WILEY | Hindawi

Research Article

Retama raetam Extract for Testicular Health in Type 2 Diabetic Rats: Insight View on the Steroidogenesis, Antioxidants, and Molecular Docking Scores of Bioactive Compounds against Bax

Mohammed Ali Alshehri [iD],[1] **Mohamed Ali Seyed** [iD],[2] **Abdulrahman Alasmari**,[1] **Chellasamy Panneerselvam**,[1] **Hamoud Hajad Alboqami**,[1,3] **Lamya Ahmed Alkeridis**,[4] **Daklallah A. Almalki**,[5] **Samy M. Sayed** [iD],[6,7] **Mustafa Shukry** [iD],[8] and **Ahmed A. Aioub** [iD][9]

[1]*Department of Biology, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia*
[2]*Department of Biochemistry, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia*
[3]*Ministry of Health, Riyadh, Saudi Arabia*
[4]*Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia*
[5]*Biology Department, Faculty of Science and Arts, Al-Mikhwah, Al-Baha University, Al Bahah, Saudi Arabia*
[6]*Faculty of Agriculture, Cairo University, Giza 12613, Egypt*
[7]*Department of Science and Technology, Raniah-Colleague University, Taif University, Taif, Saudi Arabia*
[8]*Department of Physiology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh 33516, Egypt*
[9]*Plant Protection Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt*

Correspondence should be addressed to Mohammed Ali Alshehri; ma.alshehri@ut.edu.sa and Mustafa Shukry; mostafa.ataa@vet.kfs.edu.eg

The exponential growth of obesity rates is a pressing issue, as it is now firmly established as a primary driver behind the development of metabolic disorders. Natural products are crucial in drug discovery, prompting an increasing need for further research on bioactive compounds to understand molecular and pharmacological mechanisms and expand available clinical treatments for various diseases. We set out to investigate the therapeutic potential of *Retama raetam* (RR) extract, a natural compound, in alleviating testicular degeneration and improving sperm quality and quantity in high-fat diet (HFD)-induced type 2 diabetes mellitus (T2DM) and streptozotocin (STZ)-induced type 2 diabetes mellitus. An eight-week high-fat diet (HFD) was administered to type 2 diabetic rats, and then a modest dose of 35 mg/kg of STZ was injected intraperitoneally. The experiment planned to assess the influence of RR extract on the steroidogenesis pathway. For twelve weeks, the rats were given medications orally. The testicular degeneration caused by HFD/STZ is shown by decreasing the accessory sexual glands while reducing the quality of sperm characters and testosterone levels with significantly increased pad fat and leptin levels. Furthermore, administration of RR extract effectively counteracted HFD/STZ-induced oxidation through enhancing the antioxidant status of the testicular tissue through significantly increased SOD and GSH accompanied by decreasing MDA. HFD/STZ rats showed decreased expression of CYP17, STAR, 3βHSD, and BCL2 genes, along with increased BAX gene expression. However, treatment with RR in HFD/STZ rats led to decreased BAX expression and increased expression of CYP17, STAR, 3βHSD, and BCL2 genes, indicating a restoration of gene expression values similar to the control group. The binding site of Bax showed a strong affinity for several bioactive compounds found in *R. raetam*. The findings from this study suggest that *Retama raetam* (RR) has the potential as a therapeutic adjunct for managing complications related to type 2 diabetes mellitus (T2DM). These findings afford credence to the idea that RR can be useful in treating infertility associated with type 2 diabetes.

1. Introduction

Diabetes, especially type 2 diabetes mellitus (T2DM), and obesity are two interlinked metabolic disorders that have become significant global health concerns. Over the past few decades, both have become more common, posing substantial global challenges for public health systems. This introduction will provide an overview of obesity, focusing on their association and the role of Wistar fatty rats as an experimental model [1]. A body mass index of over 30 is considered obese. Genetics, lifestyle decisions, and environmental factors, including the availability of high-calorie foods and sedentary behavior, all impact the condition [2].

Type 2 diabetes, on the other hand, is characterized by impaired insulin production and insulin resistance, leading to elevated blood glucose levels. Insulin facilitates the control of blood sugar levels. For those with type 2 diabetes, insufficient glucose absorption and elevated blood sugar levels result from cells' resistance to the actions of insulin. Obesity, a sedentary lifestyle, poor eating habits, and genetic susceptibility are threat influences for type 2 diabetes. When insulin is not produced correctly, it can lead to type 2 diabetes, which is increased by obesity. The abundance of fatty tissue in the body, especially visceral fat (fat around internal organs), produces hormones and inflammatory chemicals that interfere with insulin signaling and affect glucose metabolism. Type 2 diabetes is facilitated by persistent low-grade inflammation linked to fat, which worsens insulin resistance [3].

Between 90% and 95% of diabetes cases are type 2 diabetes, with low-income nations having the highest incidence of the disease [4]. Type 2 diabetes is exemplified by persistently high blood sugar concentrations that result in chronic hyperglycemia, which in turn causes oxidative injury and the activation of inflammatory cytokines. As a result of consistently high blood glucose levels, persons with type 2 diabetes develop advanced glycation end products (AGEs). This, in turn, triggers the release of ROS and activates inflammatory pathways. These processes are serious in the progression and exacerbation of diabetes type 2 mellitus [5]. Regrettably, various oral hypoglycemic medications have demonstrated efficacy in treating diabetes by targeting different biochemical pathways, but they often come with undesirable side effects. As a result, the utilization of medicinal plants has emerged as a prominent approach in diabetes treatment, primarily due to their affordability, easy accessibility, and minimal adverse effects.

Retama raetam (R. raetam) is a native Fabaceae family plant commonly found in the Northern and Eastern Mediterranean [6]. Recent studies have shown that *R. raetam* affects blood sugar levels in healthy and diabetic rats [7]. It has been documented that the aqueous extracts of RR demonstrate properties such as hypoglycemic, antioxidative, and lipid-lowering activities [8–10].

The study aimed to investigate a high-fat diet's effects on obesity and a single dose of streptozotocin (STZ) on oxidative stress markers, specifically malondialdehyde (MDA); the study also aimed to assess insulin resistance using the homeostatic model assessment of insulin resistance (HOMA-IR). Furthermore, it sought to explore the effects of reproductive rehabilitation (RR) on testicular degeneration in rats induced by obesity and T2DM, including the underlying cellular mechanisms involved.

2. Materials and Methods

2.1. Gathering and Extracting R. raetam Shrubs.
R. raetam subsp. *gussonei* fruits were utilized (no. 15567) in the experiment, which was conducted at Al Hafirah, Saudi Arabia (latitude, 25° 47′ 30.54″N; longitude, 45° 22′ 53.71″E). Regarding plant identification, following a shaded room temperature drying period, the RR fruits were electrically blended into a fine powder. Extraction was performed per the specified protocol [11]. Following the manufacturer's guidelines, the powdered plant material was extracted with methanol at room temperature using an IKA ULTRA-TURRAX T 25 digital device. The solution was then distilled at 40°C under reduced pressure, producing a brownish semisolid residue. The solvent extraction of RR involved dissolving 10 g of finely powdered RR in 100 mL of double-distilled water, following the specified method [11].

2.2. RP-HPLC and 1H-NMR Investigation.
The 1H-NMR spectra were collected using a Bruker Advance III spectrometer running at 400 MHz, and spectral analysis was carried out using the ADC software. 200 mg of dried RR was extracted with 2 mL of deuterated methanol and sonicated to prepare the samples for 10 minutes. The solution was centrifuged at 25°C for 5 minutes at a speed of 13,000 rpm before being subjected to NMR analysis.

The chromatographic analysis involved dissolving extract samples from Scharlau, Italy, in methanol at a 50 mg/mL concentration. The analysis utilized an Agilent Infinity series 1200 chromatograph with a diode array and an Agilent/Varian 500MS mass spectrometer. A gradient elution method was employed using a column of Eclipse XDB-C18, C18 3.5 μm, 3 × 150, with the chromatograms analyzed at various wavelengths. The same extract underwent chromatography using the same system but with an Agilent Poroshell HILIC-Z column. Further details are available in Figure 1S and Table 2S.

2.3. Animals.
Fifty male Wistar rats, ten weeks old (weighing roughly 180 ± 5 g), were obtained from the National Research Center in Cairo, Egypt. Rats were held in well-ventilated plastic cages with limitless food and water access. They were subjected to a 12-hour light/dark cycle. All experimental procedures, animal handling, and management ethics followed the National Institutes of Health (NIH) standards. The animal procedures in this study adhered to the guidelines and regulations established by the Animal Ethics Committee (KFS-IACUC/133/2021) of the Faculty of Veterinary Medicine at Kafrelsheikh University. Rats were acclimated for two weeks before the experiments and were randomly subdivided into five groups, each comprising ten rats. The rats were provided with a consistent diet pellet that contained fat (3%), protein (26%), carbohydrates (54%), vitamins (17%), and minerals per 100 grams.

2.4. Type 2 Diabetes Mellitus Induction. Type 2 diabetes mellitus (T2DM) was provoked in rats with high-fat feed (HFD) containing 25% protein, 17% CHO, and 58% fat at a rate of adding up to kcal for two months. Following two months on the HFD, the obese rats underwent an overnight fast before receiving an intraperitoneal injection of STZ at 35 mg/kg body weight [12]. The STZ was dissolved in 0.1 M citrate buffer at pH 4.5 (Sigma Aldrich, USA). Diabetes confirmation was performed 72 hours after the STZ injection, with rats displaying blood glucose levels exceeding 300 mg/dL being selected for further experimentation.

2.5. Experimental Plan. The rats were randomly allocated into 5 groups using a random assignment approach. The groups included the control rats, which received saline treatment; the HFD/STZ-induced group; the HFD/STZ-RR1-treated group, where RR extract was supplemented at a dosage of 10 mg/kg [10]; the HFD/STZ-RR2-treated test group, administered RR extract at 20 mg/kg [13]; and HFD/STZ-RR3-treated test group, administered RR extract at 30 mg/kg [14]. Doses of the extract were administered to the rats in each group as indicated and orally for 12 weeks. The rats were given daily doses of extract at the same time. A normal saline solution was generated by dissolving the necessary extract concentration according to the rats' body weight.

