Hindawi
Andrologia
Volume 2023, Article ID 6385767, 10 pages
https://doi.org/10.1155/2023/6385767

Research Article

Resveratrol Mitigates Diabetic Testicular Dysfunction, Endocrine Deficits, and Insulin Resistance via Suppression of Sperm-Endocrine Aberrations and Oxidative Inflammation in Rats

Hany Elsawy[1,2], **Ademola C. Famurewa**[3,4] **and Azza Sedky**[5,6]

[1]*Department of Chemistry, College of Science, King Faisal University, PO Box 400 Al-Ahsa 31982, Saudi Arabia*
[2]*Department of Chemistry, Faculty of Science, Tanta University, Tanta, Egypt*
[3]*Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Medical Sciences, Alex Ekwueme Federal University, Ndufu-Alike, Ikwo, Ebonyi State, Nigeria*
[4]*Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal, Karnataka State, India*
[5]*Department of Biological Sciences, College of Science, King Faisal University, PO Box 400, 31982 Al-Ahsa, Saudi Arabia*
[6]*Department of Zoology, Faculty of Science, Alexandria University, Alexandria, Egypt*

Correspondence should be addressed to Hany Elsawy; hmostafa@kfu.edu.sa
and Ademola C. Famurewa; ademola.famurewa@funai.edu.ng

Received 6 October 2022; Revised 19 October 2022; Accepted 25 October 2022; Published 6 February 2023

Academic Editor: Vikas Kumar Roy

Diabetes mellitus (DM) provokes reproductive impairments through endocrine disturbance, sperm deficits, and testicular oxidative inflammation. The study investigated the reproductive protective effects of resveratrol (RSV) against testicular oxidative inflammation, sperm/endocrine deficits, and insulin resistance in streptozotocin- (STZ-, 65 mg/kg) induced DM rat model. Male rats were randomly divided into 4 groups ($n = 6$): control, DM, RSV (150 mg/kg bw, orally), and RSV+DM group (21 days). The nontreated DM rats showed marked decreases in serum insulin, reproductive hormones (T, LH, and FSH), and lipid profile levels compared to control. The homeostatic index of insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI) were adversely modulated. Sperm count and motility were profoundly decreased, whereas sperm abnormality was significantly increased. The testicular activities of catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), and malondialdehyde (MDA) level, along with inflammatory cytokines (TNF-α, IL-6, IL-4, and IL-10) were significantly dysregulated. The DM induced histopathological lesions compared to control rats. Interestingly, the RSV administration to DM rats attenuated the altered reproductive parameters, restored antioxidant mechanism, and anti-inflammatory responses with improved insulin resistance. RSV could prevent DM-induced reproductive deficits and insulin resistance via modulating oxidative stress-mediated testicular inflammation in rats.

1. Introduction

Diabetes is a global health challenge in various countries of the world with prevalence estimated 463 million adults [1]. It has been projected that, by 2045, the endocrine disease may affect 700 million adults [1, 2]. The pathophysiology of the frequently diagnosed type 2 diabetes mellitus is complicated and defined by 50% pancreatic cell loss at diagnosis, reduced insulin sensitivity of target tissues, and decreased insulin production. As a consequence, chronic hyperglycemia promotes reactive oxygen species (ROS) accumulation, oxidative damage, and impaired systemic antioxidant mechanism leading to organ damage [3, 4]. During hyperglycemic milieu, self-oxidation of glucose is accelerated provoking

excessive production of ROS and lipid peroxidation resulting into oxidative stress mechanism [5]. The chronic hyperglycemia-mediated oxidative stress mechanism adversely affects reproductive apparatus leading to spermatogenesis decline and testicular damage [6, 7]. Diabetic reproductive impairment therefore is a major complication which has been reported in a considerable number of diabetic patients. In the testis oxidative stress, ROS impairs cell membrane and DNA integrity and activates overexpression of genes related to regulatory cascades of testicular inflammation, including cytokines and cyclooxygenases [5]. Given the role of oxidative stress in the pathogenesis of diabetic testicular complication, spermatogenic cells may undergo natural apoptosis, structural damage, and decrease in sperm count and motility [7, 8]. Furthermore, studies have reported the deleterious effect of diabetes on endocrine physiology, steroidogenic signaling, and lipid metabolism [9–11]. For example, remarkable decreases in the concentrations of testosterone and gonadotropins-follicle-stimulating hormone (FSH), gonadotropin releasing hormone (GnRH), and luteinizing hormone (LH) have been reported in published papers [10, 12, 13].

However, the pharmacological management of diabetes mellitus (DM) is currently unsatisfactory and is associated with some considerable side effects [14]. Natural products have been recognized as an effective and safer approach for therapeutics development. Accumulating evidence suggests that natural compounds could improve glucose homeostasis in DM and consequently mitigate development and progression of diabetic complications, including DM testicular damage [15–18]. Hence, the search for antioxidant natural products is to scavenge ROS and curtail hyperglycemia-mediated reproductive impairments.

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a bioactive natural compound belonging to the group of polyphenolic derivatives of stilbene. Resveratrol (RSV) is abundant in grape, grape juice, peanuts, wine, mulberries, and blackcurrants [19]. Among many stilbene compounds, RSV has distinctively demonstrated several beneficial biological activities like antioxidant, anti-inflammatory, antiobesity, anticancer, neuroprotective, hepatoprotective, and cardioprotective effects in various models as well as mitigation against diabetic nephropathy and organ toxicities [14, 19–22]. Although there are reports that RSV may improve glucose homeostasis and reverse insulin resistance in DM, the reported data are inconsistent [23]. In addition, the molecular effect of RSV particularly on DM-induced testicular damage and endocrine dysfunction remains to be profoundly elucidated [24]. The current study was therefore investigated to elucidate the role RSV in DM-induced testicular oxidative inflammation, endocrine deficits, sperm characteristics, and insulin resistance in rats.

2. Materials and Methods

2.1. Chemicals. Resveratrol, STZ, buffered saline solution, and other reagents were procured from Sigma-Aldrich Chemical, Shanghai, China. The standard lipid peroxide kits for ELISA assays for MDA (Cat No: MD2528, Bio-Diagnostic, Egypt), CAT (Cat No: CA2517), SOD (Cat. No: SD2521), and GPx (Cat. No: GP2529) activities were obtained from Bio-Diagnostics, Giza, Egypt. Commercial kits for determination of luteinizing hormone (LH: Cat. No. LH-010) and follicle-stimulating hormone (FSH: Cat. No. E-El-M0511) were purchased from Shibayagi Co., Ltd., Shibukawa-Gunma, Japan, and testosterone (Cat. No: KT29533) was purchased from K-assay, WA, USA. Rat ELISA kit (Cat. No: E-EL-R3034) for serum insulin was purchased from Elabscience, Texas, USA, while glucose assay kit (Cat No: ab65333) was from Abcam, UK.

