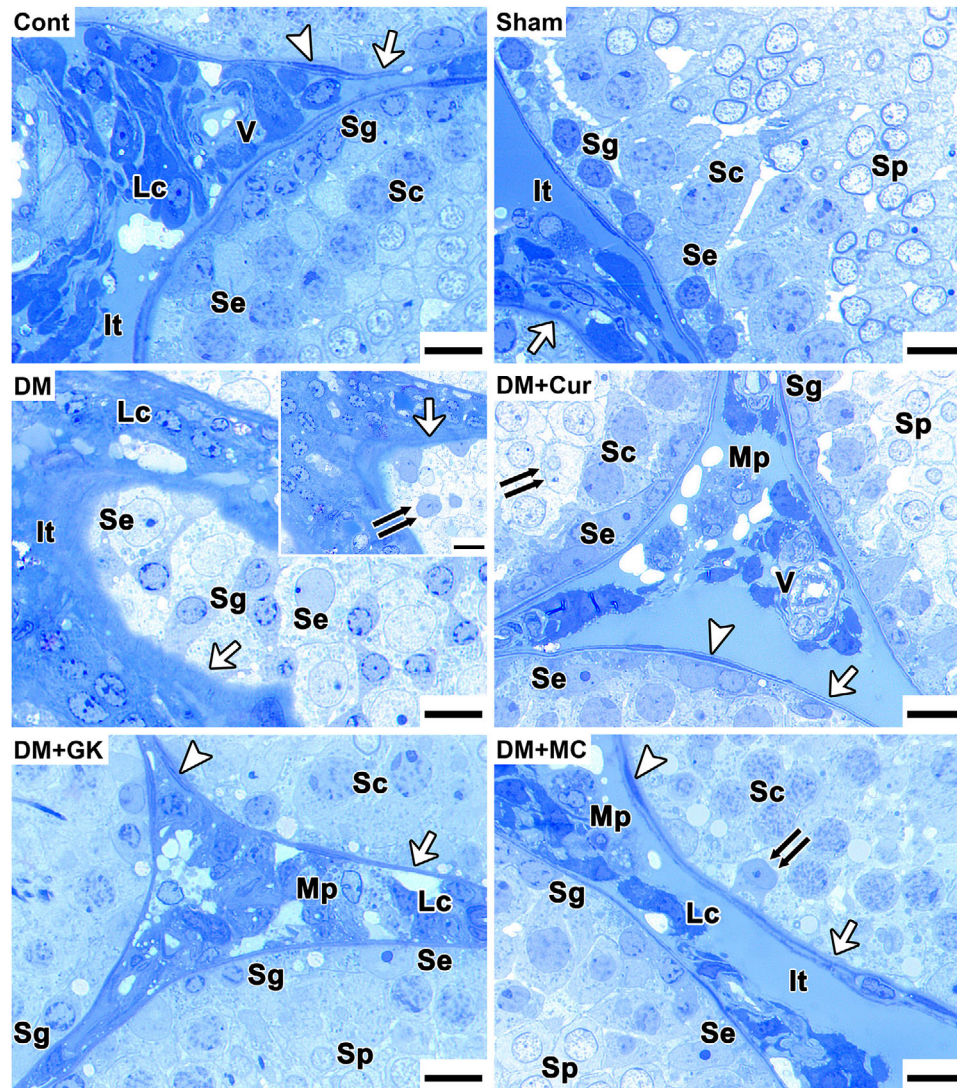


FIGURE 8 | Representative images obtained from semi-thin sections of testicular tissue. **(Cont)** Distinct nuclear and cytoplasmic boundaries are observed. The basement membrane (arrow) and myoid cells (arrowhead) display normal morphology, and the interstitial tissue shows rich vascularization with healthy Leydig cells. **(Sham)** The basement membrane (arrow) appears thin and well preserved. Germ cells and Sertoli cells exhibit normal morphology, with locally increased intercellular spaces in the epithelium. No degenerative changes are observed in the interstitial tissue. **(DM)** The seminiferous epithelium shows loss of epithelial integrity associated with pronounced cellular degeneration and the presence of dark-stained degenerated cells (black arrows). Increased fibrosis and reduced vascularization are observed in the interstitial tissue, along with an increased number of Leydig cells. **(DM+Cur)** The basement membranes (white arrow) are thin and continuous, and myoid cells (arrowhead) appear normal. Most germ and Sertoli cells exhibit preserved morphology, although some degenerated cells are present (double black arrows). Leydig cells show partial morphological alterations, and macrophages are observed in the interstitial tissue. **(DM+GK)** The basement membrane (arrow) and myoid cells (arrowhead) exhibit normal morphology. Spermatogenic cell layers are regularly arranged, and epithelial cells display distinct nuclear and cytoplasmic boundaries. The organization of the interstitial tissue is partially preserved, with macrophages present. **(DM+MC)** Spermatogenic