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Protective effects of gilaburu (*Viburnum opulus* L.) fruit extract on testicular damage in streptozotocin induced diabetic rats

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Diabetes mellitus (DM) causes the impairment of male reproductive functions by inducing oxidative stress and apoptosis in testicular tissues. Despite various therapeutic approaches, the need for effective strategies to reduce diabetes-related testicular damage remains a significant issue. In this study, the potential protective effects of Gilaburu fruit extract, known for its potent antioxidant and anti-inflammatory properties, were investigated against streptozotocin (STZ)-induced testicular damage. Subjects were divided into control, diabetes (STZ, 50 mg/kg), and treatment (DM+Glb; 200 mg/kg Gilaburu) groups. While serum testosterone levels were measured, malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase 1 (GPx1) levels, along with histopathological parameters and immunohistochemical markers (Bax, Bcl-2, PCNA, and Sirt6), were evaluated in testicular tissues. In the diabetes group, testosterone, SOD, and GPx1 levels significantly decreased, while MDA levels and the Bax/Bcl-2 ratio increased. These biochemical changes were accompanied by a narrowing of seminiferous tubule diameters and a decrease in PCNA and Sirt6 expressions. Gilaburu treatment significantly improved these impairments by reducing oxidative stress, inhibiting apoptosis, and increasing cellular proliferation and Sirt6 levels. In conclusion, Gilaburu extract may demonstrate potential as a supportive phytotherapeutic agent against diabetes-related testicular damage by regulating the oxidative stress-apoptosis-proliferation axis.

Keywords: Phytotherapy, Spermatogenesis, DNA Repair, Antioxidants, Infertility

Diabetes mellitus (DM) is a metabolic disease characterized by chronic hyperglycemia that causes serious complications in numerous organs. According to the International Diabetes Federation (2025) data, approximately 590 million people worldwide have diabetes, and this number is expected to increase by 46% by 2050[1,2]. Among these complications, male reproductive system dysfunction stands out as a significant problem, as persistent hyperglycemia leads to oxidative stress (OS), adversely affecting spermatogenesis[3,4]. he increase in reactive oxygen species (ROS) and the decrease in antioxidant defense mechanisms in diabetic testes cause protein oxidation, DNA damage, and mitochondrial mutations, ultimately triggering germ cell apoptosis[5].

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Although the potential effects of various antioxidants in reducing these damages have been demonstrated, natural phytotherapeutic agents are attracting increasing interest due to their multi-target effects[6-8]. *Viburnum opulus* L. (Guelder rose, Gilaburu, Glb) belongs to the Caprifoliaceae family and is known for its rich phenolic content and potent antioxidant, anti-inflammatory, and anti-apoptotic properties[9-11]. It has been reported that high concentrations of chlorogenic acid, quercetin, and anthocyanins found in gilaburu fruit exhibit vasorelaxant, antiproliferative, and potent anti-inflammatory effects in various metabolic diseases[12,13]. Although previous studies have reported the protective effects of gilaburu in various diseases, its specific effect on diabetic testicular damage has not yet been fully elucidated[14-17].

The molecular mechanisms of diabetic testicular damage often involve the disruption of key

pathways involved in the regulation of cell survival and genomic stability[18]. Sirtuin 6 (Sirt6), a histone deacetylase, plays a critical role in DNA repair and metabolic regulation, while Proliferating Cell Nuclear Antigen (PCNA) is an essential marker for cellular proliferation and DNA synthesis[19,20]. The role of sirtuins, particularly Sirt6, in maintaining cellular homeostasis and reducing DNA damage under hyperglycemic conditions has become an important focus in reproductive biology studies in recent years[21,22]. Furthermore, maintaining the balance between pro-apoptotic Bcl-2-associated X protein (Bax) and anti-apoptotic B-cell lymphoma 2 (Bcl-2) plays a vital role in sustaining germ cell integrity[23,24].

Elucidating the interaction mechanisms of natural extracts such as gilaburu with protective and apoptotic proteins may contribute to the development of new therapeutic approaches for diabetic complications. Accordingly, this study aimed to evaluate the potential protective effects of *Viburnum opulus* L. extract on streptozotocin (STZ)-induced diabetic testicular damage in terms of its effects on the modulation of oxidative stress, apoptosis, and proliferative/repair pathways.

Materials and Methods

This study was submitted to the Dicle University Health Sciences Application and Research Center Animal Experiments Local Ethics Committee (DÜHADEK) and received ethical approval with the decision number 02 dated 30.04.2024.