2.2. Animals. Twenty-four (24) adult male rats weighing 180-200 g (8 weeks old) were purchased from the animal house of the Faculty of Science, King Faisal University, Saudi Arabia. Ethical statement—all experimental procedures were done according to the Institutional Animal Care and Use Committee (IACUC) of the King Faisal University (Reference number: KFU-REC-2022-OCT-ETHICS196). The rats were housed in plastic cages, floored with soft wood shaving that was changed three times per week. The animals were acclimatized for 2 weeks prior the study and were maintained under 12 h light/dark cycle at ($25 \circ C \pm 2 \circ C$), with free access to water and rat chow.

2.3. Induction of Diabetes Mellitus. Healthy male rats were fasted overnight and a single dose of streptozotocin (STZ) (65 mg/kg bw), newly prepared in citrate buffer, pH 4.5, was injected by intraperitoneal route to induce type 2 diabetes mellitus. Diabetes was confirmed 72 hours after STZ injection via estimating plasma glucose levels from blood samples (3 ml each) collected through lateral tail vein. Rats with blood glucose levels of 250 mg/dl or higher were considered diabetic and used for this study. Rats that appeared unhealthy were identified and separated out from the study.

2.4. Experimental Design and Treatment Protocols. After the two-weeks acclimatization, all rats were randomly divided into 4 groups with six rats in each group. Group 1 was the control administered only water solution of carboxymethyl cellulose (CMC, 2 ml/kg bw) by gavage for 21 days. Group 2 rats were diabetic rats and were administered CMC by gavage for 21 consecutive days. Group 3 contained nondiabetic rats administered only RSV (150 mg/kg) by gavage for 21 consecutive days [25]. RSV was dissolved in water solution of CMC. Group 4 contained diabetic rats administered RSV (150 mg/kg) by gavage for 21 consecutive days. After the experimental treatment, rats were fasted overnight, anesthetized, dissected, and both trunk blood and testis samples were collected for biochemical and histological studies. The animals were euthanized by anesthetic exsanguination using the combination of 10 mg/kg xylazine and 100 mg/kg ketamine HCl. Whole blood samples were collected in clean dry centrifuge tubes, containing no anticoagulation factors and allowed to clot for a minimum of 30 minutes at 37°C before centrifuged to obtain serum (769 × g for 15 minutes) and stored at -20°C until the analysis. The testes were weighed and homogenized (1 : 5 w/v) with a Potter-Elvehjem homogenizer attached to a Teflon plunger in ice

cold phosphate buffer (50 mM, pH 7.5). The homogenates were centrifuged to obtain supernatant samples (11,000 × g for 20 minutes). The supernatant samples divided into aliquots stored at -20°C for different assays.

2.5. Biochemical Estimations

2.5.1. Estimation of Fasting Serum Insulin and Glucose Concentration. Fasting serum insulin levels were measured by an Insulin ELISA Kit (Cat. No: E-EL-R3034) according to the manufacturer's procedures. Serum glucose was determined using the glucose oxidase method [26].

2.5.2. Homeostatic Index of Insulin Resistance (HOMA-IR). The homeostatic index of insulin resistance (HOMA-IR) was estimated as a surrogate of insulin sensitivity [27]. HOMA-IR was calculated by using the homeostasis model assessment equation below

$$\text{HOMA-IR} = [\text{fasting glucose(mg/dl)} \times \text{fasting insulin}(\mu\text{IU/ml})] \div 405 \quad (1)$$

2.5.3. Quantitative Insulin Sensitivity Check Index (QUICKI). Quantitative insulin sensitivity check index (QUICKI) was determined as a surrogate of insulin sensitivity [28]. QUICKI was calculated by using the inverse of the sum of the logarithms of fasting insulin and fasting glucose as follows:

$$\text{QUICKI} = 1 \div \log\,[\text{fasting glucose(mg/dl)}] + \log\,[\text{fasting insulin}\,(\mu\text{IU/ml})] \quad (2)$$

2.6. Determination of Serum Lipid Profile Parameters. Total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) levels were determined by using commercial kits (TC, Cat. No: CH 1220; TG, Cat. No: TR 2030, HDL-C, Cat. No: CH 1230; Bio-Diagnostics, Giza, Egypt). Very-low-density lipoprotein cholesterol (VLDL-C) and low-density lipoprotein cholesterol (LDL-C) were calculated using Friedewald's formula [29].

2.7. Determination of Reproductive Hormones. Serum testosterone levels were determined using the K-Assay Testosterone ELISA Kit (Cat No. KT29533) following Sakuma [30] method. Luteinizing hormone levels were estimated with the Japanese Shibayagi Company Rat ELISA Kit (Cat No. LH-10) according to Shioya and Wakabayashi [31] method, while follicle-stimulating hormones were assessed using commercial kits (Cat No. E-EI-M0511) based on the method of Teerds et al. [32] protocols from BioVendor, Tokyo, Japan.

2.8. Determination of Sperm Count, Motility and Abnormality. The suspension from testis epididymis was used for assessing sperm quality. Sperm count was done using hemocytometer following the method of Freud and Carol (1964). The technique of Evans and Maxwell [33] was adopted for sperm abnormality study. Whereas, the assessment of sperm motility was done according to Morrissey et al. (1988) as stated in our recent paper by Alturki et al. [34].

2.9. Determination of Testicular Oxidative Stress Markers. Supernatant homogenate samples were used for the oxidative stress analysis. Testis tissues were homogenized (1 : 5 w/v) with a Potter-Elvehjem homogenizer attached to a Teflon plunger in ice cold phosphate buffer (50 mM, pH 7.5). The homogenates were centrifuged to obtain supernatant samples (11,000 × g for 20 minutes). Malondialdehyde levels were measured following the method of Ohkawa et al. [35]. Testicular catalase activity was determined by Aebi [36]. The activity of SOD was determined by Masayasu and Hiroshi [37]. Glutathione peroxidase (GPx) activity was estimated by the method of Paglia and Valentine [38].

2.10. Quantification of Testicular Proinflammation and Anti-Inflammation. The testicular levels of TNF-α (Cat No: EA100365), IL-4 (Cat No: MBS162452), IL-10 (Cat No: MBS764911), and IL-6 (Cat No: MBS726707) were measured with rat standard-ELISA kits procured from OriGene Technologies Inc., Rockville, MD and MyBioSource, Inc., San Diego, USA, following the manufacturers' directions.

2.11. Testicular Histopathological Analysis. Testis tissue samples were removed and immediately fixed in 10% buffered formalin. Dehydration was carried out with graded ethanol and embedded in paraffin. Testis sections were stained with haematoxylin and eosin (H and E) for microscopic histopathological lesions according to Bancroft and Gamble [39]. The prepared slides were examined under light microscope. Testicular lesions were semiquantitatively graded as follows: 0: intact histoarchitecture (normal), 1: mild lesions, 2: moderate lesions, and 3: severe damage [34].

2.12. Statistical Analyses. Data were compared using one-way ANOVA followed by LSD multiple range test. Significant differences were obtained at $P < 0.05$. Statistical tests were performed using SAS statistical software (SAS v.9.2, SAS Institute, Inc.).