and Sertoli cell boundaries are well preserved within the seminiferous tubule epithelium. The basement membrane (white arrow) is thin and continuous, and surrounding myoid cells (white arrowhead) show normal morphology. Some Sertoli cells appear reduced in size and darkly stained (double black arrows). Interstitial tissue components appear preserved, with macrophages present. It, interstitial tissue; Lc, Leydig cell; Mp, macrophage; Sc, spermatocyte; Se, Sertoli cell; Sg, spermatogonium; Sp, spermatid; V, vessel. Toluidine blue-stained semi-thin resin sections, section thickness 0.5 μm . Bars: 15 μm .

the DM+MC group. Cur has been shown to improve testicular histology and spermatogenesis in various experimental models [25, 52, 57] while studies on MC have reported both protective and potentially reproductive toxic effects depending on the dose, extract type, and target organ. In a chronic stress model, MC fruit has been shown to reduce caspase 3 expression due to its antioxidant properties, protecting against sexual dysfunction and testicular damage caused by stress-related apoptosis [58];

conversely, MC seeds have been shown to exhibit antispermato-genic effects, and these effects are suggested to arise through disruptions in Leydig and Sertoli cell functions, which play a role in the histophysiological events of spermatogenesis [59]. In DM+Cur, the Sertoli cell volume ratio did not differ from DM, whereas the spermatocyte and spermatid volume ratios increased significantly. Since Sertoli cells are postmitotic cells in adulthood, no numerical or volumetric increase in these cells is expected