Preparation of *Viburnum opulus* L.fruit extract

Viburnum opulus L. ripe fruits were collected in 2023 in the borders of Kayseri province. The taxonomic identification of the plant species was performed by Dr. Betül Büyükkılıç Altınbaşak. A voucher specimen has been deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Kocaeli Health and Technology University for future reference. The collected fruits were dried. They were crushed and turned into powder before the extraction process. 100 g of fruit sample was extracted by maceration method in 70% methanol at 1:10 (w/v) ratio at 36°C in a shaking water bath, changing the solvent every 24 hours for 3 days. The obtained extract was concentrated in rotavapor and turned into dry extract form by lyophilization method. The prepared extract was stored at 4°C for experimental studies and content analysis.

Analysis of phenolic antioxidants by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

The phenolic compound content of the extract was analyzed by Agilent 6460 Triple Quadrupole LC-MS/MS system within the scope of service procurement within the scope of Ataturk University Eastern Anatolian High Technology Application and Research Center (DAYTAM). In the analyses, the electrospray ionization (ESI) source was operated in positive ion mode, gradient elution was applied and quantitative analysis was performed by the Multiple Reaction Monitoring (MRM) method. Calibration curves were created using standard solutions prepared in methanol at a concentration of 100 µg/mL. Detailed device parameters are presented in a limited manner due to the analysis performed within the scope of service procurement.

Animals and experimental treatment

The study was conducted on 21 young adult male *Wistar albino* rats with similar biological and physiological characteristics obtained from Dicle University Prof. Dr. Sabahattin Payzın Health Sciences Application and Research Center (DUSAM). The animals were kept under standard laboratory conditions (22±1°C, 12-hour light/12-hour dark cycle) and standard pellet feed and fresh drinking water were provided ad libitum. Subjects were randomly divided into three groups according to their body weights (n=7). Only citrate buffer (0.1 M, pH 4.5) was administered intraperitoneally (i.p.) to the control group. In the diabetes group, rats were administered a single intraperitoneal dose of 50 mg/kg STZ (Sigma-Aldrich, USA) dissolved in citrate buffer to model Type 1 diabetes[14]. In the treatment group, 200 mg/kg of Gilaburu extract dissolved in phosphate buffer saline (PBS) was administered intraperitoneally throughout the experimental period, starting one week before the diabetes model was established[15]. This group was also administered a single intraperitoneal dose of 50 mg/kg STZ simultaneously with the diabetes group to model diabetes.

In order to confirm the occurrence of diabetes, blood samples were taken from the tail vein 48 hours after the STZ injection and glucose levels were measured using a glucometer. Animals with fasting blood glucose levels of 250 mg/dL and above were considered[14]. Blood glucose levels were also monitored regularly every week during the experimental period. The body weights of the animals in all groups were also recorded weekly and at the end of the experiment.

On the 37th day of the experimental protocol, testicular tissues were surgically excised from the subjects under anesthesia. The biopsy materials obtained were subjected to routine tissue follow-up procedures for biochemical, light microscopy and immunohistochemical analyses. Additionally, urine and feces samples were collected from all animals before sacrifice.

Biochemical analysis

Biochemical analyses were performed to evaluate oxidative stress and hormonal parameters in testicular tissue. All analyses were performed using commercially available enzyme-linked immunosorbent assay (ELISA) kits and samples were prepared according to the manufacturer's instructions. For ELISA analyses, homogenates were prepared from testicular tissues stored at -80°C. Testicular tissues were removed from the freezer, placed in a glass tube containing PBS at a ratio of 1:10 (w/v, pH: 7.2), and homogenized on ice at 30,000 g for 60 seconds using a tissue homogenizer (Bandelin, UW 2070, Sigma, St. Louis, MO). Homogenates were placed in centrifuge tubes and centrifuged at 4,200 g for 10 min at 4°C. The supernatants were transferred to an Eppendorf tube, and the precipitates were discarded. Testosterone (ATLAS Biotechnology Inc., Turkey; Catalog Number: ABT10175Ra) and Malondialdehyde (MDA), levels (ATLAS Biotechnology Inc., Turkey; Catalog Number: ABT10165Ra), Superoxide Dismutase (SOD) levels (Shanghai YL Biotechnology Inc., China; Catalog Number: YLA0115RA) and Glutathione Peroxidase 1 (GPx1) enzyme activities (ATLAS Biotechnology Inc., Turkey; Catalog Number: ABT2557Ra) were measured in testicular tissue supernatants by ELISA using specific ELISA kits according to the manufacturer's instructions. The supernatant was read spectrophotometrically at 450 nm using a microplate reader (Biochrom, Anthos Zenyth 200). Results were given as ng/mL or pg/mL.