3. Results

3.1. Effect of RSV on Testes of Rats. The diabetic status of rats led to a drastic reduction in testis weights. The percentage of testes weight loss was 59.68% in the DM group compared to the standard control group ($P < 0.05$). On the other hand, RSV at the administered dose considerably increased the testis weights compared with the DM group ($P < 0.05$; Table 1).

3.2. Effect of RSV on Serum Glucose, Insulin, and Insulin Resistance Markers. Table 2 presents the effect of RSV on serum glucose, insulin, HOMA-IR, and QUICKI in STZ-induced diabetic rats. In DM rats, serum glucose level and homeostatic index of insulin resistance (HOMA-IR) were significantly increased compared to control ($P < 0.05$), whereas insulin level and quantitative insulin sensitivity check index (QUICKI) were significantly decreased compared to control ($P < 0.05$). In contrast, the administration

Table 1: Effect of RSV on testis weight of rats.

Group	Testis weight (g)
Control	3.77 ± 0.03
DM	1.52 ± 0.08*
RSV	3.74 ± 0.11
DM+RSV	2.33 ± 0.09#

Data are displayed as mean ± SEM of $n = 6$ rats/group. *$P < 0.05$: significant compared to control in the same column. #$P < 0.05$: significant when compared to DM in the same column.

Table 2: Effect of RSV on serum glucose, insulin, and markers of insulin resistance in DM rats.

Group	Glucose (mg/dl)	Insulin (μIU/ml)	HOMA-IR	QUICKI
Control	81.32 ± 0.23	10.38 ± 0.12	2.08 ± 0.02	0.34 ± 0.01
DM	373.58 ± 0.32*	4.20 ± 0.01*	3.89 ± 0.03*	0.31 ± 0.01
RSV	80.62 ± 0.21	11.02 ± 0.09	2.19 ± 0.01	0.34 ± 0.03
DM +RSV	175.70 ± 0.24#	6.38 ± 0.06#	2.76 ± 0.02#	0.33 ± 0.02

Data are displayed as mean ± SEM of $n = 6$ rats/group. *$P < 0.05$: significant compared to control in the same column. #$P < 0.05$: significant when compared to DM in the same column. HOMA-IR: homeostatic index of insulin resistance; QUICKI: quantitative insulin sensitivity check index.

Table 3: Effect of RSV on serum lipid profile in DM rats.

Group	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Control	54.6 ± 0.22	36.2 ± 0.20	29.2 ± 0.20	17.1 ± 0.20
DM	114.7 ± 0.20*	76.2 ± 0.20*	17.6 ± 0.11*	56.5 ± 0.14*
RSV	54.7 ± 0.12	36.5 ± 0.21	28.9 ± 0.10	17.4 ± 0.21
DM +RSV	66.2 ± 0.30#	48.7 ± 0.13#	20.3 ± 0.10#	29.6 ± 0.13#

Data are displayed as mean ± SEM of $n = 6$ rats/group. *$P < 0.05$: significant compared to control in the same column. #$P < 0.05$: significant when compared to DM in the same column.

Table 4: Effect of RSV on serum reproductive hormones in DM rats.

Group	T (pg/ml)	LH (ng/ml)	FSH (ng/ml)
Control	8.72 ± 0.12	1.50 ± 0.05	0.87 ± 0.01
DM	4.91 ± 0.01*	0.69 ± 0.01*	0.36 ± 0.01*
RSV	8.72 ± 0.09	1.40 ± 0.00	0.89 ± 0.00
DM+RSV	6.38 ± 0.06#	0.90 ± 0.02#	0.57 ± 0.01#

Data are displayed as mean ± SEM of $n = 6$ rats/group. *$P < 0.05$: significant compared to control in the same column. #$P < 0.05$: significant compared to DM in the same column. T: Testosterone; LH: leutenizing hormone; FSH: follicle-stimulating hormone.

of RSV to DM rats markedly decreased glucose level and suppressed insulin resistance as shown by reduced HOMA-IR compared to DM group. Also, the insulin level significantly increased as well as insulin sensitivity check index (QUICKI) in comparison to the DM group.

Table 5: Effect of RSV on sperm parameters in DM rats.

Group	Sperm count (10^6 cells/ml)	Motility (%)	Abnormality (%)
Control	97.4 ± 0.80	83.4 ± 0.83	8.0 ± 0.44
DM	65.4 ± 0.72*	57.2 ± 1.01*	42.2 ± 1.02*
RSV	97.2 ± 0.91	85.2 ± 0.84	8.2 ± 0.42
DM +RSV	74.0 ± 1.00#	79.6 ± 0.74#	23.8 ± 0.71#

Data are displayed as mean ± SEM of $n = 5$ rats/group. *$P < 0.05$: significant compared to control in the same column. #$P < 0.05$: significant compared to DM in the same column.

3.3. Effect of RSV on Serum Lipid Profile. Table 3 presents the effect of RSV on serum levels of TC, TG, HDL-C, and LDL-C in diabetic rats. It was observed that the diabetic condition of the rats considerably increased serum levels of TC, TG, and LDL-C, whereas HDL-C reduced compared to the non-diabetic control group ($P < 0.05$). In DM+RSV group, the levels of the lipid profiles were reversed significantly ($P < 0.05$) when the levels were compared to the DM group.

3.4. Effect of RSV on Reproductive Hormones. The results of levels of serum reproductive hormones are presented in Table 4. The levels of T, LH, and FSH were prominently declined in nontreated DM group in comparison to nondiabetic control group ($P < 0.05$). However, the RSV administration in DM+RSV group markedly increased the hormones appreciably compared to DM group in this study.

3.5. Effect of RSV on Sperm Count, Motility, and Abnormality. Table 5 depicts the effect of RSV on the sperm count, sperm motility, and abnormality of rats with STZ-induced DM. In DM group, it was found that the sperm count, motility, and abnormality were increased significantly compared to control ($P < 0.05$). Interestingly, in the DM +RSV group, the count, motility, and abnormality were profoundly prevented by RSV in comparison to DM group.

3.6. Effect of RSV on Testicular Oxidative Stress. Figures 1–4 show the effect of RSV and diabetes on testicular antioxidant enzyme activities and lipid peroxidation marker, MDA, in diabetic rats. In nontreated diabetic rats, testicular activities of the antioxidant enzymes, CAT, SOD, and GPx were significantly reduced, whereas the level of MDA markedly increased ($P < 0.05$) compared to control. Contrarily, the administered RSV in DM+RSV group significantly enhanced the enzyme activities and reduced MDA level in comparison to DM group in this study.

