Phytomedicine Plus 5 (2025) 100808

Contents lists available at ScienceDirect

Phytomedicine Plus

journal homepage: www.sciencedirect.com/journal/phytomedicine-plus

Protective effects of flavonoid-rich extracts from *Hibiscus sabdariffa* leaves on streptozotocin-induced testicular damage in rats

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ARTICLE INFO

Keywords:
Hibiscus sabdariffa
Dehydrogenase
Biochemical parameters
Testis

ABSTRACT

Objective: This study investigated the protective effects of flavonoid-rich extracts from *Hibiscus sabdariffa* leaves on testicular cumulative reproductive function biomarkers in streptozotocin (STZ)-induced diabetic rats.
Methods: Rats were induced with 40 mg/kg body weight (bwt) STZ intraperitoneally (i.p.), after which they were administered either a low (150 mg/kg bwt) or a high (300 mg/kg bwt) dose of flavonoid-rich extracts from *H. sabdariffa* leaves. Testicular redox biomarkers, reproductive hormone biomarkers, and relative gene expression levels of PDE-5 and iNOS were assessed. Additionally, histological data, glucose levels, G6PDH activity, and hydroxysteroid dehydrogenase activities (3β-HSD and 17β-HSD) were analyzed.
Results: H. sabdariffa leaf extract was promising for restoring redox balance, normalizing reproductive hormone levels, modulating gene expression, and regulating various biochemical parameters related to testicular function.
Conclusion: These data suggest a potential therapeutic role of *H. sabdariffa* leaves in mitigating the testicular dysfunction associated with diabetes.

1. Introduction

Diabetes mellitus, a chronic metabolic disorder characterized by sustained hyperglycemia, has emerged as a global health concern with far-reaching implications (Zakir et al., 2023). Among its diverse complications, the impact on reproductive health, particularly male fertility, has garnered increasing attention (Maresch et al., 2018). Diabetes-induced oxidative stress, inflammation, and hormonal imbalances can significantly compromise testicular function, leading to impaired spermatogenesis and reproductive dysfunction (Rato et al., 2019). Additionally, chronic hyperglycemia generates excessive reactive oxygen species (ROS), leading to oxidative stress (He et al., 2021). This damage damages testicular cells, including Leydig cells (responsible for testosterone production) and Sertoli cells (which support spermatogenesis). Oxidative stress also disrupts mitochondrial function in spermatogonia, causing increased apoptosis and impaired spermatogenesis

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https://doi.org/10.1016/j.phyplu.2025.100808

Available online 30 April 2025

(He et al., 2021).

Numerous strategies have been explored to mitigate the adverse effects of diabetes on the male reproductive system, and one such avenue under investigation is the utilization of natural compounds with potential antioxidant and anti-inflammatory properties (Adewoyin et al., 2017; Aziz, et al., 2018). *Hibiscus sabdariffa*, a plant widely recognized for its high flavonoid content, has various pharmacological activities, including antioxidant (Ajiboye et al., 2016, 2024), anti-inflammatory (Garbi et al., 2017) and antidiabetic effects (Hamadjida et al., 2023). While previous studies have explored the potential benefits of *H. sabdariffa* in the context of diabetes, the specific impact on male reproductive function remains an area of interest (Kasim et al., 2021; Orororo et al., 2022).

The present study aimed to elucidate the effects of flavonoid-rich extracts obtained from *H. sabdariffa* leaves on testicular biomarkers in streptozotocin-induced diabetic rats. Streptozotocin, a diabetogenic agent, induces insulin deficiency, leading to a diabetic state in experimental animals and mimicking certain aspects of human diabetes (Radenković et al., 2016). The chosen biomarkers encompass a comprehensive array of parameters, including redox status, reproductive hormone levels, gene expression, histological architecture, sialic acid, transferrin concentrations, ion concentrations, glucose metabolism, and steroidogenic enzyme activities (Ricardo, 2018; Owumi et al., 2022; Sahoo and Chainy, 2023). The investigation spans multiple dimensions of testicular health, providing a holistic understanding of the potential protective effects of *H. sabdariffa* in the context of diabetes-induced testicular dysfunction.