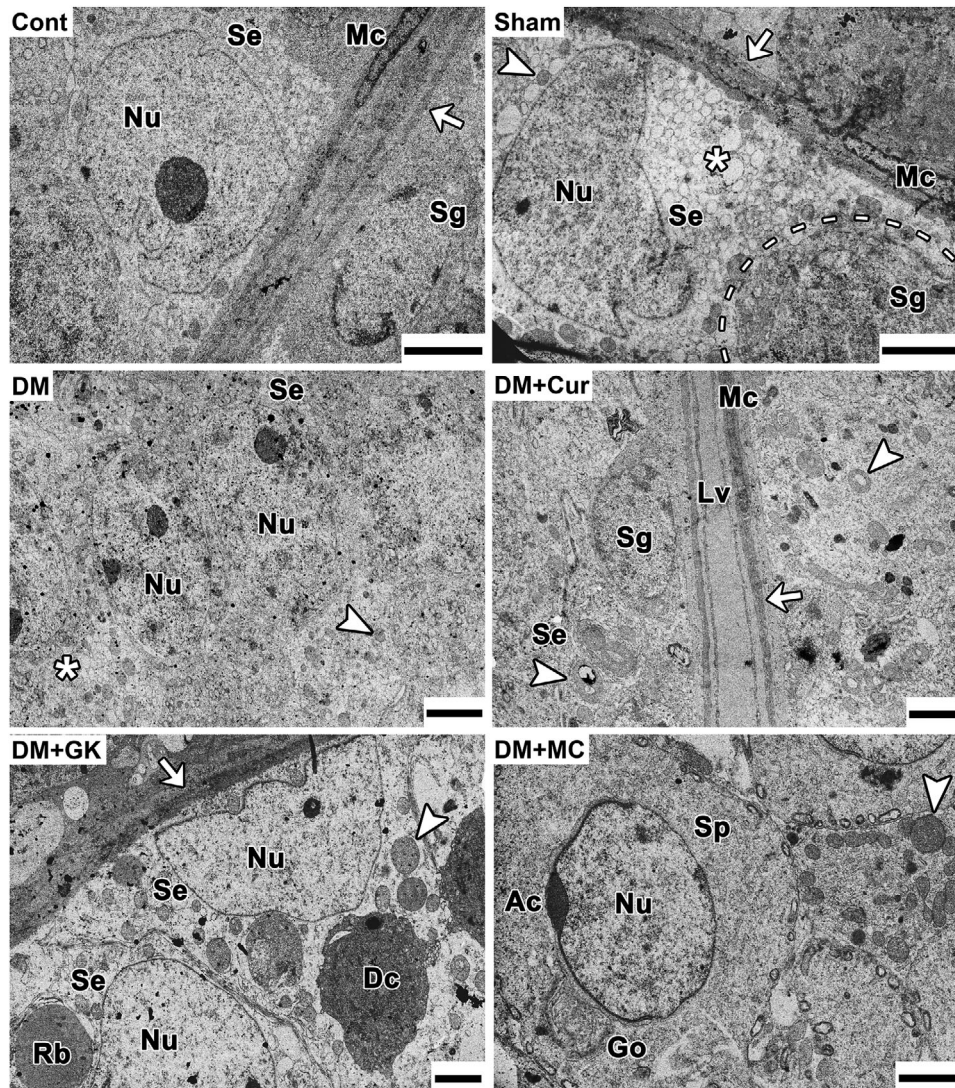


FIGURE 9 | Representative transmission electron micrographs of testicular tissue stained with uranyl acetate and lead citrate. **(Cont)** Sertoli cells and spermatogonia are observed. Nuclear boundaries of the cells are distinct. The basement membrane (arrow) exhibits normal morphology. **(Sham)** The nuclear boundaries of the Sertoli cell are clearly defined and appear normal. The cytoplasm contains healthy mitochondria (arrowhead) and abundant smooth endoplasmic reticulum (asterisk). The spermatogonium, outlined by dashed lines, shows loss of distinct nuclear and cytoplasmic boundaries. **(DM)** Although nucleoli of Sertoli cells are partially discernible, the nuclear envelope shows focal loss of integrity. Cytoplasmic boundaries are indistinct. The cytoplasm contains a limited number of mitochondria (arrowhead) and smooth endoplasmic reticulum profiles (asterisk). **(DM+Cur)** Seminiferous tubules and the intervening interstitial tissue are observed. The lymphatic vessel within the interstitial tissue appears structurally intact, with a clearly defined endothelium. The spermatogonial cells within the tubular epithelium exhibit normal morphology, with well-preserved nuclear boundaries. Damaged mitochondria (arrowhead) are evident in the cytoplasm of the Sertoli cell. The basement membrane is thin and continuous. **(DM+GK)** The basement membrane (arrow) is well preserved, and Sertoli cells display distinct nuclear boundaries. In the cytoplasm, healthy mitochondria (arrowhead) are observed, along with residual bodies and remnants of degenerated cells. **(DM+MC)** Spermatids exhibit normal morphology, with well-preserved nuclear and cytoplasmic boundaries. A prominent Golgi apparatus is present in the cytoplasm. The acrosomal cap, a darkly stained structure at one pole of the spermatid nucleus, shows normal morphology. In one spermatid, mitochondria of varying sizes with well-preserved structures are clearly observed (arrowhead). Ac, acrosomal cap; Dc, degenerated cell; Go, Golgi apparatus; Lv, lymphatic vessel; Mc, myoid cell; Nu, nucleus; Rb, residual body; Se, Sertoli cell; Sg, spermatogonium; Sp, spermatid. Thin sections obtained from resin blocks, section thickness 90 nm. Bars: Cont, Sham, DM (3 μ m); DM+Cur, DM+GK, DM+MC (2 μ m).

after chronic hyperglycemia. It is thought that Cur administration improves support of germ cells by preserving the functional integrity of Sertoli cells rather than their number, thereby providing volumetric recovery, especially in spermatocytes and spermatids. Total seminiferous tubule volume did not differ among groups, indicating that within the experimental period, diabetes

and antioxidant treatments primarily affected cellular and functional parameters while the tubular framework remained largely preserved.