3.7. Effect of RSV on Testicular Inflammation. In the current study, we evaluated proinflammatory and anti-inflammatory markers in the testis of diabetic rats. Figures 5–8 present the effect of RSV on testicular proinflammatory markers (TNF-α and IL-6) and anti-inflammatory markers (IL-4 and IL-10). In DM group, the levels of TNF-α and IL-6 increased

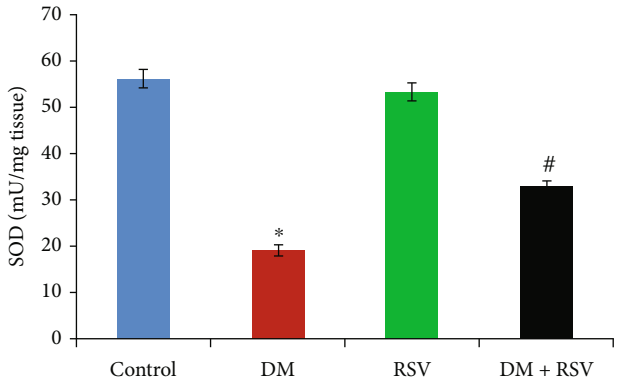

Figure 1: Effect of RSV on testicular superoxide dismutase activity in DM rats. Data are expressed as mean ± SEM ($n = 6$). *Significant when compared to control ($P < 0.05$); #significant compared to DM group ($P < 0.05$).

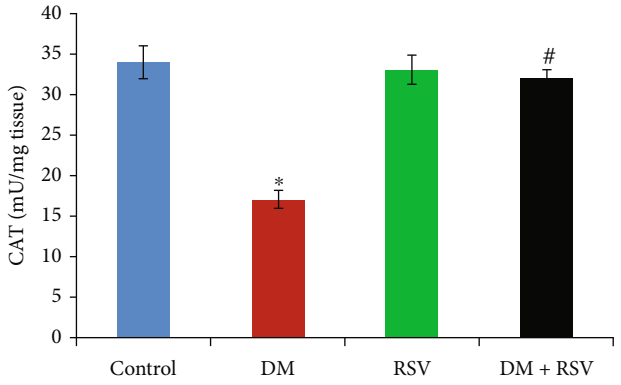

Figure 2: Effect of RSV on testicular catalase activity in DM rats. Data are expressed as mean ± SEM ($n = 6$). *Significant when compared to control ($P < 0.05$); #significant compared to DM group ($P < 0.05$).

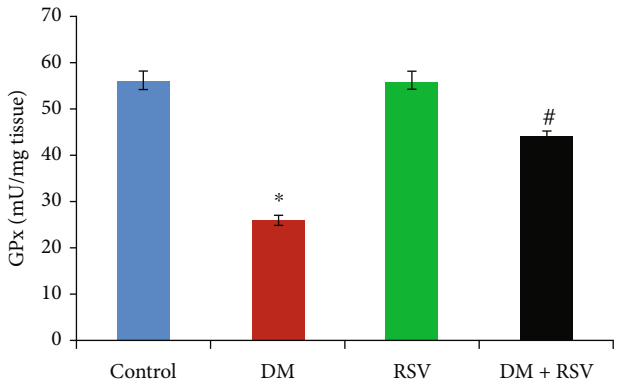

Figure 3: Effect of RSV on testicular glutathione peroxidase activity in DM rats. Data are expressed as mean ± SEM ($n = 6$). *Significant when compared to control ($P < 0.05$); #significant compared to DM group ($P < 0.05$).

significantly while IL-4 and IL-10 levels decreased significantly in comparison to control. However, it was interesting to observe that the RSV administration to the DM rats

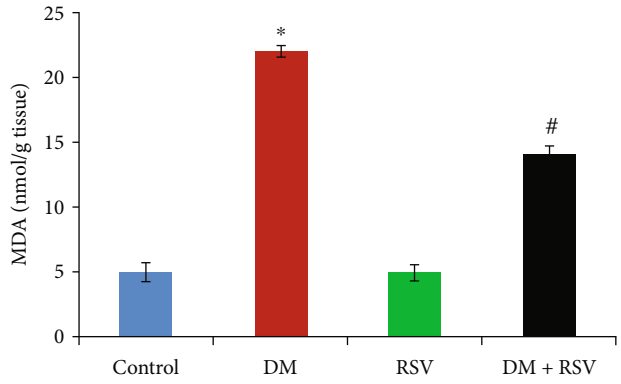

Figure 4: Effect of RSV on testicular malondialdehyde level in DM rats. Data are expressed as mean ± SEM ($n = 6$). *Significant when compared to control ($P < 0.05$); #significant compared to DM group ($P < 0.05$).

reduced TNF-α and IL-6 levels and increased IL-4 and IL-10 levels significantly compared to DM group.

3.8. Effect of RSV on Testis Histopathology. The histopathological lesions observed in the testis samples were presented in Figure 9. Control group with normal seminiferous tubules (indicated by the star) with complete spermatogenic series and separated by interstitial cells indicated by arrow. The DM group revealed wide interstitial space (arrow) between irregular/distorted tubules (ST) and depletion of sperms (asterisks). RSV group showed normal architecture in which tubules were separated by narrow interstitial tissue (circle) with aggregation of spermatozoa in the lumen (S). RSV +DM group showed mild improvement in the seminiferous tubules (ST) and restoration of spermatogenesis (asterisks).

4. Discussion

Diabetes mellitus is a metabolic disease that affects about 8.4% of world population [40]. The hallmark of DM pathogenesis is hyperglycemia and impairment in insulin secretion and/or action. Reproductive damage is a debilitating complication of DM. Resveratrol is a natural polyphenol associated with health-promoting effects in both animals and humans [22]. In the present study, we have reported the effect of RSV on DM-induced testicular damage and endocrine dysfunction.

The dose of STZ (65 mg/kg bw) used for DM induction has been widely reported in experimental models of DM. It is a cytotoxic destroyer of pancreatic β cells via free radical oxidative damage and attack on GLUT-2 receptors which are abundant on the β cells. This damage results in β cell deficient capacity to secrete insulin and thus hyperglycemia [3]. This underscores the observation of significantly elevated levels of serum glucose in the DM group in comparison to control (Table 2). The rats in RSV group showed glucose levels comparable to control in this study. The administration of RSV in RSV+DM group significantly reduced the glucose level when compared to DM. By implication, RSV possesses antihyperglycemic effect in agreement with the existing reports [14,

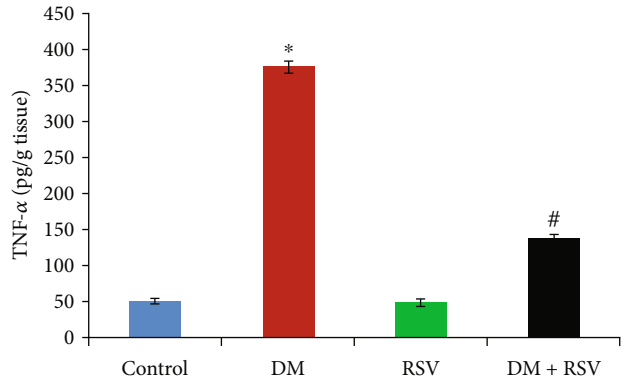

Figure 5: Effect of RSV on testicular tumor necrosis factor-alpha level in DM rats. Data are expressed as mean ± SEM ($n = 6$). *Significant when compared to control ($P < 0.05$); #significant compared to DM group ($P < 0.05$).