Histopathology

After routine histological processing, the fixed testis tissues were embedded into paraffin. Sections, 5 µm in thickness, were prepared and subsequently stained with Hematoxylin & Eosin (H&E). Morphological integrity and degenerative changes were evaluated under a light microscope (Carl Zeiss Axiolab 5, Carl Zeiss, Oberkochen, Germany). Digital images were obtained using ZEN 3.7 software and analyzed with ImageJ (version 1.53, NIH, USA). Ten different areas were measured for each slide, and scale bars were added. All measurements were performed blindly by two independent histologists according to previously described methods in the literature[3].

Immunohistochemistry

Testicular tissue sections (5 µm) prepared for immunohistochemical analysis were incubated at 56°C overnight, deparaffinized with toluene, and rehydrated through a graded alcohol series. Antigen retrieval was performed in 10 mM citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked with 3% H_2O_2, and nonspecific binding was prevented using a blocking solution. Sections were incubated overnight at +4°C with primary antibodies against Bax (Santa Cruz Biotechnology, sc-7480), Bcl-2 (Affinity Biosciences, AF6139), PCNA (Thermo Scientific, sc-2528), and Sirt6 (Affinity Biosciences, DF12739). After incubation with a biotinylated secondary antibody and HRP-conjugated streptavidin, staining was visualized with DAB and counterstained with hematoxylin. Immunohistochemical evaluations were performed using a Zeiss Axiolab 5 microscope (Carl Zeiss, Oberkochen, Germany). Digital images were obtained with ZEN 3.7 software and analyzed using ImageJ (version 1.53, NIH, USA), where measurements were performed and scale bars were added. Immunoreactivity measurements were conducted blindly by two expert histologists according to previously described methods in the literature[3].

Statistical analysis

Statistical analyses of all data were performed using SPSS 20.0 software. Non-parametric Kruskal-Wallis variance analysis test was applied to determine the differences between the groups. In cases where significant differences were detected, Mann-Whitney U test was used for multiple comparisons between groups. Results were expressed as mean ± standard deviation (SD). $P<0.05$ was considered statistically significant.

Results

Phenolic compound profile in *Viburnum opulus* L. Extract (LC-MS/MS)

The phenolic profile of *Viburnum opulus* L. extract was characterized using LC-MS/MS analysis. The identified compounds are presented in Table 1 in

Table 1 — Phenolic compound profile of *Viburnum opulus L*. extract determined by LC-MS/MS

Compound Name	RT (min)	Response	Concentration (ng/mL)
Chlorogenic acid	10.744	1,170.424	44,386.0710
Quinic acid	2.344	51,401	19,584.3418
Catechin	10.935	17,932	2,013.8361
Hesperidin	11.552	19,852	1,725.6226
Fumaric acid	3.971	755	428.8363
Epicatechin	11.327	4,680	375.2564
Quercetin	13.427	7,395	123.1611
Cyanidin-3-O-glucoside	10.496	226	47.3785
4-Hydroxybenzoic acid	11.232	1,646	37.0486
Ferulic acid	12.432	168	9.0888
Gallic acid	5.548	530	2.7995
ND / <LOQ			Pyrogallol, Keracyanin chloride, Peonidin-3-O-glucoside, Epigallocatechin gallate, Caffeic acid, Vanillic acid, Syringic acid, Vitexin, Naringin, Ellagic acid, p-Coumaric acid, Sinapic acid, Taxifolin, Rosmarinic acid, Vanillin, Myricetin, Resveratrol, Luteolin, Apigenin, Naringenin, Isorhamnetin, Chrysin, Galangin, Curcumin

[Phenolic compound profile of Viburnum opulus L. extract determined by LC-MS/MS, showing retention times (RT), response values, and concentrations. Non-detected (ND) or below limit of quantification (<LOQ) compounds are also listed]

decreasing order of concentration. The most abundant compounds were chlorogenic acid, quinic acid, catechin, and hesperidin, which constituted a significant proportion of the total phenolic content. Additionally, secondary phenolic compounds such as epicatechin, quercetin, cyanidin-3-O-glucoside, and ferulic acid were identified, while other phenolic compounds remained below the detection limit (Table 1).

Chromatographic analysis revealed a distinct and symmetrical peak for chlorogenic acid at a retention time (RT) of 10.744 min. A high signal intensity (1,170,424 counts) was obtained during the MRM transition (m/z 354.9→190.9). Quinic acid showed a narrow peak at an RT of 2.344 min, with a response of 51,401 counts recorded in the 190.9→85.0 m/z MRM transition. Catechin was detected at an RT of 10.935 min with 17,932 counts (289.0→244.9 m/z). Hesperidin was identified by a strong signal of 19,852 counts at an RT of 11.552 min, corresponding to the 609.9→300.9 m/z MRM transition (Fig. 1).