Phosphodiesterase-5 (PDE-5) and inducible nitric oxide synthase (iNOS) are pivotal players in testicular function and influence various aspects of reproductive physiology. PDE-5, which is responsible for cGMP hydrolysis, is integral in testicular signal transduction (Sofikitis et al., 2021). Within the testes, cGMP signaling regulates smooth muscle tone and vasodilation in blood vessels, which are crucial for proper erectile function (Samidurai et al., 2023). Inhibiting PDE-5, as with medications such as sildenafil, counteracts dysfunction, enhancing the erectile response. Conversely, iNOS, which catalyzes nitric oxide (NO) production, plays a multifaceted role in regulating processes such as spermatogenesis and blood flow in the testes (Kedia et al., 2020; Dutta, et al., 2021). Dysregulation of iNOS and NO production, which are prevalent in inflammatory conditions, induces oxidative stress, which adversely affects sperm quality. The convoluted interplay between PDE-5 and iNOS involves NO-mediated activation of soluble guanylate cyclase, increased cGMP levels, and subsequent cGMP degradation by PDE-5 (Bisht and Dada, 2017; Krishnan et al., 2018). This balance is pivotal for maintaining normal vascular function and the erectile response. Dysfunction in PDE-5/iNOS interplay is implicated in conditions such as erectile dysfunction, which impacts vascular function and penile erection (Khodamoradi et al., 2023). In individuals with testicular dysfunction associated with diabetes or inflammation, alterations in PDE-5 and iNOS activities may contribute to impaired spermatogenesis; therefore, an in-depth understanding of the mechanisms underlying their functions and dysregulation is vital for developing targeted therapeutic interventions to address male reproductive health issues (Kaya-Sezginer and Gur, 2020).

Given the increasing prevalence of diabetes and its associated complications, identifying novel therapeutic approaches that address the multifaceted nature of the disease is of paramount importance (Pearson, 2019). The exploration of natural compounds, such as those present in *H. sabdariffa*, holds promise for the development of adjunctive therapies that not only target glycemic control but also mitigate the secondary complications affecting male reproductive health (de Arruda et al., 2016; Kasim et al., 2021). This study contributes to the growing knowledge in the field and may pave the way for further research aimed at harnessing the therapeutic potential of plant-derived compounds in the context of diabetes-associated reproductive dysfunction.

2. Materials and methods

2.1. Plant materials

Hibiscus sabdariffa leaves were purchased from Oja-Oba Market, Ado-Ekiti, Ekiti State, Nigeria. with voucher number FHI:113742, obtained from the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria;

2.2. Chemicals and enzyme kits

All the chemicals used in this study were produced by Sigma Aldrich (Germany), and all the reagents used were of analytical grade. Additionally, the enzyme kits used were produced by Randox Laboratory (Crumlin, United Kingdom).

2.3. Flavonoid-rich extract of Hibiscus sabdariffa

The leaves were dried for 14 days at room temperature. The samples were then ground into powder, and a specific gram was defatted in 80 % methanol and filtered. The obtained filtrate was concentrated via a rotary evaporator. Hence, 200 mL of 10 % H_2SO_4 was used to dissolve 20 g of the residue, which was subsequently hydrolyzed at 100 °C for 30 min in a water bath. The mixture was placed on ice for 15 min for the precipitation of the flavonoid aglycones. The flavonoid aglycones were dissolved in 50 mL of 95 % ethanol, filtered into a 100 mL volumetric flask, which was filled with 95 % ethanol and concentrated in a rotary evaporator. The filtrate was subsequently precipitated via concentrated ammonium hydroxide. The solution was allowed to precipitate and rinsed with dilute ammonium hydroxide to obtain the flavonoid extracts (Obafemi et al., 2017; Ajiboye et al., 2022).

2.4. Experimental animals and induction

The Male Wistar rats used in this study were obtained from the Gold Animal House Idofin, Oye-Ekiti, Ekiti State, Nigeria. They were housed in a conventional laboratory at 22 ± 20 °C with a 12 h light/dark cycle. The animals were acclimatized for 7 days.