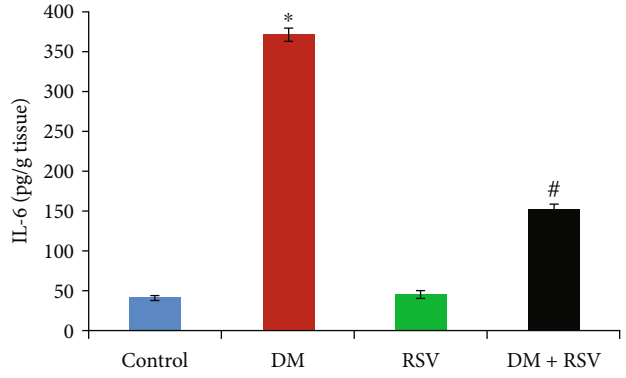

Figure 6: Effect of RSV on testicular interleukin-6 level in DM rats. Data are expressed as mean ± SEM ($n = 6$). *Significant when compared to control ($P < 0.05$); #significant compared to DM group ($P < 0.05$).

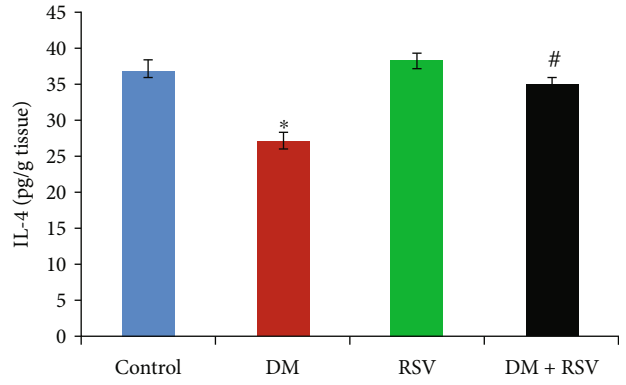

Figure 7: Effect of RSV on testicular interleukin-4 level in DM rats. Data are expressed as mean ± SEM ($n = 6$). *Significant when compared to control ($P < 0.05$); #significant compared to DM group ($P < 0.05$).

23, 41]. The antioxidant property of RSV may play a role in this effect via exerting therapeutic effect on the pancreatic β cells for insulin secretion. In addition, RSV might have enhanced the transport of blood glucose molecules across

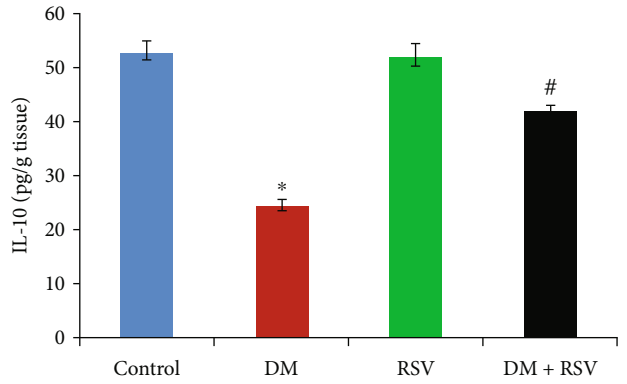

Figure 8: Effect of RSV on testicular interleukin-10 in DM rats. Data are expressed as mean ± SEM ($n = 6$). *Significant when compared to control ($P < 0.05$); #significant compared to DM group ($P < 0.05$).

the cell membranes of peripheral tissues. The resultant antihyperglycemic effect of RSV could be associated with the marked increase levels of insulin in RSV+DM group. In the first instance, the STZ-induced DM (DM group) caused a considerable reduction in insulin levels. Expectedly, the cytotoxic attack of STZ as well as the glucotoxicity of hyperglycemia in DM exerted β-cell damage and impairment of insulin secretion [3]. Therefore, it could be suggested that RSV treatment in RSV+DM group provoked insulin secretion through its protection for the active β-cells that produced insulin and ameliorated the glucotoxicity [42]. Moreover, the antidiabetic action of RSV in the present study was further evident by the improved insulin resistance (HOMA-IR) and/or insulin sensitivity (QUICKI) indices. These indices are known reliable clinical and epidemiological tools for assessment of insulin resistance [43]. The HOMA-IR index value prominently increased in the nontreated DM group, whereas the QUICKI index decreased insignificantly compared to control. Insulin action is crucial to the maintenance of blood glucose level; it initiates the transmembrane signaling cascades via glucose receptors. Insulin resistance index is a calculated value from fasting serum glucose and insulin to reveal the resistance of the peripheral tissue to membrane influx of glucose. Thus, the values for HOMA-IR and QUICKI show that insulin resistance developed in the experimental groups. Our findings herein are in consonance with the reports of previous studies [18, 42, 43]. In contrast, RSV administration to the DM rats evidently restored the insulin action and sensitivity demonstrated by the considerably reduced HOMA-IR and insignificantly increased QUICKI values. Hence, present results indicate that RSV may have improvement effects on DM in relation to β-cell function, insulin sensitivity, insulin secretion, and reduction of insulin resistance de novo [23, 42].

Besides hyperglycemia, hyperlipidemia is a critical metabolic derangement in DM. Glucolipid metabolism is complicated, and usually triggered by pancreatic β-cell deficiency in optimal insulin secretion [44]. The clinical characteristics of diabetic dyslipidemia are elevated serum LDL-C, TG, and depressed HDL-C levels which are biochemical precursors to hypertension, hepatic steatosis, and cardiovascular