The solutions were administered to the rats via oral gavage. Following the trial period, the rats were subjected to an overnight fast, weighed, and then anesthetized using sodium pentobarbital (100 mg/kg, i.p.). Testicular tissues were carefully dissected, washed with cold saline, frozen in liquid nitrogen, and stored at −80°C for further examination. Before anesthesia, blood samples were collected, and the serum was kept at −20°C. The testes, seminal vesicles, prostates, and epididymis were isolated and weighed individually, and the ratio of organ weight to body weight was evaluated. The testicles and epididymal fat pads were eliminated, rinsed in cold saline solution, and weighed.

2.6. Insulin and HOMA-IR Evaluation. Glucose levels were quantitatively and colorimetrically determined using diagnostic kits from Spectrum Kits, Germany (catalog no. 250001). Serum insulin was assessed using an ELISA technique (MyBioSource, Co., San Diego, CA, USA, catalog no. MBS281388). The following formula was employed to calculate HOMA-IR: fasting plasma insulin (IU mg/l in the fasting state) split by 405, multiplied by plasma glucose (mg/dl). Additionally, a β-cell function was determined using the subsequent formula: (HOMA-β) % = (360 × fasting insulin (μIU/ml))/(fasting glucose (mg/dl) − 63) [15].

2.7. Semen Evaluation. The procedure involved dissecting and incising the epididymis to extract semen by gently squeezing it onto a clean, warmed slide. Two drops of warm 2.9% sodium citrate were added to the semen and mixed using a cover slip. Progressive sperm motility was then visually assessed at 400x magnification, with three fields examined in each sample for estimation [16]. The number of sperm was determined utilizing a hemocytometer at 40x magnification. Eosin nigrosin was used to produce and stain seminal smears to assess sperm viability (ratio of alive to dead sperm) and sperm cell abnormality [17]. Following Johnsen [18], the score was assessed.

2.8. Testicular Markers and Leptin Analysis. Testicular protein concentration was measured following Bradford [19]. Additionally, the acid phosphatase and alkaline phosphatase levels in the testicular homogenate were determined following the methods in [20, 21] using Clinical and Applied Chemistry Laboratory in Amposta, Tarragona, Spain, and Biolabo SA in Maizy, France. The serum leptin levels were measured following Considine et al. [22] using the active Leptin ELISA Kit from DSL (Diagnostic Systems Laboratory, Inc.).

2.9. Assessment of Antioxidant Parameters in Testicular Tissue. GSH level is assessed as follows [23]. Malondialdehyde (MDA) levels were measured according to Preuss et al. [24]. The activity of SOD was assessed following Nishikimi et al. [25]; this method evaluates SOD's ability to stop the phenazine methosulphate-mediated reduction of nitro blue tetrazolium (NBT) dye, with photometric measurements taken at 560 nm. Following the specified procedure, nitric oxide concentration in the testicular homogenate was ascertained by calculating the total nitrite-to-nitrate ratio [26]. MDA, SOD, and GSH levels were measured spectrophotometrically using Bio-diagnostic kits, Egypt, with CAT nos. MD2529, SD2521, and TA2511, respectively.

2.10. Histopathological Analysis. Histopathological analysis was conducted using the protocol described by Bancroft and Gamble [27]. Testicular samples from four rats in each group were collected immediately after euthanasia and sliced into small pieces of 0.4–0.5 cm thickness. After fixation in Bouin's solution for 18 hours, the tissue samples underwent dehydration using a gradient of ethanol concentrations (from 70% to absolute alcohol) and clearing in xylene. Subsequently, the samples were processed for histological examination. Thin sections, approximately 4-5 μm thick, were stained with hematoxylin and eosin for histopathological analysis.

Detection of circumferences of the seminiferous tubules (in micrometers): The average circumferences of the seminiferous tubules in the tested groups were determined by measuring the lining of a cross section of the tubules. This measurement was conducted on six samples in each field, using a magnification of 50x. It is worth noting that longitudinal cuts were not included in the analysis. Total count of maturing sperm cells: The count of spermatogenic cells was performed, encompassing all stages from spermatogonia to spermatozoa. Following Johnsen [18], the score was assessed.

2.11. Gene Expressions (qPCR). TRIzol (Invitrogen, Carlsbad, USA) was utilized for extraction of the total RNA from 100 mg of testicular tissue, with subsequent RNA

concentration determination using a Nanodrop UV-Vis spectrophotometer Q5000/Quawell, focusing on samples with an A260/A280 ratio of 1.8 or higher. These RNA samples were further employed for cDNA synthesis utilizing a kit from Fermentas. For cDNA amplification, SYBR Green Master Mix from Roche and specific primers listed in Table 1S were exploited in a final reaction volume of 20 μl. The housekeeping gene GAPDH served as a reference during amplification. Melting curve analysis validated the PCR products, and the Rotor-Gene Q instrument from Qiagen facilitated automatic data collection and analysis of the threshold cycle value (Ct). The $2^{-\Delta\Delta Ct}$ method was then applied to evaluate the amplification data [28].

2.12. Molecular Docking Assessment

2.12.1. Instruments and Tools. Protein and ligand retrieval, as well as molecular docking, was conducted using the AlphaFold database (https://alphafold.ebi.ac.uk/) and the Molecular Operating Environment (MOE 2015.10, Chemical Computing Group, Montreal, QC, Canada).

2.12.2. Ligand Preparation. Three-dimensional structures of *R. raetam's* bioactive compounds were retrieved from the LOTUS database (https://lotus.naturalproducts.net/) in SDF format and opened in MOE software for energy minimization and docking with target proteins.

2.12.3. Protein Preparation. Bax 3D structures were obtained from the UniProt database and applied to rats (https://www.uniprot.org/uniprotkb/Q63690/entry). The target protein was prepared for docking using MOE software by removing water and ligand molecules present in the protein structures along with target protein energy minimization.

2.12.4. Molecular Docking Analysis and Visualization. Target proteins were docked with ligands using MOE software by identifying the binding site and docking with the induced fit model. Ultimately, the protein-ligand interactions were imagined by the same software.

2.13. Data Analysis. The data are shown as mean ± SEM for each group. The data normality and homoscedasticity were evaluated using Shapiro–Wilk and Bartlett's tests. One-way ANOVA, with the Tukey–Kramer postanalysis test, was utilized for multiple comparisons at $P < 0.05$, using GraphPad Prism 8 software (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. RR Quells the Relative Weight and Accessory Glands of HFD/STZ Rats. A substantial reduction in the relative weight of the testes and other accessory glands, such as the prostate, seminal vesicle, and epididymis, was observed in HFD/STZ rats, demonstrating a decrease linked to the other groups as indicated in Table 1 ($P < 0.05$). A notable disparity

did not exist in the relative accessory gland weight between the control one and the RR-supplemented rats. Furthermore, the epididymal fat pads and total body weight were notably boosted in the HFD group linked to the other relevant groups.

Identifying an effective treatment for hyperglycemia is a critical aspect of diabetes research. As such, our objective was to examine whether RR could potentially exert an anti-hyperglycemic effect in diabetic rats. Upon analyzing the data in Table 2 and comparing the results with the control group, we observed a substantial upsurge in fasting blood glucose, serum insulin, and HOMA-IR level following the induction of hyperglycemia using HFD/STZ throughout the study period ($P < 0.05$). In contrast, healthy rats maintained fasting blood glucose levels within the normal range. The heightened fasting glucose levels in diabetic rats underscore the severity of the hyperglycemic condition.

The administration of RR decreased insulin and HOMA-IR levels in HFD/STZ-treated rats linked to untreated HFD/STZ rats ($P < 0.05$). Additionally, a significant decline in beta-cell function was noticed in the HFD/STZ-induced rats linked to the treated groups ($P < 0.01$), and concerning the control group, rats treated with standard diabetes treatments exhibited improved beta-cell activity ($P < 0.05$). Notably, the RR-treated groups demonstrated a substantial enhancement in beta-cell function linked to the control one, with the RR20 rats showing a particularly noteworthy elevation in beta-cell function ($P < 0.05$).

3.2. Effect of RR Administration on Sperm Parameters of HFD/STZ-Treated Rats. According to the findings in Table 3, the rats induced with HFD/STZ exhibited a drop in concentration of sperm, sperm motility %, and viability and a boost in sperm cell abnormalities associated with the other groups. Nonetheless, the group treated with RR presented a considerable improvement in sperm concentration, sperm motility percentage, and viability while also experiencing decreased sperm cell abnormalities.

3.3. Effect of RR Administration on Testicular Enzyme Marker and Leptin of HFD/STZ-Treated Rats. This study observed a notable decrease in testicular enzyme marker (ACP and ALP) levels in the HFD/STZ group associated with the treated rats. Conversely, control and RR-administration groups confirmed a significant rise ($P < 0.05$) in ACP and ALP concentrations in the HFD/STZ rats. Figure 1 depicts a meaningful decline in testosterone concentration (serum and tissue) in the HFD/STZ rats concerning treated groups ($P < 0.01$). Conversely, RR groups exhibited considerable improvement in both testicular and serum testosterone concentrations associated with the HFD/STZ groups, with increased leptin levels in the HFD group concerning other treated groups (Figure 2).

3.4. Impact of RR Administration on Oxidative Injury Marker of HFD/STZ-Treated Rats. The HFD/STZ rats exhibited a noteworthy upsurge ($P < 0.05$) in MDA (testicular)

TABLE 1: The impact of RR supplementation on the relative weight of the testes, seminal vesicle, epididymis, and prostate gland across various treated groups.

	Body weight (g)	Testes (g)	Seminal vesicle (g)	Epididymis (g)	Prostate glands (g)	Fat pads (g)
Control	325.2 ± 5.1^d	1.44 ± 0.1^a	0.95 ± 0.01^a	0.96 ± 0.01^a	0.48 ± 0.01^a	4.94 ± 0.19^e
HFD/STZ	422.5 ± 7.1^a	0.71 ± 0.2^b	0.40 ± 0.04^c	0.40 ± 0.01^c	0.20 ± 0.01^c	7.84 ± 0.12^a
HFD/STZ + RR10	385.4 ± 5.2^b	1.05 ± 0.1^c	0.65 ± 0.01^b	0.66 ± 0.02^b	0.33 ± 0.01^b	6.34 ± 0.11^b
HFD/STZ + RR20	367.5 ± 6.1^c	1.22 ± 0.2^a	0.87 ± 0.02^a	0.90 ± 0.01^a	0.45 ± 0.01^a	5.47 ± 0.39^d
HFD/STZ + RR30	384.5 ± 8.1^b	1.15 ± 0.1^{ab}	$0.78 \pm 0.01ab$	0.79 ± 0.04^{ab}	$0.39 \pm 0.02ab$	6.01 ± 0.39^c

Values are represented as mean ± SEM. Means with distinct letter superscripts within the same column indicate significance at $P < 0.05$ ($N = 10$).