Evaluation of multiple BTB-associated tight junction proteins together provides more comprehensive information about BTB

integrity. Claudin-11 is the main structural component of tight junctions between Sertoli cells, while occludin is a functional component that primarily regulates permeability [60]. Intense expression and altered localization of linkage proteins have been associated with increased permeability of the BTB due to structural and functional damage and impaired spermatogenesis [61–63]. Conversely, some studies have shown that increased expression of various proinflammatory cytokines and chemokines, including TGF- β 3, TNF- α , MCP-1, IL-1, and IL-6, leads to decreased expression of occludin and claudin-11, as well as to oxidative stress in Sertoli cells, thereby facilitating BTB permeability [64–66]. The term abnormal expression may refer to either increased or decreased expression levels. Accordingly, BTB permeability is influenced not only by the abundance of tight junction proteins but also by their correct localization and functional organization [67, 68]. In this context, it can be suggested that antioxidant applications increase protein expression in response to barrier alterations, but optimal localization has not yet been achieved.

Caspase-3 immunoreactivity showed clear differences among the antioxidant-treated groups. The DM group exhibited intense nuclear caspase-3 positivity in Sertoli cells and across germ cell lineages, indicating increased apoptotic activity. Previous studies have shown that DM-related oxidative stress, inflammation, and mitochondrial dysfunction activate the caspase-3-mediated apoptotic pathway in testicular germ cells [64–66, 69]. In the Control group, low-intensity caspase-3 staining reflected physiological germ cell apoptosis, a process required for normal spermatogenesis. More than half of germ cells die during normal developmental processes [70]. In the Sham group, increased staining at the spermatocyte and spermatid levels was likely related to acute gavage-associated stress. Increased glucocorticoids during stress can alter Sertoli and Leydig cell function and increase germ cell apoptosis [71]. Additionally, stress can increase sympathetic activity in the testis, altering vascular tone and affecting testicular perfusion [72].

Among antioxidant treatments, GK showed a highly pronounced qualitative suppression of apoptosis, with only low-intensity cytoplasmic caspase-3 staining in a limited number of germ cells. This finding indicates that GK provided the lowest level of residual apoptotic damage. In studies, Cur has been reported to improve fertility in STZ-induced diabetic rats by affecting sperm parameters, testicular antioxidant enzyme activities, and testosterone levels, and alleviating diabetes-related organ damage by reducing lipid peroxidation, oxidative stress, and inflammation [29, 73, 74].

Cur has been reported to inhibit the activation of the caspase cascade under diabetic conditions through its anti-apoptotic effects, thereby protecting testicular cells from mitochondrial and ER-dependent apoptotic cell death induced by cellular stress [23]. In an experimental varicocele model, administration of Cur at 100 mg/kg for 2 months significantly reduced caspase-3 immunoreactivity [75]. In the present study, Cur treatment reduced apoptotic damage compared with the DM group; however, caspase-3 activity was not completely suppressed, particularly in spermatocytes and spermatids, which may be related to the applied dose, treatment duration, or the severity of the diabetic model.

In contrast, MC showed prominent caspase-3 expression in spermatogonia and Sertoli cells, indicating persistent apoptosis despite partial protective effects. It has been reported that MC increases Bcl-2 expression, decreases Bax and caspase-3 expression, and reduces apoptotic activity in testicular tissue, along with a decrease in the Bax/Bcl-2 ratio [33]. However, it may cause testicular dysfunction or have antispermatogenic effects on the male reproductive system [59, 76, 77]. This suggests that the effect of MC depends on the type of extract used and the experimental conditions.