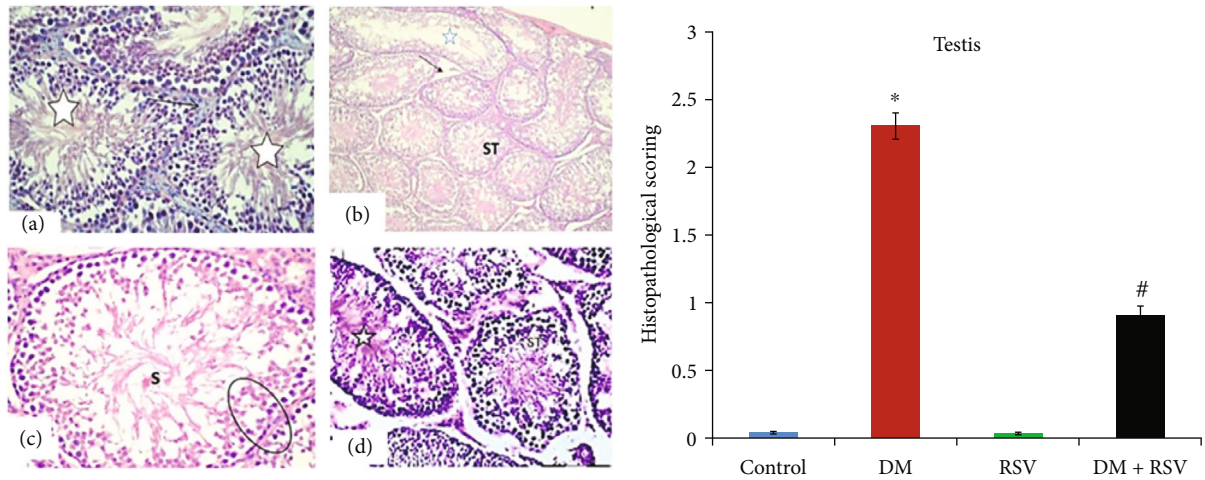

Figure 9: Photomicrographs of DM rat testis administered with RSV (H&E stain). Control (a) DM (b) RSV (c), and RSV+DM (d). Control group with normal seminiferous tubules (star) with interstitial cells (arrow). The DM group revealed interstitial space (arrow), irregular/distorted tubules (ST), and depletion of sperms (asterisks). RSV group showed normal tubules, separated by narrow interstitial tissue (circle) with aggregation of spermatozoa in the lumen (S). RSV+DM group showed mildly damaged seminiferous tubules (ST) and restoration of spermatogenesis (asterisks). Values are expressed as mean ± SEM ($n = 3$). *Significant when compared to control ($P < 0.05$); #significant compared to DM group.

diseases [18, 45]. In our study herein, the experimental DM-induced dyslipidemia evident by considerable elevation in TC, TG, and LDL-C serum levels, while HDL-C level was markedly decreased compared to control (Table 3) [9, 46, 47]. However, on the contrary, the RSV mitigated the dyslipidemia and restored the lipid profiles comparable to control.

In the present study, DM-induced endocrine disruption observed by the altered levels of T, LH, and FSH. Literature has shown that DM is an endocrine disruptor that impairs spermatogenesis and steroidogenesis [10, 11]. It was observed in the DM group that T, LH, and FSH serum levels prominently reduced in comparison to control (Table 4). The depressed levels of these reproductive hormones suggest deficits in testicular testosterone synthesis which can be endocrinologically associated with dysregulations in the gonadotrophic hormones found in this study [34]. Furthermore, insulin resistance, as observed in the DM rats has been linked to reduced secretion of T by Leydig cells [7]. Consequent upon the hormonal deficits in DM rats, the sperm count, mobility, and structural integrity were adversely affected (Table 5) as reported in the earlier systematic publications [24, 41, 48]. The reduction in sperm count was due to reduced spermatogenesis which could be ascribed to reduction in serum T level and/or DM-induced ROS generation. In consonance with the published reports therefore, our study also confirms that DM can disrupt endocrine axis and spermatogenesis [41]. Interestingly, RSV mediated the alterations and caused restoration in the status of the hormones and sperm characteristics. The LH, T, and FSH levels along with sperm count, mobility, and abnormality were significantly restored in RSV+DM group in comparison to DM group. Studies indicate RSV potential to modulate diabetic complications and preserve the testicular integrity [24, 48]. In the study [24], RSV improved the sperm count, motility, viability, and sperm abnormalities as found in our study herein. The antioxidant protection of RSV on spermatocytes and its capacity to increase T levels could contribute to the observed beneficial effects.

Robust body of evidence has implicated elevated ROS generation in the pathophysiology of DM-induced testicular damage [5–7]. The status of imbalance between ROS generation and antioxidant defense results into oxidative stress. The impaired glucose metabolism in DM is a critical mechanism to induce oxidative stress. Herein, DM produced oxidative stress leading to impaired antioxidant milieu in the testes of rats. This was demonstrated by considerable diminution in the testicular activities of CAT, SOD, and GPx, while MDA level conspicuously increased (Figures 1–4) compared to control. The antioxidant enzymes, CAT, SOD, and GPx are the guardian of cellular antioxidant homeostasis. Physiologically, SOD detoxifies superoxide radicals; CAT catalyzes the reaction that drives the lysis of H_2O_2 to H_2O and molecular O_2. Whereas, GPx, a tripeptide peroxidase mediates the neutralization of peroxyl radicals through the reducing action of its glutathione (GSH) moiety [49]. Therefore, DM-induced depression in the testicular activities of SOD, CAT, and GPx would consequently result into testicular oxidative stress [24, 47] and degeneration of testicular ultrastructure which might have contributed to decreased testis weight herein (Table 1). And the elevated level of MDA in the testis unarguably reveals the oxidative stress-mediated damage in the testis of DM rats [8, 47, 50]. The histology of testis confirmed the oxidative damage. The histopathological lesions of the testis were consistent with wide interstitial space, irregular/distorted tubules, and depletion of spermatogenesis in seminiferous tubules, which were earlier reported by Aly [24]. However, the oxidative deterioration in the testis triggered proinflammatory process which caused marked elevation in IL-6 and TNF-α in comparison to control (Figures 5–8). Intriguingly, the

significantly reduced levels of anti-inflammatory cytokines, IL-4, and IL-10 (Figures 7 and 8), further confirmed the DM-induced oxidative stress-mediated testicular proinflammation in the current study. DM and ROS are known triggers of nuclear transcription of a number of inflammatory genes that translate into several cytokine proteins, including TNF-α, IL-6, and IL-1β [4, 51]. Thus, it can be concluded that the induced inflammation was associated with DM oxidative stress in the testicular tissue. RSV is a bioactive antioxidant polyphenol and its potency to combat oxidative stress has been documented [19–22]. We confirm in this study of the antioxidant prowess of RSV in that it significantly elevated the SOD, CAT, and GPx activities in comparison to DM group rats. Antioxidant effect of RSV might have scavenged and blocked ROS production, thereby spearing the enzymes from being consumed and/or overwhelmed by the ROS spurts; hence the markedly reduced MDA level. In consequence to this mechanism, the expressions of the inflammatory cytokines were abated and manifested in the testes as considerably modulated levels of IL-4, IL-6, IL-10, and TNF-α. However, the mild effect of oxidative stress was still observed in the histopathological tissue.

In conclusion, the study findings suggest that RSV could combat redox imbalance, inflammation, sperm-endocrine dysfunctions, and insulin resistance via its antioxidant, antihyperglycemic, and anti-inflammatory mechanisms. Our results add to the existing paucity of data on the effect of RSV on DM-induced testicular complications.

Data Availability

The data used will be provided upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

The authors acknowledge the Deanship of Scientific Research, King Faisal University, Vice Presidency for Graduate Studies and Scientific Research in Saudi Arabia, for financial support under the annual funding track [Grant 1886].

References

[1] P. Saeedi, I. Petersohn, P. Salpea et al., "Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the International Diabetes Federation Diabetes Atlas, 9th edition," *Diabetes Research and Clinical Practice*, vol. 157, article 107843, 2019.

[2] E. Korkus, G. Dąbrowski, M. Szustak et al., "Evaluation of the anti-diabetic activity of sea buckthorn pulp oils prepared with different extraction methods in human islet EndoC-betaH1 cells," *NFS Journal*, vol. 27, pp. 54–66, 2022.