The rats were fed rat pellets and given 20 % (w/v) fructose and standard feed for one week, except for those in the control group, as previously described by Salau et al. (2021). The rat feeds were removed from their respective cages at exactly 12 h before 40 mg/kg body weight of streptozotocin (STZ) was induced. Thereafter, animals with fasting blood glucose levels greater than 250 mg/dL at 3 days of induction were considered diabetic. All experimental protocols were approved by the FUOYE Faculty of Science Ethics Committee on October 21, 2022, with the ethics number FUOYEFSC 201122-REC2022/008.

2.5. Animal treatment

The animals were grouped into five groups (n = 8) as follows:

Group I: Rats that were not induced (normal control);
Group II: Diabetic rats without treatment (diabetic control);
Group III: Diabetic rats were administered a low dose (150 mg/kg body weight) of *Hibiscus sabdariffa* flavonoid-rich extract leaf (LDHDFL);
Group IV: Diabetic rats were administered a high dose (300 mg/kg body weight) of *Hibiscus sabdariffa* flavonoid-rich extract leaf (HDHDFL); and
Group V included diabetic rats that were administered 200 mg/kg metformin (MET).

The administration was conducted for 21 days between 9 a.m. and 10 a.m. daily. The doses used were selected on the basis of a previous study conducted by Ajiboye et al. (2024).

2.6. Tissue collection and processing

The animals were euthanized with ketamine on the 22nd day of the experiment. Prior to this, they were fasted overnight. Blood samples were immediately withdrawn via cardiac puncture into the plain bottle. This mixture was centrifuged for a period of 5 min at 1,500 rpm to obtain a serum sample, which was stored in a refrigerator. The animal samples were collected, homogenized with 0.1 M potassium phosphate buffer, and centrifuged at 4000 rpm for 15 min. The obtained filtrate was used for various biochemical assays.

2.7. Biomarker parameters studied

The levels of the oxidative stress biomarkers testosterone (T), luteinizing hormone (LH), follicle stimulating hormone (FSH), transferrin, sialic acid, testis electrolyte, glucose, glucose-6-phosphate dehydrogenase, cholesterol, 3β-hydroxysteroid dehydrogenase, and 17β-hydroxysteroid dehydrogenase were determined via commercial kits.

2.7.1. Relative gene expression of PDE-5 and iNOS

Total RNA was isolated from the testes via a Quick-RNA MiniPrep™ Kit (Zymo Research). One (1 μg) of DNA-free RNA was subsequently converted to cDNA via reverse transcriptase with the aid of a cDNA synthesis kit based on ProtoScript II first-strand technology (New England BioLabs). Thereafter, polymerase chain reaction (PCR) for the amplification of the gene of interest was carried out with OneTaqR2X Master Mix (NEB) using the following primers (Inqaba Biotec, Hatfield, South Africa) (Table 1).

2.7.2. Histopathological examination

This was carried out via hematoxylin and eosin (H&E) staining methods. Freshly excised testis tissue was fixed in a 10 % formalin solution for 12 h, after which it was embedded in paraffin wax. The wax blocks were cut on a microtome to yield 4 μm thick slices of paraffin containing the tissues. The sample slices were placed on a microscope slide, air dried, and heated. The residual paraffin was dissolved by rinsing with an acid−alcohol solution followed by rinsing with water to remove the acid−alcohol.

2.8. Data analysis

The results were from 8 replicates with standard deviations (SDs). GraphPad Prism 7 software was used to analyze the results. One-way ANOVA and the Tukey post hoc test were used to determine differences, and differences were considered significant at $p < 0.05$.