TABLE 2: The impact of RR on the serum levels of glucose, insulin, HOMA-IR, and HOMA-β in HFD/STZ-treated rats.

	Fasting glucose (fasting) (mg/dl)	Fasting insulin (μIU/ml)	HOMA-IR	HOMA-β
Control	95.26 ± 1.2^c	25.16 ± 1.2^d	5.92 ± 0.14^e	280.77 ± 5.2^d
HFD/STZ	450.36 ± 10.1^a	175.5 ± 4.5^a	195.21 ± 4.1^a	163.14 ± 3.2^e
HFD/STZ + RR10	108 ± 2.4^b	85 ± 1.4^b	22.67 ± 1.4^b	$680 \pm 7.4c$
HFD/STZ + RR20	78 ± 2.4^e	72 ± 1.1^c	13.87 ± 1.2^d	1728 ± 9.1^a
HFD/STZ + RR30	88 ± 3.1^d	78 ± 1.6^b	16.95 ± 1.2^c	1123.2 ± 8.2^b

The values are presented as mean ± SEM, and differences indicated by distinct letters within the same column are considered significant at $P < 0.05$ ($N = 10$).

TABLE 3: Effect of RR administration on sperm cell concentration, motility, abnormalities, and sperm cell viability of HFD/STZ-treated rats.

	Sperm cell concentration ($\times 10^6$/ml)	Motility (%)	Abnormalities (%)	Sperm viability (%)
Control	251 ± 5.1^a	82 ± 1.2^a	10.4 ± 0.8^d	82.3 ± 0.5^a
HFD/STZ	145 ± 4.2^d	36 ± 0.5^c	35.6 ± 1.2^a	45.5 ± 0.4^d
HFD/STZ + RR10	215 ± 3.5^c	65 ± 0.8^c	16.5 ± 0.7^b	68.4 ± 1.1^c
HFD/STZ + RR20	235 ± 2.5^b	74 ± 0.8^b	12.3 ± 0.4^d	74.5 ± 0.7^b
HFD/STZ + RR30	223 ± 2.1^c	67 ± 0.9^c	14.4 ± 0.5^c	70.4 ± 1.5^c

The values are presented as mean ± SEM, and differences indicated by distinct letters within the same column are considered significant at $P < 0.05$ ($N = 10$).
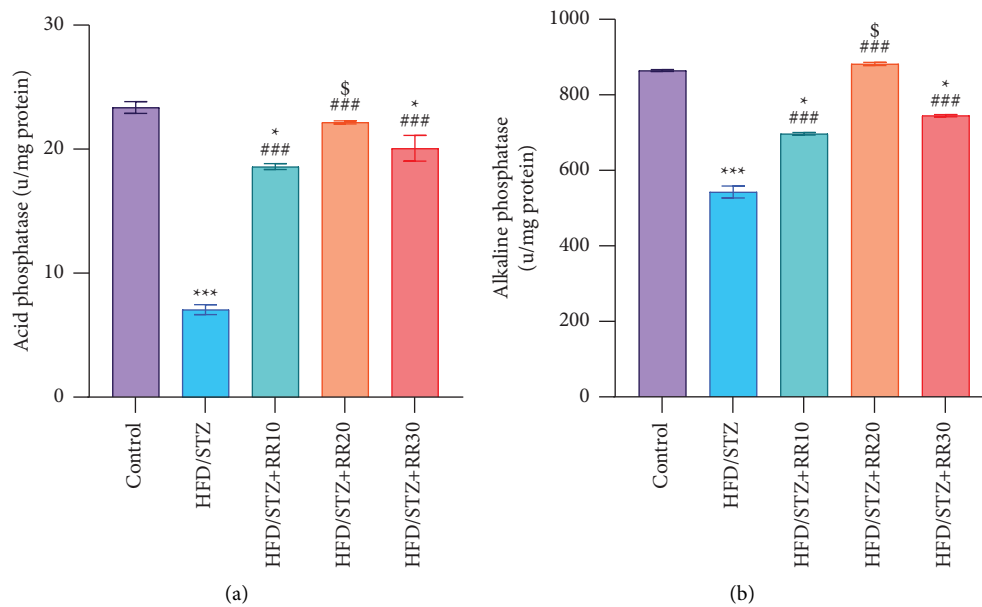

FIGURE 1: Effect of EE supplementation on enzyme markers of the testicular acid phosphatase (a) and alkaline phosphatase (b). Values are represented as mean ± SEM. $^*P < 0.05$, or $^{***}P < 0.001$, compared with the treated control group; $^{###}P < 0.001$, compared with the HFD/STZ group; $^\$P < 0.05$, compared with the RR20 treated group ($N = 10$).
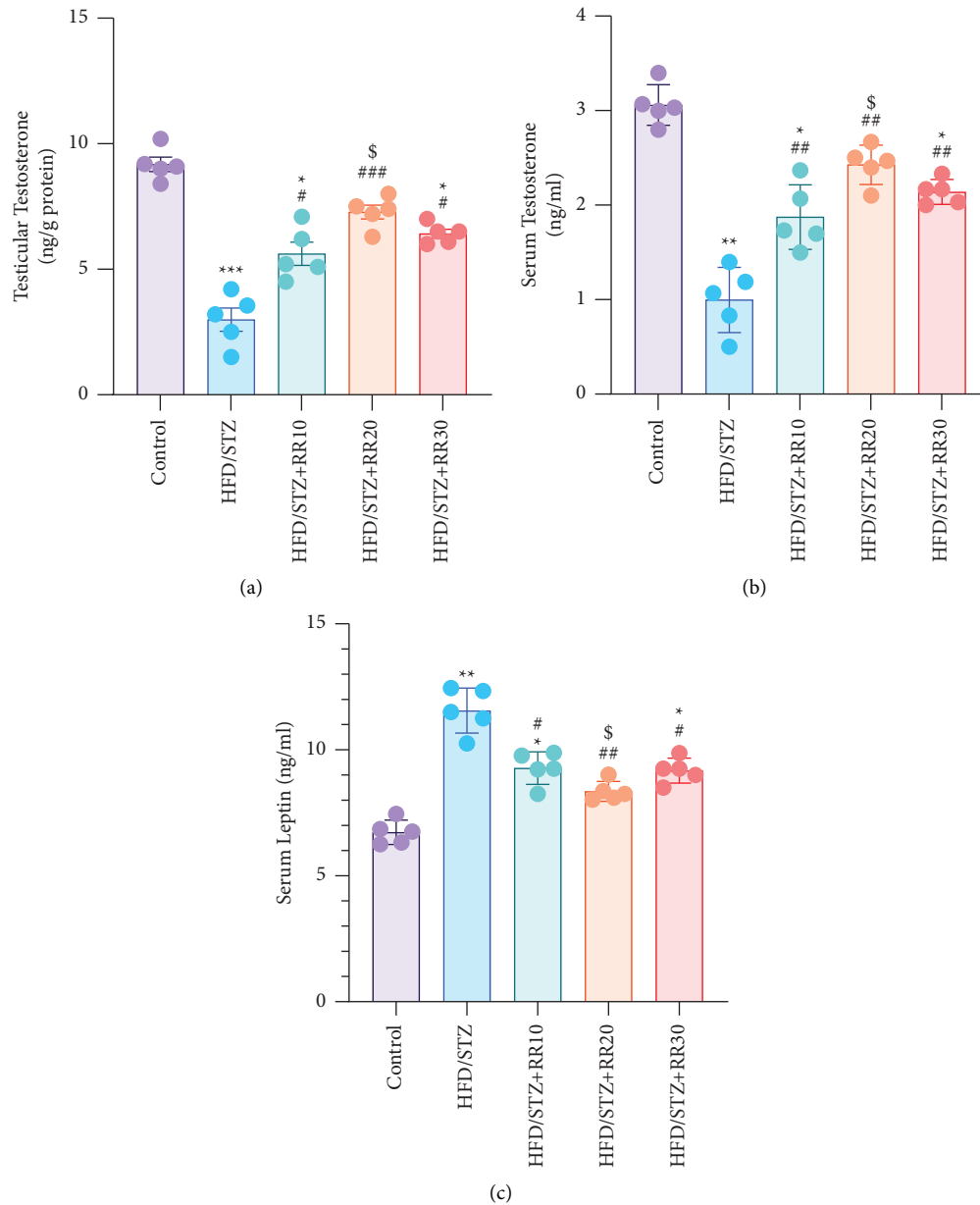
(a)

(b)

(c)

Figure 2: The impact of RR supplementation on the enzyme markers of testicular testosterone (a), serum testosterone (b), and leptin (c) is expressed as mean ± SEM. Significance levels are denoted by, $^*p < 0.05$, $^{**}p < 0.01$, or $^{***}p < 0.001$, compared with the treated control group; or $^\#p < 0.05$, $^{\#\#}p < 0.01$, or $^{\#\#\#}p < 0.001$, compared with the HFD/STZ group; and $^\$p < 0.05$ compared with the RR20 treated group ($N = 10$).

quantity concerning the control group. However, treatment with RR at doses of RR10, RR20, and RR30 led to a meaningful reduction ($P < 0.05$) in testicular MDA quantity in the HFD/STZ-treated rats. Additionally, GSH levels in the HFD/STZ rat's testicles were substantially reduced ($P < 0.05$), as shown in Figure 3. However, RR supplementation prevented the decrease in GSH levels (testicular). Furthermore, the testicular SOD levels were markedly reduced in the HFD/STZ rats when matched with the control one. The RR administration to the HFD/STZ rats led to a marked improvement in testicular SOD levels.