A combined evaluation of the light microscopic and ultrastructural findings demonstrated that diabetes mellitus induces profound disruption of testicular architecture at both structural and subcellular levels. Severe degeneration of the seminiferous epithelium, basement membrane irregularities, Sertoli cell damage, and alterations in sperm flagellar organization observed in the DM group collectively indicate impaired spermatogenic support and compromised BTB integrity. These findings were consistently reflected at the ultrastructural level by loss of organelle organization, mitochondrial damage, and discontinuity of basal membrane structures. Among the antioxidant-treated groups, GK exhibited the most pronounced protective profile, preserving seminiferous tubule organization, Sertoli cell ultrastructure, and Leydig cell morphology to a near-normal extent. Cur provided partial protection, with noticeable improvement in epithelial organization and Sertoli cell integrity; however, persistent mitochondrial damage and residual apoptotic features indicated incomplete recovery. In contrast, although MC largely preserved seminiferous epithelial architecture and early spermiogenic structures, sustained macrophage infiltration, interstitial edema, and focal ultrastructural abnormalities suggested ongoing inflammatory activity. Taken together, these findings indicate that while all tested antioxidants mitigated DM-related testicular injury to varying degrees, their protective efficacy and underlying cellular targets differed substantially, highlighting the importance of dose, treatment duration, and inflammation-related mechanisms in modulating testicular recovery.

A major strength of this study lies in its comprehensive evaluation of diabetic testicular injury beyond oxidative stress and hormonal alterations, with a particular focus on the structural and functional integrity of the BTB. Under identical diabetic conditions and treatment protocols, the effects of Cur, GK, and MC were assessed using an integrated stereological, biochemical, immunohistochemical, light-microscopic, and ultrastructural approach, which is currently underrepresented in existing literature. The combined interpretation of claudin-11 and occludin expression with ultrastructural findings provides novel insight into the cellular and structural basis of barrier dysfunction in the diabetic testes. Moreover, the comparative analysis of three phytotherapeutic agents at the same dose reveals distinct protective profiles: GK shows pronounced morphological and barrier-preserving effects, Cur primarily attenuates oxidative stress, and MC exerts more limited and variable protection.

5 | Conclusions

STZ-induced diabetes caused substantial testicular injury associated with disruption of seminiferous organization, BTB integrity,

and cellular homeostasis. The findings of this study demonstrate that antioxidant-based interventions can provide partial and differential protection against diabetes-related testicular damage, supporting the relevance of targeting oxidative stress, barrier integrity, and apoptotic pathways in this context.

Several limitations of the study should be acknowledged. The applied doses and treatment duration were based on previous literature and may not represent optimal regimens for all antioxidants. The vehicles were not identical due to the different solubility profiles of the compounds; Cur and MC were administered in an oil phase, whereas GK was dissolved in distilled water, given its hydrophilic nature. Although this solvent variation was a methodological necessity, it remains a limitation. Additionally, selecting a uniform dose for all extracts standardized the comparison under identical experimental conditions. This weight-based approach, however, does not account for differences in absorption and chemical potency, so identical dosing may not yield equivalent biological effects. The immunohistochemical evaluation of tight junction and apoptotic proteins was qualitative and descriptive and was not complemented by molecular validation. Because this investigation focused on the stereological, histopathological, and biochemical parameters of male rats, direct functional fertility outcomes, such as mating performance or pregnancy rates, were not evaluated. Finally, the severe STZ model may not fully reflect milder or diet-induced forms of diabetes. Future studies focusing on dose optimization, extended treatment periods, alternative diabetic models, and complementary molecular analyses are warranted to better define the translational relevance of these findings.

Author Contributions

Ayşenur Kaya Mutlu: conceptualization, investigation, methodology, formal analysis, data curation, visualization, Writing – original draft, Writing – review and editing. **Süleyman Kaplan:** supervision, conceptualization, methodology, visualization, validation, project administration, resources, funding acquisition, writing – original draft, writing – review and editing.

Acknowledgments

The authors thank Prof. Dr. Özgür Korhan Tunçel for granting permission to perform the biochemical analyses, and Dr. Sebati Sinan Ürkmez, Dr. Okan Güleröğlu, and Dr. Ebru Kalaycı for their assistance in carrying out these analyses at the Department of Medical Biochemistry, Ondokuz Mayıs University.

Funding

This study was supported by the Ondokuz Mayıs University Scientific Research Projects Coordination Unit (Suleyman Kaplan; BAP04-B-2024-5057).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets used during the current study are available from the corresponding author on reasonable request.

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