3. Results

3.1. Effect of H. sabdariffa flavonoid-rich extract on testis redox biomarker levels/activities in STZ-induced diabetic rats

In the testis, the effects of flavonoid-rich extracts from *H. sabdariffa* leaves on redox biomarkers in streptozotocin-induced diabetic rats were assessed (Fig. 1). There was a significant ($p < 0.05$) increase in the MDA level resulting from lipid peroxidation in untreated diabetic rats compared with normal control rats. The activities of the antioxidant enzymes SOD (superoxide dismutase), CAT (catalase), GPx (glutathione peroxidase), and GST (glutathione-S-transferase), as well as the level of GSH (reduced glutathione), were significantly ($p < 0.05$) lower than normal (Fig. 1). However, these marked abnormalities were significantly ($p < 0.05$) attenuated in the diabetic group treated with 150 mg/kg bwt and 300 mg/kg bwt *H. sabdariffa* leaf flavonoid-rich extracts as well as in the standard group (200 mg/kg metformin) compared with the diabetic untreated group, indicating an improvement in antioxidant defense mechanisms.

Table 1
Primer sequences.

Enzyme	Forward primer	Reverse primer
PDE-5	5'- CAGTCCACCATCCGAGGAGT -3'	5'-CATGCATTGACCATGTCTCTCG-3'
iNOS	5'- CACCACCCTCCTTGTTCAAC-3'	5'-CAATCCACAACTCGCTCCAA -3'
GAPDH	**5'-**GCAAGGATACTGAGAGCAAGAG-**3'**	**5'-**CATCTCCCTCACAATTCCATCC-**3'**

3.2. Effects of H. sabdariffa flavonoid-rich extract on testicular levels of reproductive hormone biomarkers in STZ-induced diabetic rats

The levels of reproductive hormones in rats with STZ-induced testicular damage were assessed, as shown in Fig. 2. There was a significant ($p < 0.05$) decrease in the levels of testosterone (T), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) in diabetic rats compared with those in normal rats. However, these effects were reversed and consequently increased ($p < 0.05$) after treatment with *H. sabdariffa* flavonoid-rich extracts (150 mg/kg bwt and 300 mg/kg bwt), indicating the potential role of the extracts in restoring the hormonal balance under diabetic conditions.

3.3. Effect of H. sabdariffa flavonoid-rich extract on testis relative gene expression in STZ-induced diabetic rats

Fig. 3 shows the relative gene expression levels of phosphodiesterase-5 (PDE-5) and inducible nitric oxide synthase (iNOS) in the testis. There was a significant ($p < 0.05$) increase in the relative expression of these genes in untreated diabetic rats compared with that in normal rats. However, compared with those in the DC group, the expression of PDE-5 and iNOS in both the LDHSFL and HDHSFL groups was significantly ($p < 0.05$) lower, suggesting potential modulation of these genes by *Hibiscus sabdariffa* leaf extracts.

3.4. Effects of H. sabdariffa flavonoid-rich extract on testis histoarchitecture in STZ-induced diabetic rats

As shown in Fig. 4, histological analysis of testis sections stained with hematoxylin and eosin revealed distinct patterns among the groups. In diabetic untreated rats, there was a significant ($p < 0.05$) deleterious change in testicular morphology and physiology, with a special emphasis on areas such as the germinal epithelium, lumen, Leydig cells and myoid cells. Notably, the LDHSFL and HDHSFL groups presented preservation of normal testicular architecture compared with the severe degeneration observed in the DC group. The metformin (MET) group presented a moderate loss of germinal epithelium cells, emphasizing the testicular protective effects of the flavonoid-rich extracts.

3.5. Effects of H. sabdariffa flavonoid-rich extracts on testis sialic acid and transferrin concentrations in STZ-induced diabetic rats

Fig. 5 shows the assessment of sialic acid and transferrin concentrations in the testis as indicators of glycoprotein metabolism. There was a significant ($p < 0.05$) decrease in the levels of sialic acid and transferrin in untreated diabetic rats compared with those in normal control (NC) rats. However, both the LDHSFL and HDHSFL groups demonstrated a noteworthy ($p < 0.05$) increase in these concentrations compared with the DC group, suggesting a potential increase in glycoprotein synthesis.