Testosterone and antioxidant results

Table 2 summarizes the levels of testosterone, MDA, GPx1, and SOD across all experimental groups. Testosterone levels were significantly lower in the diabetes group compared to the control group (*P<0.001*). While lower testosterone levels were also observed in the treatment group compared to the control group (*P=0.042*), the difference between the diabetes and treatment groups was not statistically significant (*P=0.068*). Regarding antioxidant enzymes, a significant decrease in SOD activity was observed in the diabetes group compared to the control group (*P=0.004*). However, no significant difference was found between the diabetes and treatment groups (*P=0.181*) or between the treatment and control groups (*P=0.166*). GPx1 levels were significantly decreased in the diabetes group compared to both the treatment (*P=0.014*) and control (*P=0.003*) groups. No significant difference in GPx1 was observed between the treatment and control groups (*P=0.766*). MDA levels were significantly increased in the diabetes group compared to the control group (*P=0.001*). No statistically significant difference was observed between the diabetes and treatment groups (*P=0.088*) and between the treatment and control groups (*P=0.109*).

Blood glucose levels and morphometric analysises

In this study, blood glucose levels in the diabetes group significantly increased compared to the control and DM+Glb groups. While a partial decrease in blood glucose levels was observed in the DM+Glb group compared to the diabetes group, this difference was not statistically significant. Regarding morphometric analyses, the mean seminiferous tubule diameter (MSTD) in the diabetes group was significantly lower than those in both the control and DM+Glb groups. Additionally, Johnsen's score showed a significant decrease in the diabetes group compared to the control group, while it was