[3] F. A. Al-Abbasi, "Potential antidiabetic activity of barbigerone on glucose and inflammatory cytokine levels in streptozotocin activated diabetic rats," *Journal of King Saud University - Science*, vol. 34, no. 7, article 102249, 2022.

[4] L. Dong, L. Yu, A. Liu, T. A. Alahmadi, H. S. Almoallim, and K. Durairaj, "Ononin mitigates streptozotocin-induced diabetic nephropathy in rats via alleviating oxidative stress and inflammatory markers," *Journal of King Saud University-Science*, vol. 34, no. 6, article 102029, 2022.

[5] B. Singh, A. Kumar, H. Singh et al., "Zingerone produces antidiabetic effects and attenuates diabetic nephropathy by reducing oxidative stress and overexpression of NF-κB, TNF-α, and COX-2 proteins in rats," *Journal of Functional Foods*, vol. 74, article 104199, 2020.

[6] S. F. Akomolafe, I. A. Odeniyi, F. L. Oyetayo, and O. B. Ajayi, "African star apple fruit pulp-supplemented diet modulates fertility-related biomolecules in the testis and epididymis of high-fat diet/streptozotocin- induced diabetic rats," *Journal of Food Biochemistry*, vol. 43, no. 9, article e12969, 2019.

[7] V. U. Nna, A. B. Bakar, A. Ahmad, and M. Mohamed, "Down-regulation of steroidogenesis-related genes and its accompanying fertility decline in streptozotocin-induced diabetic male rats: ameliorative effect of metformin," *Andrology*, vol. 7, no. 1, pp. 110–123, 2019.

[8] M. Mohasseb, S. Ebied, M. A. Yehia, and N. Hussein, "Testicular oxidative damage and role of combined antioxidant supplementation in experimental diabetic rats," *Journal of Physiology and Biochemistry*, vol. 67, no. 2, pp. 185–194, 2011.

[9] C. Eleazu, C. E. Ekeleme, A. Famurewa et al., "Modulation of the lipid profile, hepatic and renal antioxidant activities, and markers of hepatic and renal dysfunctions in alloxan-induced diabetic rats by virgin coconut oil," *Endocrine, Metabolic & Immune Disorders-Drug Targets*, vol. 19, no. 7, pp. 1032–1040, 2019.

[10] P. Song, C. Sun, J. Li et al., "*Tiliacora triandra* extract and its major constituent attenuates diabetic kidney and testicular impairment by modulating redox imbalance and pro-inflammatory responses in rats," *Journal of the Science of Food and Agriculture*, vol. 101, no. 4, pp. 1598–1608, 2021.

[11] Z. Wang, Q. Wu, H. Wang et al., "Diosgenin protects against podocyte injury in early phase of diabetic nephropathy through regulating SIRT6," *Phytomedicine*, vol. 104, article 154276, 2022.

[12] Y. Chen, Y. Wu, X. Gan et al., "Iridoid glycoside from *Cornus officinalis* ameliorated diabetes mellitus- induced testicular damage in male rats: involvement of suppression of the AGEs/RAGE/p38 MAPK signaling pathway," *Journal of Ethnopharmacology*, vol. 194, pp. 850–860, 2016.

[13] K. P. Reddy, M. N. Rao, J. Murthy, and P. S. Reddy, "Lead aggravates the diabetic-induced reproductive toxicity in male Wistar rats," *Toxicology Research*, vol. 5, no. 5, pp. 1465–1476, 2016.

[14] T. Szkudelski and K. Szkudelska, "Resveratrol and diabetes: from animal to human studies," *Biochimica et Biophysica Acta*, vol. 1852, no. 6, pp. 1145–1154, 2015.

[15] R. K. Al-ishaq, M. Abotaleb, P. Kubatka, K. Kajo, and D. Büsselberg, "Flavonoids and their anti-diabetic effects: cellular mechanisms and effects to improve blood sugar levels," *Biomolecules*, vol. 9, no. 9, pp. 430–464, 2019.

[16] N. Jiao, Y. Chen, Y. Zhu et al., "Protective effects of catalpol on diabetes mellitus-induced male reproductive damage via suppression of the AGEs/RAGE/Nox4 signaling pathway," *Life Sciences*, vol. 256, article 116736, 2020.

[17] C. Wen, C. Liu, Y. Li et al., "Ameliorative potentials of the ethanolic extract from *Lycium chinense* leaf extract against diabetic cardiomyopathy. Insight into oxido-inflammatory and

apoptosis modulation," *Biomedicine & Pharmacotherapy*, vol. 154, article 113583, 2022.

[18] Z. Yong, W. Ruiqi, Y. Yanan et al., "Laurolitsine ameliorates type 2 diabetes by regulating the hepatic LKB1-AMPK pathway and gut microbiota," *Phytomedicine*, vol. 106, article 154423, 2022.

[19] E. Rintz, K. Pierzynowska, M. Podlacha, and G. Węgrzyn, "Has resveratrol a potential for mucopolysaccharidosis treatment?," *European Journal of Pharmacology*, vol. 888, article 173534, 2020.

[20] S. H. El-Din, N. M. El-Lakkany, M. B. Salem, O. A. Hammam, S. Saleh, and S. S. Botros, "Resveratrol mitigates hepatic injury in rats by regulating oxidative stress, nuclear factor-kappa B, and apoptosis," *Journal of Advanced Pharmaceutical Technology & Research*, vol. 7, no. 3, pp. 99–104, 2016.

[21] F. Wang, R. Li, L. Zhao, S. Ma, and G. Qin, "Resveratrol ameliorates renal damage by inhibiting oxidative stress-mediated apoptosis of podocytes in diabetic nephropathy," *European Journal of Pharmacology*, vol. 885, article 173387, 2020.

[22] E. Yadav, P. Yadav, M. M. Khan, H. Singh, and A. Verma, "Resveratrol: a potential therapeutic natural polyphenol for neurodegenerative diseases associated with mitochondrial dysfunction," *Frontiers in Pharmacology*, vol. 13, article 922232, 2022.

[23] X. Zhu, C. Wu, S. Qiu, X. Yuan, and L. Li, "Effects of resveratrol on glucose control and insulin sensitivity in subjects with type 2 diabetes: systematic review and meta-analysis," *Nutrition and Metabolism*, vol. 14, no. 1, p. 60, 2017.

[24] H. A. A. Aly, "Mitochondria-mediated apoptosis induced testicular dysfunction in diabetic rats: ameliorative effect of resveratrol," *Endocrinology*, vol. 162, no. 4, pp. 1–12, 2021.

[25] C. Bia, J. Alizargar, C. Peng, and J. Wu, "Combination of exercise training and resveratrol attenuates obese sarcopenia in skeletal muscle atrophy," *The Chinese Journal of Physiology*, vol. 63, no. 3, pp. 101–112, 2020.