3.6. Effects of H. sabdariffa flavonoid-rich extracts on specific testis ion concentrations in STZ-induced diabetic rats

The specific ion concentrations in the testis were measured (as shown

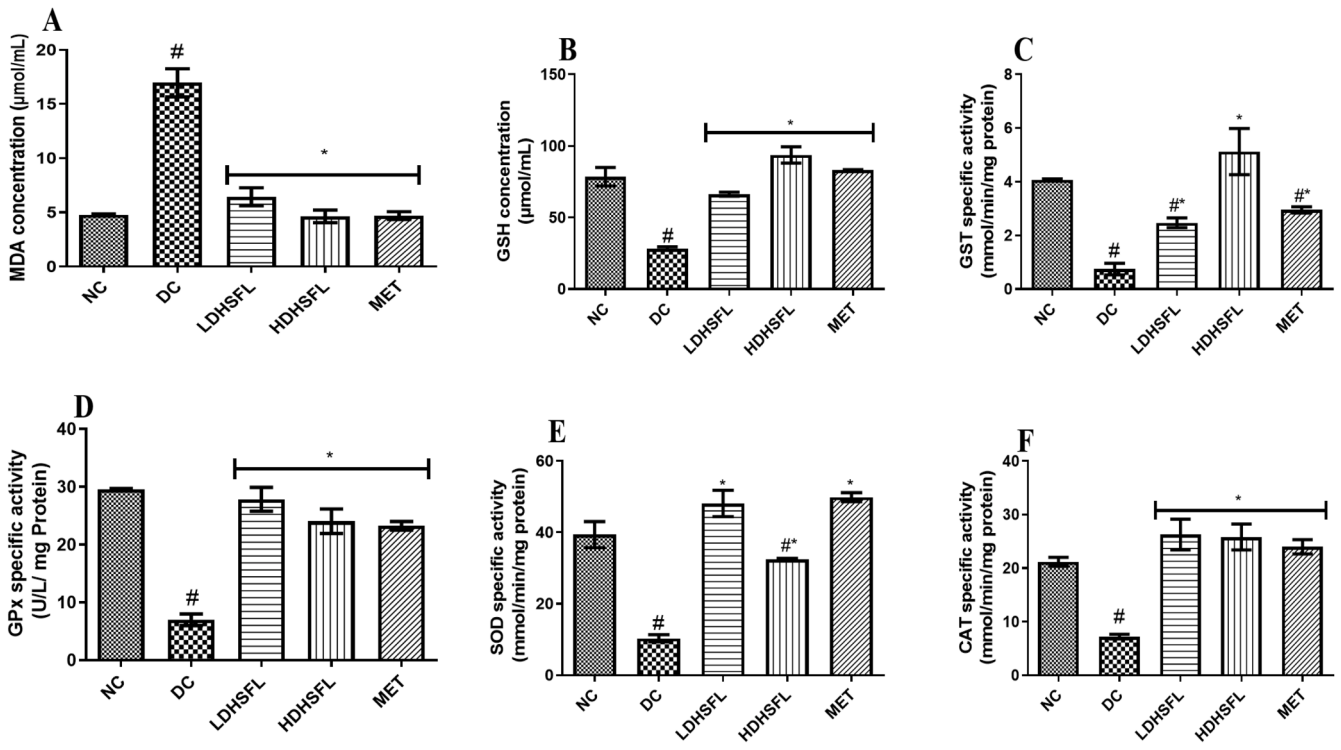

Fig. 1. Testis redox biomarkers of flavonoid-rich extracts from *Hibiscus sabdariffa* leaf in streptozotocin-induced diabetic rats. Each value is a mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. DC. Legend: NC: Normal Control, DC: Diabetic Control, LDHSFL: Diabetic rats administered low dose (150 mg/kg body weight) of flavonoid-rich extract of *Hibiscus sabdariffa,* HDHSFL: Diabetic rats administered high dose (300 mg/kg body weight) of flavonoid-rich extract of *Hibiscus sabdariffa*, MET: Diabetic rats administered 200 mg/kg of metformin, MDA: Malondialdehyde, GSH: Reduced glutathione GST: Glutathione-S-Transferase, CAT: Catalase, GPx: Glutathione Peroxidase, and SOD: Superoxide dismutase.