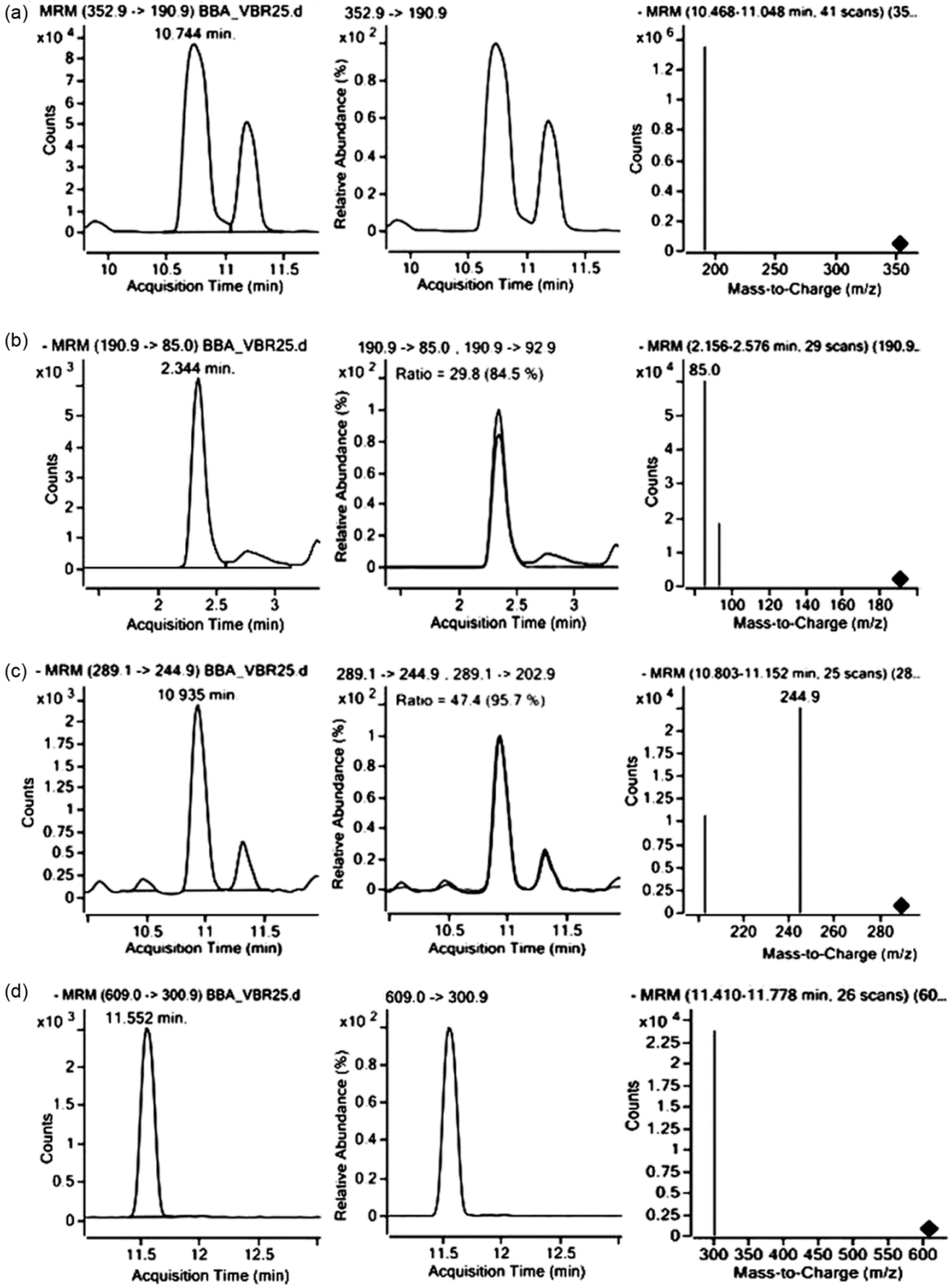

Fig. 1 — Representative LC-MS/MS chromatograms of dominant phenolic compounds in *Viburnum opulus* L. extract: (a) Chlorogenic acid (RT: 10.744 min), (b) Quinic acid (RT: 2.344 min), (c) Catechin (RT: 10.935 min), and (d) Hesperidin (RT: 11.552 min). MRM transitions and corresponding peak intensities are shown for each compound (The LC-MS/MS analysis and the provided figure were obtained through service procurement from the DAYTAM, Atatürk University, Erzurum, Türkiye).

Table 2 — Levels of testis Testosterone, SOD, GPx and MDA in all groups

	Control	DM	DM+Glb
Testosterone (ng/mL)	1.31±0.03	1.23±0.03[a]	1.27±0.03[b]
SOD (ng/mL)	0.78±0.14	0.34±0.28[c]	0.56±0.22
GPx1 (pg/mL)	0.12±0.0	0.05±0.05[d,e]	0.11±0.03
MDA (ng/mL)	1.12±0.04	1.25±0.08[f]	1.18±0.03

[[a]*P<0.001* Significantly decreased in the diabetes group compared with the control group. [b]*P=0.042* Also decreased in the treatment group compared with the control group. [c]*P=0.004* Significantly decreased in the diabetic group compared with the control group. [d]*P=0.003* Significantly decreased in the diabetes group compared with the control group and compared with the treatment group [e]*P=0.014*. [f]*P=0.001* Significantly increased in the diabetes group compared with the control group. Results are shown as the means ± standard deviation. SOD; Superoxide dismutase, GPx1; Glutathione peroxidase, MDA; Malondialdehyde, DM; Diabetes Mellitus, DM+Glb; Gilaburu treatment group]

Table 3 — Blood glucose levels, MSTD, and Johnsen scoring

	Initial blood glucose levels (mg/dl)	Final blood glucose levels (mg/dl)	MSTD	Johnsen scoring
Control	122.9 ± 7.1	115.3 ± 9.8	349.1 ± 22.4	9.57 ±0.53
DM	122.6 ± 9.9	500.3 ± 82.8[a]	268.7 ± 10.9[b]	7.43 ±0.54[b]
DM+Glb	119.3 ± 6.6	471.6 ± 76.5[a]	302.2 ± 9.8[c, d]	8.29±0.76[c, d]

[[a]*P<0.001* Statistically significant compared with initial levels. [b]*P<0.001* Statistically significant compared with the control group. [c]*P<0.01* Statistically significant compared with the diabetes group. [d]*P<0.05* Statistically significant compared with the control group. Results are shown as the means ± standard deviation. MSTD: Measurement of seminiferous tubule diameters. DM; Diabetes Mellitus, DM+Glb; Gilaburu treatment group]

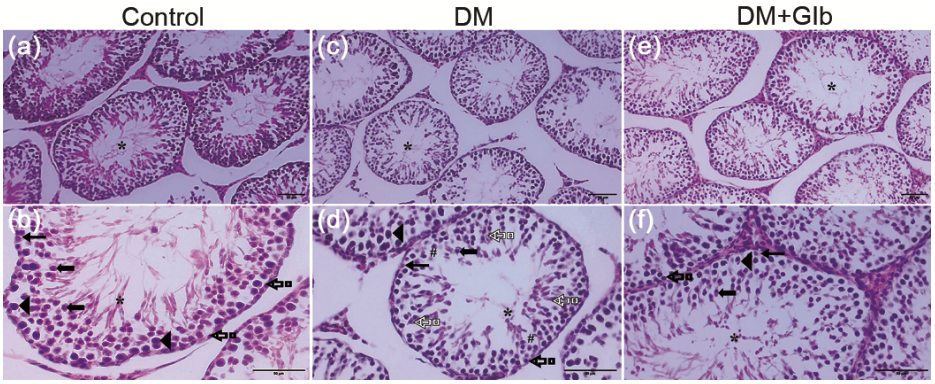

Fig. 2 — Hematoxylin and Eosin staining of testis tissues. Sperms (asteriks); Sertoli cells (thin arrows); spermatids (thick arrows); spermatogoniums (segmented arrows); primer spermatocytes (arrow heads); tissue degenerations (#); vacuolizations (white segmented arrows); DM: Diabetes Mellitus; DM+Glb: Gilaburu treatment group. Magnification: a,c,e x200 and b,d,f x400. Scale bar: 50 µm.

significantly increased in the DM+Glb group relative to the diabetes group (Table 3).