3.5. Effect of HFD/STZ-Treated Rats and RR Treatment on CYP17, STAR, 3βHSD, BAX, and BCL2 Expression. According to the findings in Figure 4, the mRNA expressions of CYP17, STAR, 3βHSD, and BCL2 substantially diminished in the HFD/STZ rats associated with the other groups. Conversely, there was substantial progress in the mRNA expression of the Bax in the HFD/STZ group. However, the HFD/STZ rats treated with RR exhibited significantly reduced BAX expression and increased mRNA expression of CYP17, STAR, 3βHSD, and BCL2 genes. These changes indicate a restoration of gene expression values comparable to control rats.
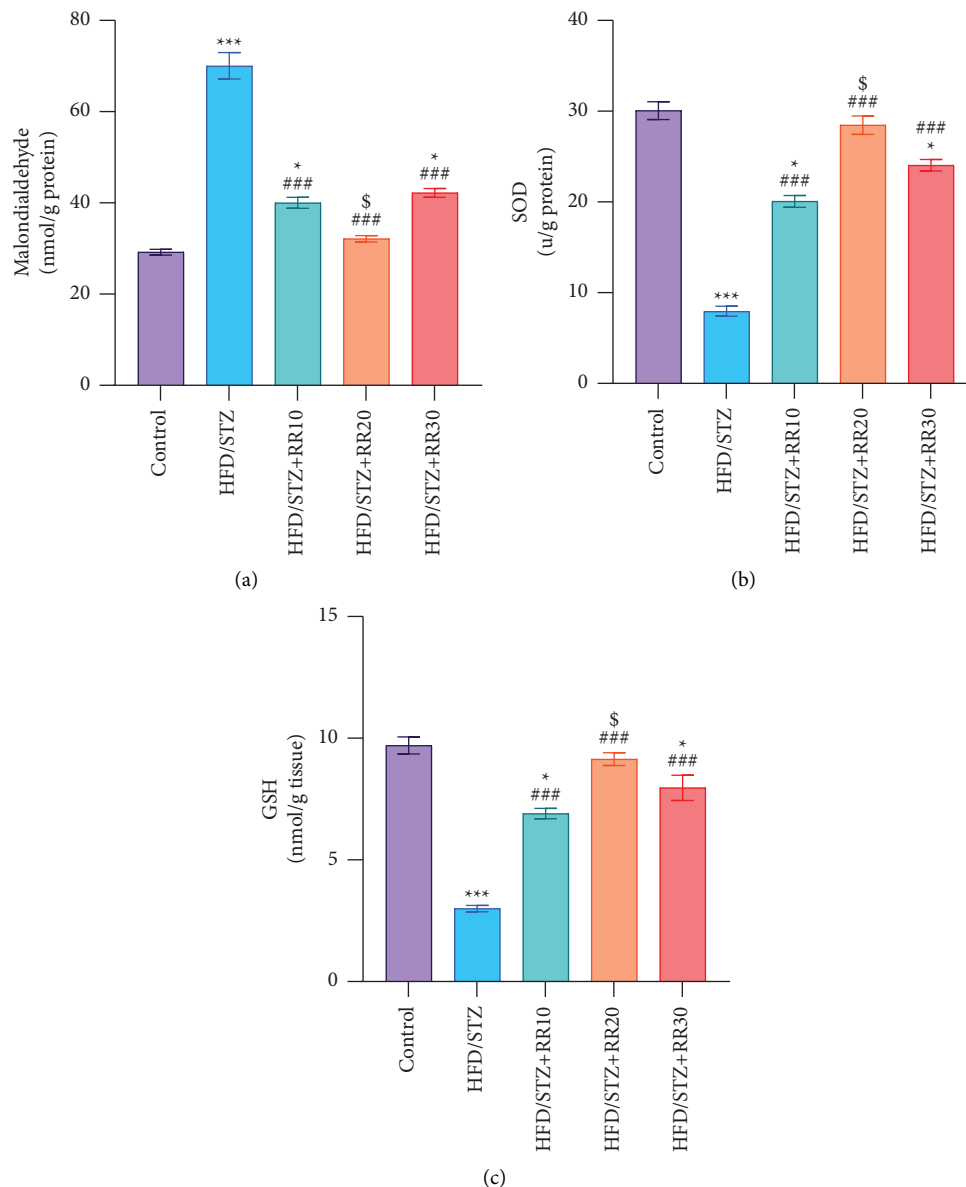
(a)

(b)

(c)

FIGURE 3: Effect of EE supplementation on the antioxidant and oxidant activity of testicular MDA (a), SOD (b), and GSH (c). Values are represented as mean ± SEM. $^{*}P < 0.05$, or $^{***}P < 0.001$, compared with the treated control group; $^{###}P < 0.001$, compared with the HFD/STZ group; $^{\$}P < 0.05$, compared with the RR20 treated group ($N = 10$).

3.6. Histopathological Examination. Normal testicular architecture was observed upon histopathological assessment of the testes of the nondiabetic groups (control and RR-treated). The seminiferous tubules had intact structure, with strata of spermatogenic cells and spermatozoa towards the lumen, separated by a thin interstitial tissue. However, the seminiferous tubules were disrupted in the HFD/STZ rats. There were decreased spermatogenic cell thickness, sloughing of cells, and reduced tubular diameter. The tubular lumen was enlarged, with interstitial tissue edema and degeneration as reported in Figure 5.

In comparison, HFD/STZ rats treated with RR showed improved testicular tissue compared to untreated rats. The treated rats exhibited increased spermatogenic cell thickness, spermatids, and spermatozoa in the tubule

lumen and mild swelling and congestion in the testicular blood vessels, especially at higher RR concentrations. These findings indicate that RR treatment positively impacted testicular tissue in HFD/STZ rats, improving spermatogenic cell thickness and mature germ cell presence while mitigating swelling/edema and interstitial tissue degeneration.

The HFD/STZ rats significantly decreased the seminiferous tubule circumferences and total sperm cell maturation count concerning the control group. Nonetheless, RR at RR10, RR20, and RR30 doses led to meaningful increases in these parameters ($P < 0.05$) in testicular MDA quantity in HFD rats. Additionally, the RR administration to the HFD/STZ rats led to a marked improvement in Johnsen scoring in HFD/STZ rats.
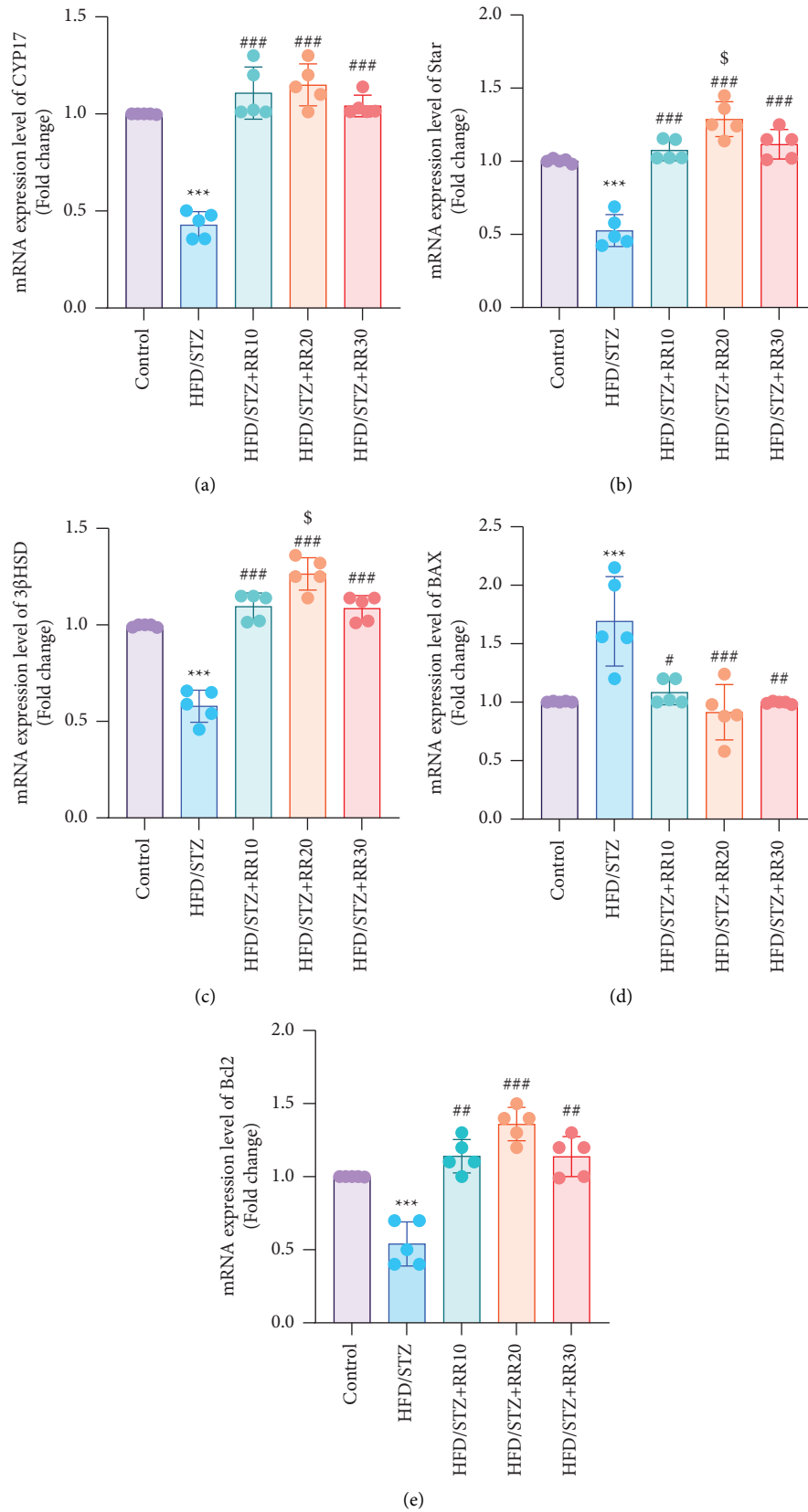
Figure 4: Effect of EE supplementation on the testicular mRNA expression of the CYP17 (a), STAR (b), 3B-HSD (c), Bax (d), and Bcl-2 (e). Values are represented as mean ± SEM. $^{***}P < 0.001$, compared with the treated control group; $^{#}P < 0.05$, $^{##}P < 0.01$ $^{###}P < 0.001$, compared with the HFD/STZ group. $^{\$}P < 0.05$ compared with the RR20 treated group ($N = 10$).
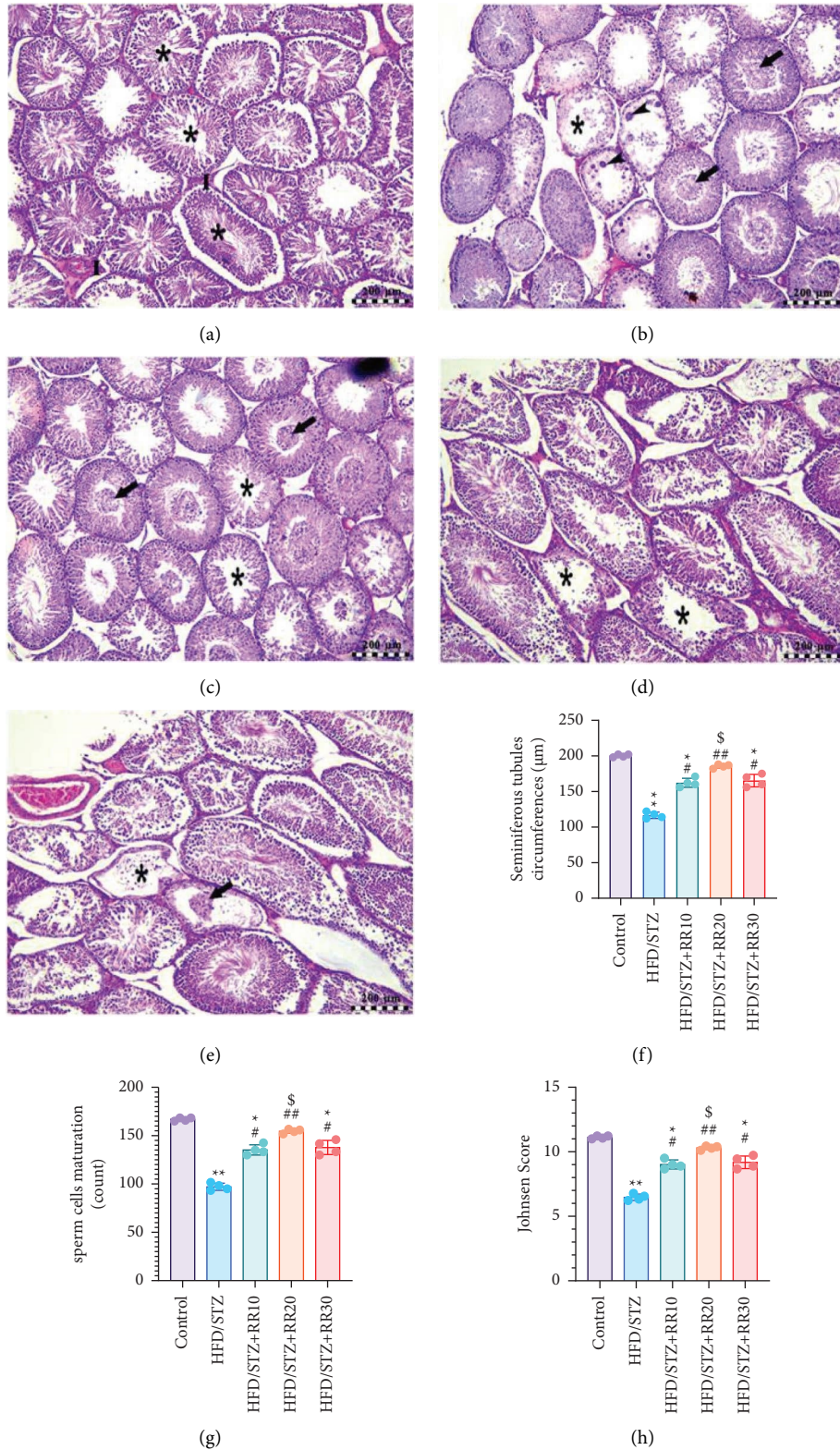
FIGURE 5: Histomicrograph of H&E-stained testicular sections of different treated groups. Control group (a) shows normal intact seminiferous tubules containing different layers of spermatogenic cells (asterisk) separated by interstitial tissue (I). The HFD/STZ group (b) shows loss of spermatids and a few layers of spermatogenic cells (asterisk), presence of giant cells (arrowheads), and accumulation of cellular debris in the lumen of some tubules (arrows). RR10 mg/kg group (c) shows loss of spermatids in some tubules (asterisk) and accumulation of cellular debris in the lumen of some tubules (arrows). RR20 mg/kg group (d) shows loss of spermatids in a few tubules (asterisk). RR30 mg/kg group (e) shows loss of spermatogenic cells in some tubules (asterisk) and accumulation of cellular debris in the lumen of some tubules (arrows). (f) Seminiferous tubule circumferences. (g) Sperm maturation count. (h) Johnsen score. Values are represented as mean ± SEM. $^*P < 0.05$, $^{**}P < 0.01$, compared with the treated control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with the HFD/STZ group; $^{\$}P < 0.05$, compared with the RR20 treated group.