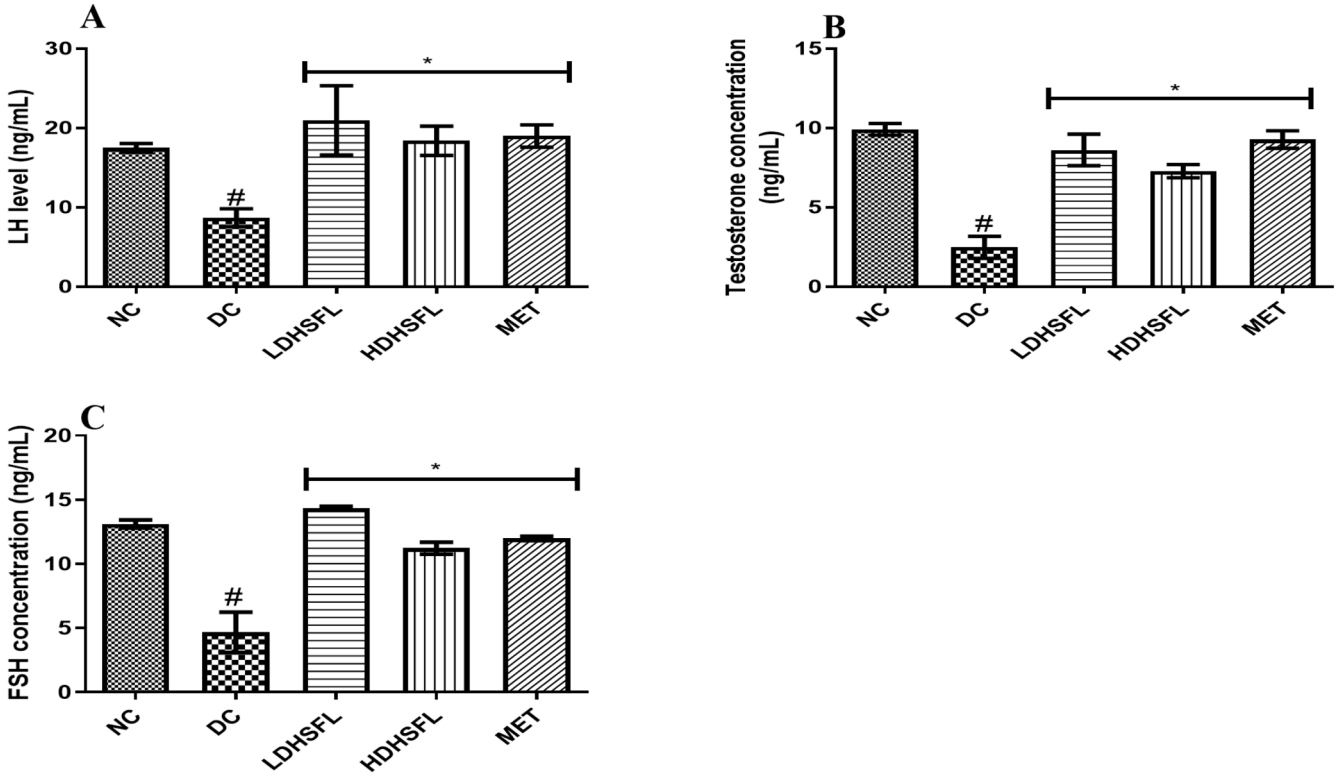

Fig. 2. Reproductive hormone biomarkers of flavonoid-rich extracts from *Hibiscus sabdariffa* leaf in streptozotocin-induced diabetic rats. Each value is a mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. DC. Legend: NC: Normal Control, DC: Diabetic Control, LDHSFL: Diabetic rats administered low dose (150 mg/kg body weight) of flavonoid-rich extract of *Hibiscus sabdariffa,* HDHSFL: Diabetic rats administered high dose (300 mg/kg body weight) of flavonoid-rich extract of *Hibiscus sabdariffa*, MET: Diabetic rats administered 200 mg/kg of metformin.