Hematoxylin and eosin findings

In the control group, the structural integrity of the seminiferous tubules was well preserved, and spermatogenic cells were arranged in an orderly fashion. Sertoli cells and spermatogonia were located along the basal membrane, while spermatocytes and spermatids were located closer to the tubule lumen (Fig 2a & b). In the diabetic group, significant histopathological changes were observed, including deformations of the seminiferous tubules, loss of cytoplasmic content, and marked cellular disorganization. Degeneration of Sertoli and spermatogenic cells led to the formation of vacuoles within the germinal epithelium and structural abnormalities in the cytoplasm of spermatogonia and spermatids (Fig 2c & d). In the DM+Glb group, these degenerative changes and cellular damage were significantly less severe compared to the diabetic group, indicating relatively preserved tubule structure (Fig 2e & f).

Immunohistochemical findings

In Bax immunoreactivity, intense cytoplasmic staining was observed in the spermatogonia, Sertoli cells, primary spermatocytes, and spermatids

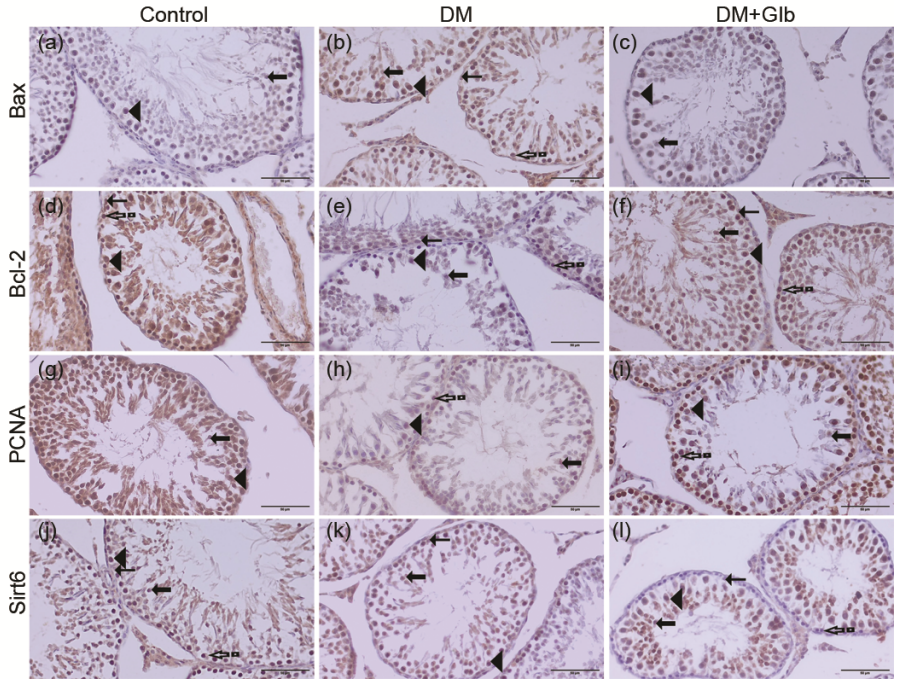

Fig. 3 — Immunohistochemical staining of tissues. Sertoli cells (thin arrows); spermatids (thick arrows); spermatogoniums (segmented arrows); primer spermatocytes (arrow heads); DM, Diabetes Mellitus; DM+Glb, Gilaburu treatment group; Bax, Bcl-2 Associated X-protein; Bcl-2, B-cell lymphoma 2; PCNA, Proliferating Cell Nuclear Antigen; Sirt6, Sirtuin 6. Magnification: x400. Scale bar: 50 µm.

of the diabetes group (Fig. 3b). In the control and treatment groups exhibited weak staining in primary spermatocytes and spermatids (Fig. 3a, c). Semi-quantitative analysis revealed that Bax immunoreactivity in the diabetes group (244.9±16.7) was significantly higher than in the control (128.6±13.2) and treatment (171.9±11.3) groups (*P<0.001*), (Fig. 4a).

For Bcl-2, intense cytoplasmic staining was present in the spermatogonia, Sertoli cells, primary spermatocytes, and spermatids of both the control and treatment groups (Fig. 3d, f). In the diabetes group showed weak staining in these cells (Fig. 3e).