3.7. Molecular Docking Assessment. Data represented in Table 4 show the molecular docking scores of *R. raetam*'s bioactive compounds against Bax. Furthermore, the top 5 compounds against Bax are illustrated in Figure 6. Kaempferol 7-o-glucoside interacted with ASP159 (two H-donors), SER55 (H-acceptor), and GLN18 (H-acceptor) residues in the binding site of Bax with an energy of −7.48 kcal/mol (Figure 6(a)). A molecule binds to the Bax protein site via hydrogen bonds with GLU61, THR42, PRO50, and ARG64, with a binding energy of −7.08 kcal/mol, indicating strong interaction (Figure 6(b)). By H-acceptor bond, vicenin 2 interacted with GLN32 in the binding site of Bax with an energy of −6.94 kcal/mol, as represented in Figure 6(c). Luteolin 7-o-glucoside binds to the binding site of Bax by H-donor (THR56) and H-acceptor (LYS21) with energy of −6.65 kcal/mol (Figure 6(d)). Finally, orientin interacted with PRO51 (H-donor) and ARG64 (H-acceptor) residues in the compelling site of Bax with an energy of −6.58 kcal/mol (Figure 6(e)).

4. Discussion

The effectiveness of *R. raetam* extract in treating diabetes using an HFD/STZ-induced animal model was examined, as this is a widely employed tool to explore the underlying mechanisms involved in T2DM. Experimental rats were supplied an HFD and received STZ, which induces insulin resistance [29]. This combination led to high blood sugar levels (hyperglycemia) and increased insulin levels (hyperinsulinemia) in rats. These findings support the previous research by Dolan [30], indicating that STZ blocks glucose metabolism. The data exhibited that the insulin resistance index was higher and fasting blood glucose and insulin levels were substantially higher in diabetic obese rats. However, when administered at different doses, reproductive rehabilitation (RR) enhanced responsiveness to insulin and glucose, leading to better glycemic management. These findings align with Maghrani et al. [10], who found that normal and diabetic rats showed substantially declined blood sugar after being administered the RR aqueous extract. The hypoglycemia impact was more noticeable in the diabetic rats than in normal rats, which is an exciting finding.

Moreover, the observation of hypoglycemic consequences in normal rats, often accompanied by indications of critical hypoglycemia, proposes that the RR extract has vital pharmacological characteristics. Glucagon, catecholamines, and cortisol are all components that work against hypoglycemia, but the RR extract kept blood sugar levels down [31]. In the context of diabetes, our findings demonstrate that RR can be a beneficial intervention for managing blood glucose levels and enhancing insulin sensitivity [32]. The antidiabetic effects of *R. raetam* (RR) infusion appear to work through mechanisms outside the pancreas. RR infusion has increased glucose excretion in urine, indicating a possible hypoglycemic activity. This effect is believed to be achieved by reducing glucose reabsorption in the renal proximal tubule. The RR extract may hinder renal glucose conveyance by influencing the activity of these transporters. The RR extract may also promote glucose uptake in

peripheral tissues, address insulin resistance, reduce glucose production within the body, or enhance glycogen production in the liver and muscles [33]. Our results indicated a reduction in the relative weight of the testes and associated glands, such as the prostate, seminal vesicle, and epididymis, compared to the other groups. This reduction in weight in the diabetic group may be attributed to testicular deterioration and a decrease in epididymis weight. It is crucial to recognize that the testes and surrounding reproductive tissues rely on testosterone, which supports the growth and secretory functions of the reproductive organs.

Consequently, this leads to an upsurge in the number and function of germinal cells in the testes, resulting in increased weight of the testes and epididymis, as highlighted in a previous study [34]. Furthermore, another study by Pourheydar et al. [35] demonstrated that the atrophic changes observed in the epididymis of diabetic rats were due to decreased tubular diameter, volume, and surface density. These findings provide further insights into the mechanisms underlying testicular and epididymal deterioration in the context of diabetes.

Research has demonstrated that being overweight can impact fertility and the quality of semen by interfering with the spermatogenesis process [36]. It has been observed that obese men often experience infertility, which is associated with metabolic disturbances and hormonal imbalances [37]. Increasing evidence suggests that obesity can lead to insulin resistance, thus influencing the advancement of various obesity-related conditions [38]. Additionally, decreased testosterone in the bloodstream is linked to insulin sensitivity [39]. Insulin resistance plays a significant responsibility in the impaired semen properties and dysfunction in spermatogenesis caused by obesity. Our research revealed a notable decline in testicular enzyme marker (ACP and ALP) levels in the HFD/STZ rats associated with the control.

Additionally, there was a substantial reduction in serum testosterone concentration and testicular testosterone in the HFD/STZ rats compared to the other groups. A sufficient level of leptin is essential for regular reproductive function, whereas excessive leptin production, leading to hormonal resistance, could be a significant factor in androgen deficiency among obese men [40]. Furthermore, elevated leptin levels correlate to insulin resistance and metabolic disorders primarily influenced by obesity [41].