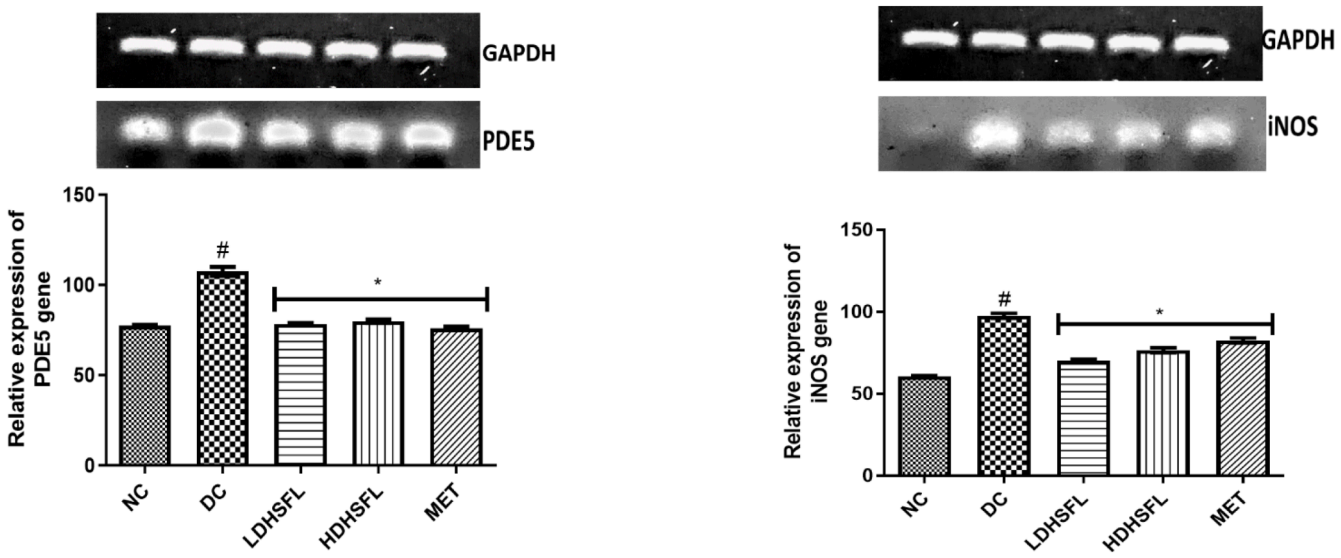

Fig. 3. Testis relative gene expressions of PDE5 and INOS of flavonoid-rich extracts from *Hibiscus sabdariffa* leaf in streptozotocin-induced diabetic rats. Each value is a mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. DC. Legend: NC: Normal Control, DC: Diabetic Control, LDHSFL: Diabetic rats administered low dose (150 mg/kg body weight) of flavonoid-rich extract of *Hibiscus sabdariffa,* HDHSFL: Diabetic rats administered high dose (300 mg/kg body weight) of flavonoid-rich extract of *Hibiscus sabdariffa*, MET: Diabetic rats administered 200 mg/kg of metformin.