Bcl-2 immunoreactivity was significantly lower in the diabetes group (137.1±24.9) compared to the control (232.9±29.5) and treatment (188.5±21.8) groups (*P<0.001*) (Fig. 4b). The Bax/Bcl-2 ratio was significantly increased in the diabetes group (1.80±0.30) compared to the control (0.56±0.11), Gilaburu treatment groups (0.92±0.05) (Fig. 4e).

PCNA immunoreactivity was strongly observed in the primary spermatocytes and spermatogonia of the control and treatment groups, with a weak reaction in spermatids (Fig. 3g, i). The diabetes group showed weak staining in spermatogonia and primary spermatocytes (Fig. 3h). PCNA levels were significantly lower in the diabetes group (35.7±9.5) than in the control (80.0±10.08) and treatment (60.7±9.9) groups (*P<0.001*) (Fig. 4c).

Sirt6 staining showed both nuclear and cytoplasmic reactions in the Sertoli cells, spermatogonia, primary spermatocytes, and spermatids of the control and treatment groups (Fig. 3j, l). The diabetes group demonstrated weak Sirt6 immunoreactivity (Fig. 3k). Sirt6 levels in the diabetes group (78.1±10.3) were significantly lower than in the control (125.1±25.6) and treatment (102.6±6.2) groups (*P<0.001*). A marked increase in Sirt6 expression was observed in the treatment group compared to the diabetes group (Fig. 4d).

Discussion

Diabetic testicular damage is a global health problem causing male infertility and hormonal imbalances. Streptozotocin-induced diabetes models are widely utilized to elucidate the pathogenesis of this damage and to evaluate novel phytotherapeutic approaches. In this study, it was determined that *Viburnum opulus* L. extract exhibited significant protective effects by maintaining spermatogenic integrity, as evidenced by the improvements observed in Johnsen scores and seminiferous tubule diameters.

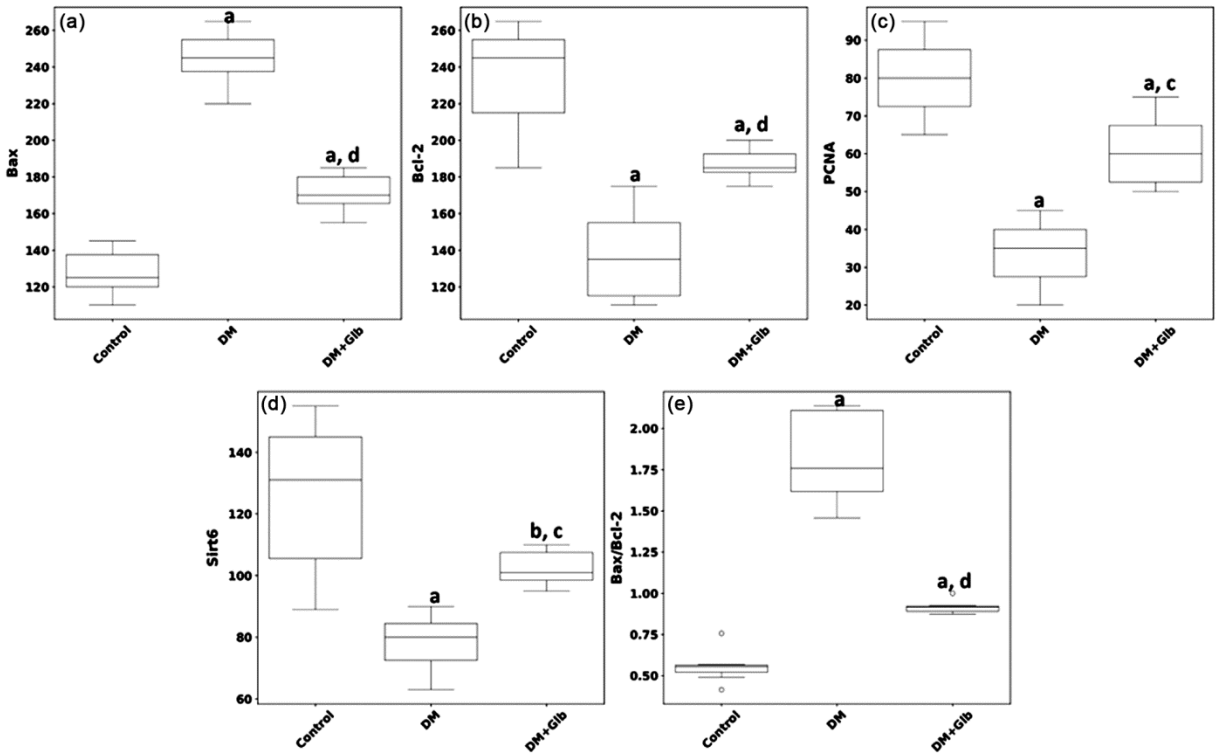

Fig. 4 — Immunoreactivities of antibodies in testis tissues. [a]*P*<0.001, Statistically significant compared with control group. [b]*P*<0.05, Statistically significant compared with control group. [c]*P*<0.01, Statistically significant compared with diabetes group. [d]*P*<0.001 Statistically significant compared with diabetes group. Results are shown as the means ± standard deviation. DM; Diabetes Mellitus, DM+Glb: Gilaburu treatment group, Bax, Bcl-2 Associated X-protein; Bcl-2, B-cell lymphoma 2; PCNA, Proliferating Cell Nuclear Antigen; Sirt6, Sirtuin 6.