The development of DM is linked to an inequality between the oxidative and antioxidative systems within cells. Excessive creation of reactive oxygen species causes this imbalance. Antioxidant enzymes play crucial roles in removing free radicals and preventing the destructive properties of oxidant molecules on tissues and cells [42]; the previous literature was inconsistent with our findings in which HFD/STZ showed a noteworthy lessening in the SOD and GSH with relevant increases in the MDA activity; this consequence was aborted by the RR supplementation in which *R. raetam* plant treatment increased SOD and glutathione peroxidase activities in the blood and decreased malondialdehyde and nitric oxide levels in the serum and plasma, respectively. The flavonoids found in medicinal
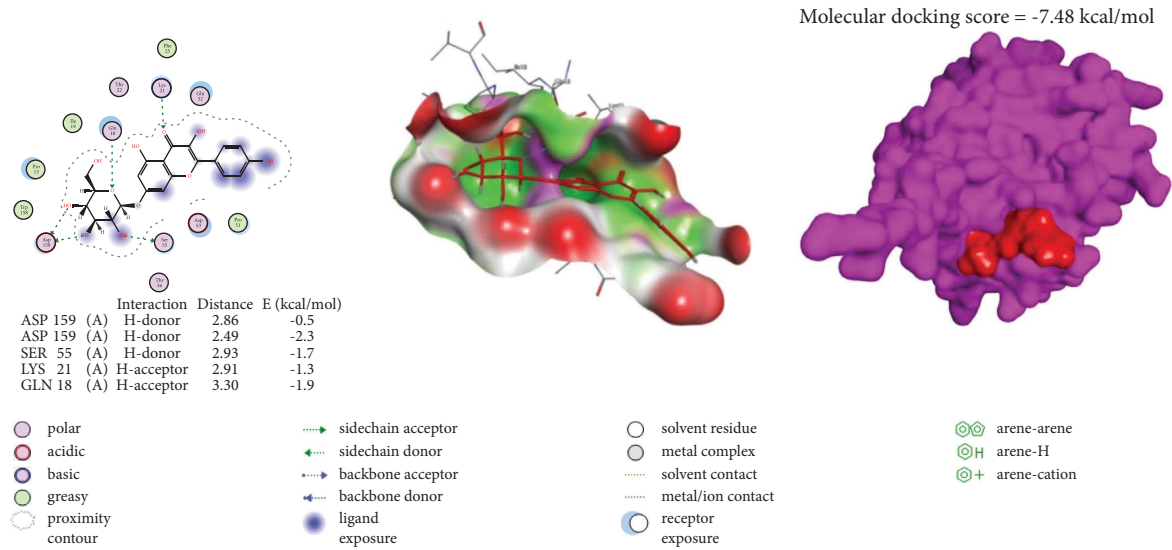
TABLE 4: Molecular docking scores of *Retama raetam's* bioactive compounds against Bax.

Compounds	Lotus ID	Scores
Kaempferol 7-o-glucoside	LTS0025882	−7.48
5-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-7-{[(2s,3r,4s,5s,6r)-3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]oxy}chromen-4-one	LTS0065615	−7.08
Vicenin 2	LTS0181160	−6.94
Luteolin 7-o-glucoside	LTS0227450	−6.65
Orientin	LTS0172349	−6.58
Stigmast-5-en-3-ol	LTS0071224	−6.57
Apigenin 7-o-β-glucoside	LTS0252743	−6.50
(1r,2s,3s,9s,10s)-7,15-Diazatetracyclo[7.7.1.0^{2,7}.0$^{1\circ,15}$]heptadecan-3-ol	LTS0031783	−6.34
Ephedroidin	LTS0212990	−6.22
(1r,2s,3s,9r,10s)-7,15-Diazatetracyclo[7.7.1.0^{2,7}.0$^{1\circ,15}$]heptadecan-3-ol	LTS0037284	−6.02
Baptifoline	LTS0154575	−5.91
17-Oxosparteine	LTS0114169	−5.85
7,15-Diazatetracyclo[7.7.1.0^{2,7}.0$^{1\circ,15}$]heptadec-10-en-3-ol	LTS0132509	−5.84
(−)-Lupanine	LTS0210779	−5.81
Sparteine	LTS0017952	−5.77
7,15-Diazatetracyclo[7.7.1.0^{2,7}.0$^{1\circ,15}$]heptadecan-3-ol	LTS0200118	−5.77
Stigmast-5-en-3-ol, octadecanoate (3β)-	LTS0204616	−5.74
(2r,10s)-7,15-Diazatetracyclo[7.7.1.0^{2,7}.0$^{1\circ,15}$]heptadecane	LTS0218994	−5.68
(1s,2r,9s)-7,15-Diazatetracyclo[7.7.1.0^{2,7}.0$^{1\circ,15}$]heptadecane	LTS0164750	−5.67
Ammodendrine	LTS0084410	−5.63
Rhombifoline	LTS0179520	−5.63
Anagyrine	LTS0112684	−5.55
(1r,9r,10r)-7,15-Diazatetracyclo[7.7.1.0^{2,7}.0$^{1\circ,15}$]heptadeca-2,4-dien-6-one	LTS0136102	−5.54
(9r,10r)-7,15-Diazatetracyclo[7.7.1.0^{2,7}.0$^{1\circ,15}$]heptadeca-2,4-dien-6-one	LTS0207526	−5.54
Lupanine l-form	LTS0101912	−5.52
(1s,9r,10r,12s)-12-Hydroxy-7,15-diazatetracyclo[7.7.1.0^{2,7}.0$^{1\circ,15}$]heptadeca-2,4-dien-6-one	LTS0078452	−5.39
Quercetin	LTS0004651	−5.32
Thermospine	LTS0069032	−5.31
Asahina	LTS0068303	−5.25
(−)-Naringenin	LTS0072900	−5.19
Kaempferol	LTS0155822	−5.18
(1r,2r,9s,10r)-7,15-Diazatetracyclo[7.7.1.0^{2,7}.0$^{1\circ,15}$]heptadecan-6-one	LTS0167136	−5.14
Hispidol	LTS0135619	−4.99
(1r,9r)-11-Methyl-7,11-diazatricyclo[7.3.1.0^{2,7}]trideca-2,4-dien-6-one	LTS0096355	−4.96
Daidzein	LTS0130369	−4.95
(9r,10s)-7,15-Diazatetracyclo[7.7.1.0^{2,7}.0$^{1\circ,15}$]heptadeca-2,4-dien-6-one	LTS0057406	−4.91
Sophoramine	LTS0114341	−4.89
Chamomile	LTS0104946	−4.83
Retamine	LTS0060917	−4.81
Sparteine	LTS0042917	−4.80
(1r,2r,9r,10s)-7,15-Diazatetracyclo[7.7.1.0^{2,7}.0$^{1\circ,15}$]heptadec-5-ene	LTS0211927	−4.69
Cytisine	LTS0079832	−4.60
6-Oxo-7,11-diazatricyclo[7.3.1.0^{2,7}]trideca-2,4-diene-11-carbaldehyde	LTS0240022	−4.60
7,11-Diazatricyclo[7.3.1.0^{2,7}]trideca-2,4-dien-6-one	LTS0030930	−4.41
(9R)-7,11-Diazatricyclo[7.3.1.0^{2,7}]trideca-2,4-dien-6-one	LTS0111966	−4.36
Choline	LTS0170307	−3.83
Trigonelline	LTS0040106	−3.81

plants may have these benefits because of their antioxidant capabilities. There have been reports of similar results by Muzes et al. [43].

Both obesity and diabetes have detrimental effects on sperm parameters and are linked to decreased testosterone levels [44]. This strengthens our finding that HFD/STZ is accompanied by reduced testosterone levels with decreased whole semen quality; this action was reversed by the RR supplementation that led to improvement in the testosterone level concerning the HFD/STZ-treated rats due to its antioxidant activity and hypoglycemic effect; it was proved that diabetes negatively impacts male sexual and reproductive function by causing adverse effects such as impaired

spermatogenesis, decreased serum testosterone levels, and reduced semen volume [45]. Androgens are essential in male sexual development, spermatogenesis, and overall sexual function. Specifically, testosterone targets Sertoli cells to maintain spermatogenesis by binding to androgen receptors and activating gene expressions that regulate the process [46]. Testosterone is synthesized through Leydig cell steroidogenesis, which relies on the passable expression of various steroidogenic enzymes, comprising STAR, P450scc, 3βHSD, 17β-HSD, and CYP17. Obesity and metabolic syndrome compromise steroidogenesis, leading to reduced levels of testosterone and progesterone in males. Previous studies have observed these effects [47, 48]. This was in

Molecular docking score = -7.48 kcal/mol

	Interaction	Distance	E (kcal/mol)
ASP 159 (A)	H-donor	2.86	-0.5
ASP 159 (A)	H-donor	2.49	-2.3
SER 55 (A)	H-donor	2.93	-1.7
LYS 21 (A)	H-acceptor	2.91	-1.3
GLN 18 (A)	H-acceptor	3.30	-1.9

polar
acidic
basic
greasy
proximity contour

sidechain acceptor
sidechain donor
backbone acceptor
backbone donor
ligand exposure

solvent residue
metal complex
solvent contact
metal/ion contact
receptor exposure

arene-arene
arene-H
arene-cation

(a)

Molecular docking score = -7.08 kcal/mol

	Interaction	Distance	E (kcal/mol)
GLU 61 (A)	H-donor	3.02	-2.2
THR 42 (A)	H-acceptor	2.88	-1.1
PRO 50 (A)	H-acceptor	3.15	-0.5
ARG 64 (A)	H-acceptor	3.70	-0.5

polar
acidic
basic
greasy
proximity contour

sidechain acceptor
sidechain donor
backbone acceptor
backbone donor
ligand exposure

solvent residue
metal complex
solvent contact
metal/ion contact
receptor exposure

arene-arene
arene-H
arene-cation

(b)

Molecular docking score = -6.94 kcal/mol

	Interaction	Distance	E (kcal/mol)
GLN 32 (A)	H-acceptor	3.55	-0.5

polar
acidic
basic
greasy
proximity contour

sidechain acceptor
sidechain donor
backbone acceptor
backbone donor
ligand exposure

solvent residue
metal complex
solvent contact
metal/ion contact
receptor exposure

arene-arene
arene-H
arene-cation

(c)

Figure 6: Continued.