[26] P. Trinder, "Determination of blood glucose using an oxidase peroxidase system with a non-carcinogenic chromogen," *Journal of Clinical Pathology*, vol. 22, no. 2, pp. 158–161, 1969.

[27] A. Ahangarpour, A. A. Oroojan, H. Heidari, G. Ehsan, and M. R. Rashidi Nooshabadi, "Effects of hydro-alcoholic extract of *Rhus coriaria (*sumac) seeds on reproductive complications of nicotinamide-streptozotocin induced Type-2 diabetes in male mice," *The World Journal of Men's Health*, vol. 32, no. 3, pp. 151–158, 2014.

[28] Y. Ma, Y. Wang, Q. Huang et al., "Impaired β cell function in Chinese newly diagnosed type 2 diabetes mellitus with hyperlipidemia," *Journal Diabetes Research*, vol. 2014, article 493039, pp. 1–6, 2014.

[29] W. T. Friedewald, R. I. Levy, and D. S. Fredrickson, "Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge," *Clinical Chemistry*, vol. 18, no. 6, pp. 499–502, 1972.

[30] Y. Sakuma, "Gonadal steroid action and brain sex differentiation in the rat," *Journal of Neuroendocrinology*, vol. 21, no. 4, pp. 410–414, 2009.

[31] N. Shioya and K. Wakabayashi, "In vivo bioactivities and kinetic parameters of rat luteinizing hormone components: discrepancy between in vitro and in vivo assays," *Endocrine Journal*, vol. 45, no. 3, pp. 307–314, 1998.

[32] K. J. Teerds, J. Closset, R. F. G. Rommerts et al., "Effects of pure FSH and LH preparations on the number and function of Leydig cells in immature hypophysectomized rats," *Journal of Endocrinology*, vol. 120, no. 1, pp. 97–NP, 1989.

[33] G. Evans and W. M. Maxwell, "Handling and examination of semen," in *Salamon's Artificial Insemination of Sheep and Goats*, W. M. C. Maxwell, Ed., pp. 93–106, Butterworths, 1987.

[34] H. A. Alturki, H. A. Elsawy, and A. C. Famurewa, "Silymarin abrogates acrylamide-induced oxidative stress-mediated testicular toxicity via modulation of antioxidant mechanism,DNAdamage, endocrine deficit and sperm quality in rats," *Andrologia*, vol. 54, no. 9, article e14491, 2022.

[35] H. Ohkawa, N. Ohishi, and K. Yagi, "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction," *Annals of Biochemistry*, vol. 95, no. 2, pp. 351–358, 1979.

[36] H. Aebi, "Catalase," in *Methods in Enzymatic Analysis*, H. U. Bergmeyer, Ed., pp. 276–286, Academic Press, 1984.

[37] M. Masayasu and Y. Hiroshi, "A simplified assay method of superoxide dismutase activity for clinical use," *Clinica Chimica Acta*, vol. 92, no. 3, pp. 337–342, 1979.

[38] D. E. Paglia and W. N. Valentine, "Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase," *Journal of Laboratory and Clinical Medicine*, vol. 70, no. 1, pp. 158–169, 1967.

[39] D. Bancroft and M. Gamble, *The Theory and Practice of Histology Technique*, Churchill Living Stone, 5th ed edition, 2002.

[40] N. H. Cho, J. E. Shaw, S. Karuranga et al., "IDF diabetes atlas: global estimates of diabetes prevalence for 2017 and projections for 2045," *Diabetes Research and Clinical Practice*, vol. 138, pp. 271–281, 2018.

[41] M. Bahmanzadeh, M. T. Goodarzi, A. R. Farimani, N. Fathi, and Z. Alizadeh, "Resveratrol supplementation improves DNA integrity and sperm parameters in streptozotocin–nicotinamide-induced type 2 diabetic rats," *Andrologia*, vol. 51, no. 8, article e13313, 2019.

[42] I. P. Kumar, P. S. Priya, R. Meenatchi et al., "Potential mechanism of *Jatropha gossypifolia* phenolic derivatives in enhancing insulin-signalling cascades GLUT 4, IR β and GSK-3β in streptozotocin nicotinamide induced type II diabetic in Wistar rat model," *Journal of King Saud University-Science*, vol. 34, no. 7, article 102223, 2022.

[43] A. Ahangarpour, H. Heidari, A. Oroojan, F. Mirzavandi, K. Esfehani, and M. Z. Dehghan, "Antidiabetic, hypolipidemic and hepatoprotective effects of *Arctium lappa* root's hydroalcoholic extract on nicotinamide-streptozotocin induced type 2 model of diabetes in male mice," *Avicenna Journal of Phytomedicine*, vol. 7, no. 2, pp. 169–179, 2017.

[44] P. Loria, A. Lonardo, and F. Anania, "Liver and diabetes. A vicious circle," *Hepatology Research*, vol. 43, no. 1, pp. 51–64, 2013.

[45] S. D. Thapa, K. C. Shiva Raj, S. Gautam, and D. Gyawali, "Dyslipidemia in type 2 diabetes mellitus," *Journal of Pathology of Nepal*, vol. 7, no. 2, pp. 1149–1154, 2017.

[46] S. Anwar, M. A. Kausar, K. Parveen et al., "Polyherbal formulation: the studies towards identification of composition and their biological activities," *Journal of King Saud University-Science*, vol. 34, no. 7, article 102256, 2022.

[47] B. S. Kumar, K. Lakshman, K. N. Jayavee et al., "Antidiabetic, antihyperlipidemic and antioxidant activities of methanolic extract of *Amaranthus viridis* Linn in alloxan induced diabetic

rats," *Experimental and Toxicologic Pathology*, vol. 64, no. 1-2, pp. 75–79, 2012.

[48] J. N. Simas, T. B. Mendes, C. C. Paccola, V. Vendramini, and S. M. Miraglia, "Resveratrol attenuates reproductive alterations in type 1 diabetes-induced rats," *International Journal of Experimental Pathology*, vol. 98, no. 6, pp. 312–328, 2017.

[49] A. C. Famurewa, K. Renu, M. A. Eladl et al., "Hesperidin and hesperetin against heavy metal toxicity: insight on the molecular mechanism of mitigation," *Biomedicine & Pharmacotherapy*, vol. 149, article 112914, 2022.

[50] Y. Zhao, W. Song, Z. Wang et al., "Resveratrol attenuates testicular apoptosis in type 1 diabetic mice: role of Akt-mediated Nrf2 activation and p62-dependent Keap1 degradation," *Redox Biology*, vol. 14, pp. 609–617, 2018.

[51] X. Chen, A. C. Famurewa, J. Tang, O. O. Olatunde, and O. J. Olatunji, "Hyperoside attenuates neuroinflammation, cognitive impairment and oxidative stress via suppressing TNF-α/NF-κB/caspase-3 signaling in type 2 diabetes rats," *Nutritional Neuroscience*, vol. 25, no. 8, pp. 1774–1784, 2022.