These findings suggest that *Viburnum opulus* may reduce structural degeneration through its potent bioactive components.

Our LC-MS/MS analysis confirmed a rich phenolic profile in Gilaburu extract, containing high concentrations of chlorogenic acid, quinic acid, catechin, and hesperidin. These compounds are known to possess potent antioxidant and pharmacological activities[7,25]. Oxidative stress, triggered by excessive ROS production and a weakened antioxidant defense system, is considered one of the primary causes of diabetic gonadal damage[26]. Flavonoids reduce this stress by scavenging free radicals and increasing endogenous antioxidant enzymes[27]. Although the extract did not significantly alter systemic blood glucose levels, its beneficial effects appear to emerge through local cytoprotective mechanisms rather than systemic glycemic control.

The marked decrease in serum MDA levels along with the increase in SOD and GPx1 activities in the treatment group suggested that these effects might stem from the action of Gilaburu. Similar protective effects have been reported for other phenolic-rich extracts, such as artichoke and *Alpinia officinarum*, which improve testicular histology and reduce lipid peroxidation in diabetic models[28,29]. Furthermore, agents such as garden cress seed oil, *Artemisia judaica*, or cyanidin-3-glucoside have also been shown to reorganize the antioxidant defense system and testosterone levels, and these findings parallel the results we obtained with Gilaburu[30-32].

One of the significant findings of this study was the regulation of the oxidative stress–apoptosis–proliferation axis. Diabetes impairs spermatogenesis by shifting the balance in favor of germ cell apoptosis. Gilaburu treatment limited cell loss by significantly reducing the Bax/Bcl-2 ratio. This anti-apoptotic effect is consistent with studies showing that natural alkaloids such as harmine and flavonoids such as diosmin protect testicular structure by regulating pro- and anti-apoptotic protein expressions[27,33]. Additionally, the decreased PCNA expression in the diabetes group increased again with Gilaburu treatment. This increased proliferative

activity suggests a potential role in tissue regeneration and repair, similar to the effects observed in icariin and carvacrol treatments[34,35].

Sirtuins, particularly Sirt6, are important epigenetic regulators involved in DNA repair and metabolic homeostasis[36]. Sirt6 is known to provide protection against oxidative stress-induced damage by activating the Nrf2 pathway and to support cellular longevity[37]. The fact that the increase in Sirt6 expression was observed alongside the rise in PCNA levels suggests a synergistic mechanism where Sirt6 supports DNA integrity and facilitates the proliferative response necessary for spermatogenic recovery. This regulation, occurring independently of glycemic control, demonstrated the role of Gilaburu in strengthening cellular defense mechanisms.

Furthermore, the significant increase observed in testosterone levels following Gilaburu administration indicates the preservation of Leydig cell function. In diabetic conditions, oxidative stress leads to erectile dysfunction[38,39]. It is thought that the antioxidant-mediated reduction in testicular MDA levels contributes to the preservation of the microenvironment required for steroidogenesis. These results are also supported by the study showing that Gilaburu fruit extract protects germ cell integrity and abnormal sperm ratios against chemotherapy-induced toxicity through free radical scavenging mechanisms [40].

Our study has several limitations. First, the findings obtained with the streptozotocin-induced rat model may not fully reflect human diabetic pathophysiology. Second, since the evaluation was limited to a single dose and duration, dose-response relationships and the long-term safety profile were not investigated. Finally, although key immunohistochemical markers were examined, comprehensive sperm function tests and a broader hormone profile were not evaluated. Future studies should focus on clinical translational models and pharmacokinetic optimization.

Conclusion

In conclusion, *Viburnum opulus* L. extract demonstrates a potent protective effect against diabetes-induced testicular damage by regulating the oxidative stress–apoptosis–proliferation axis. These restorative effects can be attributed to its rich phenolic content and its ability to enhance Sirt6 expression and antioxidant enzyme activities (SOD, GPx1) while suppressing lipid peroxidation (MDA). Importantly, these effects of Gilaburu occur independently of blood glucose levels. This suggests that Gilaburu holds potential as a supportive phytotherapeutic agent in the management of diabetes-related male infertility.

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Competing interests

The authors declare that they have no competing interests.

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