Molecular docking score = -6.65 kcal/mol

	Interaction	Distance	E (kcal/mol)
THR 56 (A)	H-donor	2.72	-2.4
LYS 21 (A)	H-acceptor	3.46	-2.6

- polar
- acidic
- basic
- greasy
- proximity contour
- sidechain acceptor
- sidechain donor
- backbone acceptor
- backbone donor
- ligand exposure
- solvent residue
- metal complex
- solvent contact
- metal/ion contact
- receptor exposure
- arene-arene
- arene-H
- arene-cation

(d)

Molecular docking score = -6.58 kcal/mol

	Interaction	Distance	E (kcal/mol)
PRO 51 (A)	H-donor	3.09	-0.8
ARG 64 (A)	H-acceptor	3.04	-0.8

- polar
- acidic
- basic
- greasy
- proximity contour
- sidechain acceptor
- sidechain donor
- backbone acceptor
- backbone donor
- ligand exposure
- solvent residue
- metal complex
- solvent contact
- metal/ion contact
- receptor exposure
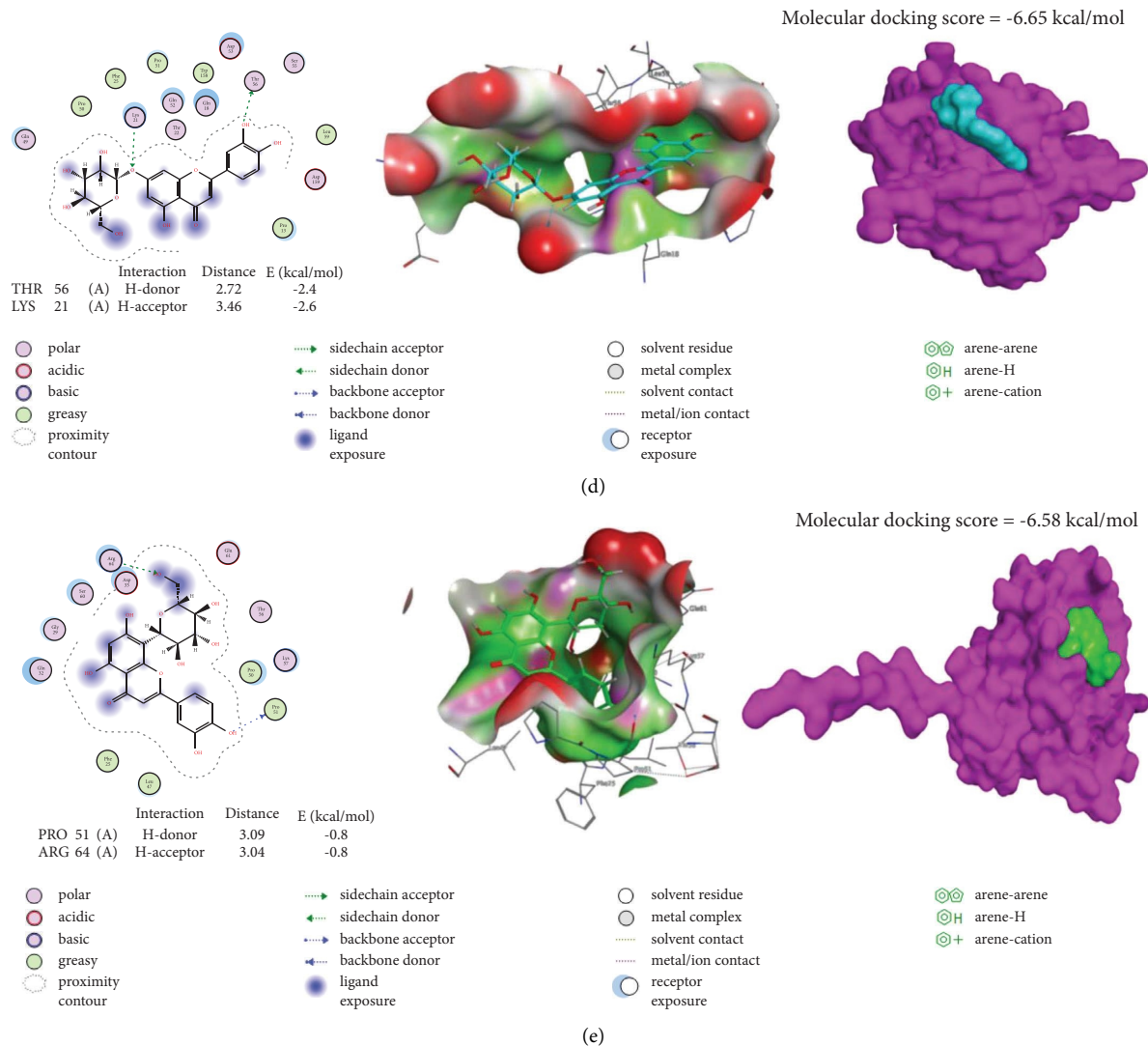- arene-arene
- arene-H
- arene-cation

(e)

FIGURE 6: Molecular docking interaction of *R. raetam*'s bioactive compounds against Bax. (a) Kaempferol 7-o-glucoside. (b) 5-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-7-{[(2s,3r,4s,5s,6r)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}chromen-4-one. (c) Vicenin 2. (d) Luteolin 7-o-glucoside. (e) Orientin.

harmony with our findings concerning the steroidogenic gene expression; in the same way, RR supplementation normalized gene expression, enhancing antioxidant and antiapoptotic effects. This led to increased BCl2 levels and decreased Bax expression. In addition, *R. raetam*'s bioactive compounds exhibited binding affinity to Bax's binding site, especially kaempferol 7-O-glucoside, 5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-7-{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}chromen-4-one, vicenin 2, luteolin 7-O-glucoside, and orientin.

The testicular tissue exhibited several changes, including spermatid loss and a decrease in spermatogenic cell layers. In addition, giant cells and an accumulation of cellular debris in the lumen of some tubules were observed. Furthermore, the seminiferous tubules showed a decreased diameter. These findings are consistent with [49] because elevated glucose levels have the potential to impact testicular function negatively and may contribute to infertility [50]. RR

supplementation resulted in seminiferous tubules with clear layers containing different stages of spermatogenic cells. However, there was a loss of spermatogenic cells in some tubules, possibly due to the RR hypoglycemic effect [10].

Experimental evidence indicates that various parts of the "*Retama*" plant contain significant secondary metabolites, including flavonoids, phenolic acids, alkaloids, glycosides, polysaccharides, stilbenes, saponins, and tannins, display promise as potential antidiabetic agents, and hold potential for pharmaceutical drug development [51]. These natural products exhibit a range of biological actions, such as antimicrobial, anti-hyperglycemic, anti-inflammatory, antiglycation, antioxidant, pain-relieving, cytocidal, hemostatic, and diuretic effects [52, 53]. Saponin steroids have been noted for their pain-relieving, anti-inflammatory, immune-modulating, immune-adjuvant, and antitumor properties [54]. Traditional medicine practitioners have also used powdered leaf extracts to treat circumcision wounds,

antiseptic wounds, pruritus, skin rashes, and microbial infections [55]. Pinitol, a compound derived from aqueous extracts, has shown potential as a mediator for reducing inflammation and blood glucose levels with hypoglycemic effects [56].

5. Conclusion

The study found that RR had multiple beneficial effects on the testes. Steroidogenesis genes were upregulated, while Bax, a cell death protein, was downregulated. Additionally, RR significantly increased aromatase activity. These outcomes propose that RR has a protective consequence on the reproductive organs like the testes and may contribute to its testicular protective effects against HFD/STZ-induced damage. *R. raetam*'s bioactive compounds, including kaempferol 7-o-glucoside, 5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-7-{[(2s,3r,4s,5s,6r)-3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]oxy}chromen-4-one, vicenin 2, luteolin 7-o-glucoside, and orientin, exhibited binding solid affinity to Bax's binding site. The antioxidant activity of RR may play a role in these protective effects, alongside its hypoglycemic effect. This study proposes the potential of RR as a therapeutic intervention for testicular protection in the context of obesity and diabetes.

Data Availability

Data are available upon request from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

Supplementary Materials

Table 1S: primer sequences. Table 2S: GC-MS analysis of *Retama raetam* extract. Figure 1S: HPLC analysis of the *Retama raetam* extract. (*Supplementary Materials*)

References

[1] G. A. Bray, K.-K. Kim, J. P. Wilding, and W. O. Federation, "Obesity: a chronic relapsing progressive disease process. A position statement of the World Obesity Federation," *Obesity Reviews*, vol. 18, no. 7, pp. 715–723, 2017.

[2] P. Ameer Arsalan Hadi, "Nutritional status and sedentary lifestyle of individuals a review," *International Journal of Modern Agriculture*, vol. 10, no. 2, 2021.

[3] C. Bleau, A. D. Karelis, D. H. St-Pierre, and L. Lamontagne, "Crosstalk between intestinal microbiota, adipose tissue and skeletal muscle as an early event in systemic low-grade inflammation and the development of obesity and diabetes," *Diabetes*, vol. 31, no. 6, pp. 545–561, 2015.

[4] M. Stumvoll, B. J. Goldstein, and T. W. Van Haeften, "Type 2 diabetes: principles of pathogenesis and therapy," *The Lancet*, vol. 365, no. 9467, pp. 1333–1346, 2005.

[5] R. Kaur, M. Kaur, and J. Singh, "Endothelial dysfunction and platelet hyperactivity in type 2 diabetes mellitus: molecular insights and therapeutic strategies," *Cardiovascular Diabetology*, vol. 17, no. 1, pp. 121–217, 2018.

[6] R. Mittler, E. Merquiol, E. Hallak-Herr, S. Rachmilevitch, A. Kaplan, and M. Cohen, "Living under a 'dormant'canopy: a molecular acclimation mechanism of the desert plant Retama raetam," *The Plant Journal*, vol. 25, no. 4, pp. 407–416, 2001.

[7] M. Maghrani, A. Lemhadri, H. Jouad, J.-B. Michel, and M. Eddouks, "Effect of the desert plant Retama raetam on glycaemia in normal and streptozotocin-induced diabetic rats," *Journal of Ethnopharmacology*, vol. 87, no. 1, pp. 21–25, 2003.

[8] F. Conforti, G. Statti, R. Tundis et al., "Antioxidant and cytotoxic activities of Retama raetam subsp. Gussonei," *Phytotherapy Research*, vol. 18, no. 7, pp. 585–587, 2004.

[9] M. Maghrani, A. Lemhadri, N. Zeggwagh et al., "Effect of Retama raetam on lipid metabolism in normal and recent-onset diabetic rats," *Journal of Ethnopharmacology*, vol. 90, no. 2-3, pp. 323–329, 2004.

[10] M. Maghrani, J. B. Michel, and M. Eddouks, "Hypoglycaemic activity of Retama raetam in rats," *Phytotherapy Research*, vol. 19, no. 2, pp. 125–128, 2005.

[11] M. M. Algandaby, H. A. Alghamdi, O. M. Ashour et al., "Mechanisms of the antihyperglycemic activity of Retama raetam in streptozotocin-induced diabetic rats," *Food and Chemical Toxicology*, vol. 48, no. 8-9, pp. 2448–2453, 2010.

[12] A. M. Mahmoud, M. M. Abdel-Rahman, N. A. Bastawy, and H. M. Eissa, "Modulatory effect of berberine on adipose tissue PPARγ, adipocytokines and oxidative stress in high fat diet/streptozotocin-induced diabetic rats," *Journal of Applied Pharmaceutical Science*, vol. 7, pp. 001–010, 2017.

[13] M. Eddouks, M. Maghrani, L. Louedec, M. Haloui, and J. Michel, "Antihypertensive activity of the aqueous extract of Retama raetam Forssk. leaves in spontaneously hypertensive rats," *Journal of Herbal Pharmacotherapy*, vol. 7, no. 2, pp. 65–77, 2008.

[14] M. M. Menshawy, A. R. H. Farrag, S. A. El Toumy, and G. S. Muhamed, "Role of retama raetam on gentamicin-induced acute kidney injury in experimental rat model," *Egyptian Academic Journal of Biological Sciences*, vol. 13, no. 2, pp. 35–45, 2021.

[15] J. P. Hosker, D. R. Matthews, A. S. Rudenski et al., "Homeostasis model assessment: insulin resistance and beta cell function in man," *Diabetologia*, vol. 28, no. 7, pp. 401–411, 1985.

[16] M. Sönmez, G. Türk, and A. Yüce, "The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats," *Theriogenology*, vol. 63, no. 7, pp. 2063–2072, 2005.

[17] R. Amann, "Use of animal models for detecting specific alterations in reproduction," *Toxicological Sciences*, vol. 2, no. 1, pp. 13–26, 1982.

[18] S. G. Johnsen, "Testicular biopsy score count–a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males," *Hormone Research in Paediatrics*, vol. 1, no. 1, pp. 2–25, 1970.

[19] M. M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," *Analytical Biochemistry*, vol. 72, no. 1-2, pp. 248–254, 1976.

[20] A. Babson and A. Read, "Determination of total acid phosphatase in serum," *American Journal of Clinical Pathology*, vol. 32, pp. 89–91, 1959.

[21] L. Babson, "Phenolphalein monophosphate methods for the determination of alkaline phosphatase," *Clinical Chemistry*, vol. 11, p. 789, 1965.

[22] R. V. Considine, M. K. Sinha, M. L. Heiman et al., "Serum immunoreactive-leptin concentrations in normal-weight and obese humans," *New England Journal of Medicine*, vol. 334, no. 5, pp. 292–295, 1996.

[23] E. Beutler, O. Duron, and B. M. Kelly, "Improved method for the determination of blood glutathione," *The Journal of Laboratory and Clinical Medicine*, vol. 61, pp. 882–888, 1963.

[24] H. G. Preuss, S. T. Jarrell, R. Scheckenbach, S. Lieberman, and R. A. Anderson, "Comparative effects of chromium, vanadium and Gymnema sylvestre on sugar-induced blood pressure elevations in SHR," *Journal of the American College of Nutrition*, vol. 17, no. 2, pp. 116–123, 1998.

[25] M. Nishikimi, N. Appaji Rao, and K. Yagi, "The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen," *Biochemical and Biophysical Research Communications*, vol. 46, no. 2, pp. 849–854, 1972.

[26] K. Sastry, R. Moudgal, J. Mohan, J. Tyagi, and G. Rao, "Spectrophotometric determination of serum nitrite and nitrate by copper–cadmium alloy," *Analytical Biochemistry*, vol. 306, no. 1, pp. 79–82, 2002.

[27] J. D. Bancroft and M. Gamble, "Theory and practice of histological techniques," *Elsevier health sciences*, 2008.

[28] K. J. Livak and T. D. Schmittgen, "Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method," *Methods*, vol. 25, no. 4, pp. 402–408, 2001.

[29] S. Shirali, S. Zahra Bathaie, and M. Nakhjavani, "Effect of crocin on the insulin resistance and lipid profile of streptozotocin-induced diabetic rats," *Phytotherapy Research*, vol. 27, no. 7, pp. 1042–1047, 2013.

[30] M. Eileen Dolan, "Inhibition of DNA repair as a means of increasing the antitumor activity of DNA reactive agents," *Advanced Drug Delivery Reviews*, vol. 26, no. 2-3, pp. 105–118, 1997.

[31] K. E. Almby, N. Abrahamsson, M. H. Lundqvist et al., "Effects of GLP-1 on counter-regulatory responses during hypoglycemia after GBP surgery," *European Journal of Endocrinology*, vol. 181, no. 2, pp. 161–171, 2019.

[32] J. E. Gerich, "Glucose counterregulation and its impact on diabetes mellitus," *Diabetes*, vol. 37, no. 12, pp. 1608–1617, 1988.

[33] R. Kinne, "Metabolic correlates of tubular transport," *Transport Organs*, pp. 529–562, 1979.

[34] L. O'donnell, R. McLachlan, N. Wreford, and D. Robertson, "Testosterone promotes the conversion of round spermatids between stages VII and VIII of the rat spermatogenic cycle," *Endocrinology*, vol. 135, no. 6, pp. 2608–2614, 1994.

[35] B. Pourheydar, F. Azarm, G. Farjah, M. Karimipour, and M. Pourheydar, "Effect of silymarin and metformin on the sperm parameters and histopathological changes of testes in diabetic rats: an experimental study," *International Journal of Reproductive BioMedicine*, vol. 19, no. 12, pp. 1091–1104, 2021.

[36] T. K. Jensen, B. L. Heitmann, M. B. Jensen et al., "High dietary intake of saturated fat is associated with reduced semen quality among 701 young Danish men from the general population," *The American Journal of Clinical Nutrition*, vol. 97, no. 2, pp. 411–418, 2013.

[37] C. H. Ramlau-Hansen, M. Hansen, C. R. Jensen, J. Olsen, J. P. Bonde, and A. M. Thulstrup, "Semen quality and reproductive hormones according to birthweight and body mass index in childhood and adult life: two decades of follow-up," *Fertility and Sterility*, vol. 94, no. 2, pp. 610–618, 2010.

[38] A. O. Hammoud, M. Gibson, C. M. Peterson, A. W. Meikle, and D. T. Carrell, "Impact of male obesity on infertility: a critical review of the current literature," *Fertility and Sterility*, vol. 90, no. 4, pp. 897–904, 2008.

[39] S. Civelek, D. Konukoglu, F. Erdenen, and H. Uzun, "Serum neurotrophic factor levels in patients with type 2 diabetes mellitus: relationship to metabolic syndrome components," *Clinical Laboratory*, vol. 59, no. 3-4, pp. 369–374, 2013.

[40] A. M. Isidori, M. Caprio, F. Strollo et al., "Leptin and androgens in male obesity: evidence for leptin contribution to reduced androgen levels," *Journal of Clinical Endocrinology and Metabolism*, vol. 84, no. 10, pp. 3673–3680, 1999.

[41] A. Esteghamati, O. Khalilzadeh, M. Anvari, A. Rashidi, M. Mokhtari, and M. Nakhjavani, "Association of serum leptin levels with homeostasis model assessment–estimated insulin resistance and metabolic syndrome: the key role of central obesity," *Metabolic Syndrome and Related Disorders*, vol. 7, no. 5, pp. 447–452, 2009.

[42] P. Newsholme, V. F. Cruzat, K. N. Keane, R. Carlessi, and P. I. H. de Bittencourt Jr, "Molecular mechanisms of ROS production and oxidative stress in diabetes," *Biochemical Journal*, vol. 473, no. 24, pp. 4527–4550, 2016.

[43] G. Müzes, G. Deak, I. Lang, K. Nekam, P. Gergely, and J. Feher, "Effect of the bioflavonoid silymarin on the in vitro activity and expression of superoxide dismutase (SOD) enzyme," *Hungarian Physiological Journal*, vol. 78, no. 1, pp. 3–9, 1991.

[44] O. Zhong, L. Ji, J. Wang, X. Lei, and H. Huang, "Association of diabetes and obesity with sperm parameters and testosterone levels: a meta-analysis," *Diabetology and Metabolic Syndrome*, vol. 13, no. 1, p. 109, 2021.

[45] J. Boivin, L. Bunting, J. A. Collins, and K. G. Nygren, "International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care," *Human Reproduction*, vol. 22, no. 6, pp. 1506–1512, 2007.

[46] G. Dohle, M. Smit, and R. Weber, "Androgens and male fertility," *World Journal of Urology*, vol. 21, no. 5, pp. 341–345, 2003.

[47] K. Leisegang, P. J. Bouic, and R. R. Henkel, "Metabolic syndrome is associated with increased seminal inflammatory cytokines and reproductive dysfunction in a case-controlled male cohort," *American Journal of Reproductive Immunology*, vol. 76, no. 2, pp. 155–163, 2016.

[48] K. Leisegang, A. Udodong, P. Bouic, and R. Henkel, "Effect of the metabolic syndrome on male reproductive function: a case-controlled pilot study," *Andrologia*, vol. 46, no. 2, pp. 167–176, 2014.

[49] Ö. Budak, "Histological investigation of the protective effect of metformin on testis and sperm parameters in obese rats with type 2 diabetes mellitus," *Journal of Surgery and Medicine*, vol. 5, no. 9, pp. 954–959, 2021.

[50] S. Kushwaha and G. Jena, "Telmisartan ameliorates germ cell toxicity in the STZ-induced diabetic rat: studies on possible molecular mechanisms," *Mutation Research, Genetic Toxicology and Environmental Mutagenesis*, vol. 755, no. 1, pp. 11–23, 2013.

[51] W. Sun and M. H. Shahrajabian, "Therapeutic potential of phenolic compounds in medicinal plants—natural health products for human health," *Molecules*, vol. 28, no. 4, p. 1845, 2023.

[52] M. B. Naili, R. O. Alghazeer, N. A. Saleh, and A. Y. Al-Najjar, "Evaluation of antibacterial and antioxidant activities of artemisia campestris (astraceae) and ziziphus lotus (rhamnacea)," *Arabian Journal of Chemistry*, vol. 3, no. 2, pp. 79–84, 2010.

[53] M. Suhaj, "Spice antioxidants isolation and their antiradical activity: a review," *Journal of Food Composition and Analysis*, vol. 19, no. 6-7, pp. 531–537, 2006.

[54] H. Edziri, M. Mastouri, I. Chéraif, and M. Aouni, "Chemical composition and antibacterial, antifungal and antioxidant activities of the flower oil of Retama raetam (Forssk.) Webb from Tunisia," *Natural Product Research*, vol. 24, no. 9, pp. 789–796, 2010.

[55] H. Tsuchiya, M. Sato, T. Miyazaki et al., "Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*," *Journal of Ethnopharmacology*, vol. 50, no. 1, pp. 27–34, 1996.

[56] N. H. González-Mauraza, A. J. León-González, J. L. Espartero, J. B. Gallego-Fernández, M. Sánchez-Hidalgo, and C. Martin-Cordero, "Isolation and quantification of pinitol, a bioactive cyclitol, in retama spp," *Natural Product Communications*, vol. 11, no. 3, 2016.