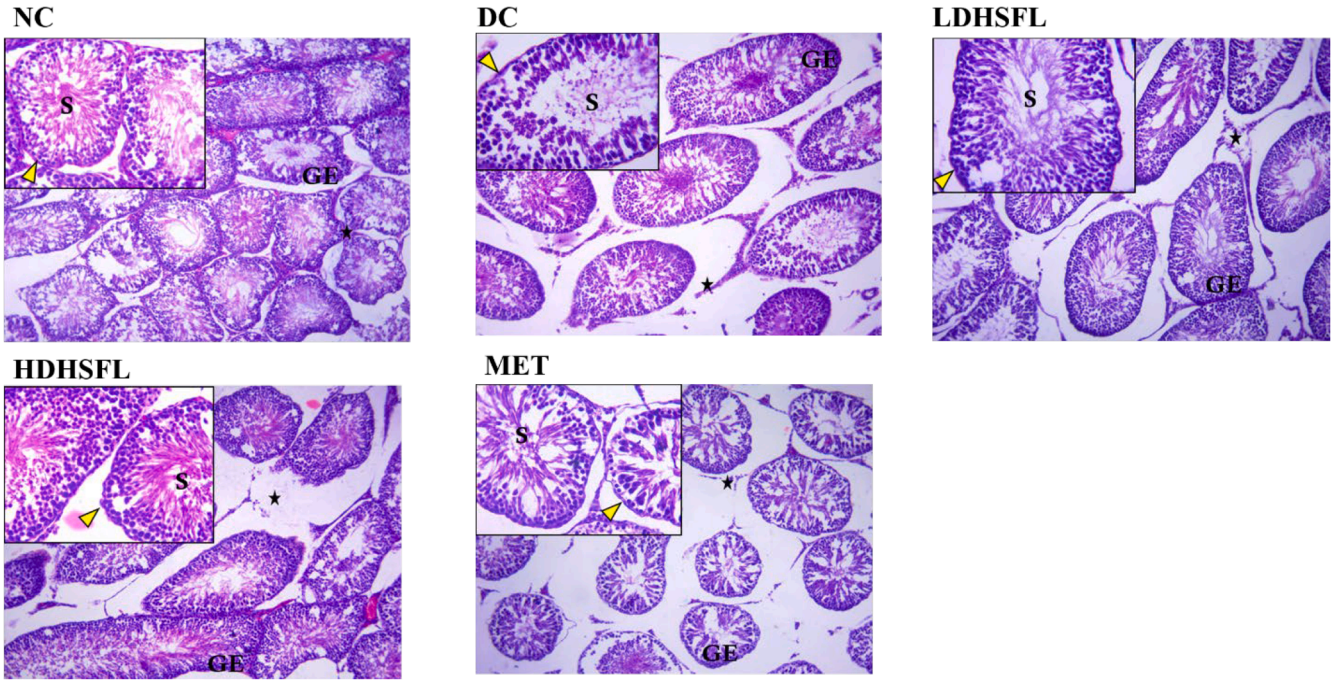

Fig. 4. Testis histoarchitecture examination of flavonoid-rich extracts from *Hibiscus sabdariffa* leaf in streptozotocin-induced diabetic rats. Stained with H&E (Mag. x200: x800). Legend: NC: Normal Control, DC: Diabetic Control, LDHSFL: Diabetic rats administered low dose (150 mg/kg body weight) of flavonoid-rich extract of *Hibiscus sabdariffa,* HDHSFL: Diabetic rats administered high dose (300 mg/kg body weight) of flavonoid-rich extract of *Hibiscus sabdariffa*, MET: Diabetic rats administered 200 mg/kg of metformin, GE: Germinal epithelium, S: Spermatids, Yellow arrowhead: myoid cell, Star: Leydig cell. NC: shows normal germinal epithelium cells, normal sperm cells at the lumen; DC: shows severe degenerated germ cells, clusters of sperm cells at the lumen; LDHSFL: shows normal architecture of the testis, with few sperm cells at the lumen; HDHSFL: shows normal germinal epithelium cells, clustered of sperm cells at the lumen; MET: shows moderate loss of germinal epithelium cells, a few sperm cells at the lumen, and loss of interstitial connective tissue and cells.

in Fig. 6), and both the LDHSFL and HDHSFL groups (compared with the standard group) presented significant ($p < 0.05$) increases in the levels of iron (Fe), copper (Cu), and zinc (Zn) ions compared with those in the DC group. Prior to this, a significant ($p < 0.05$) decrease in the levels of these ions was detected in the diabetic untreated rats compared with those in the normal group. These findings indicate the potential influence of *H. sabdariffa* leaf extracts on ion homeostasis in the testes of diabetic rats.

3.7. Effects of H. sabdariffa flavonoid-rich extract on testis glucose levels and G6PDH activity in STZ-induced diabetic rats

The glucose level and activity of glucose-6-phosphate dehydrogenase (G6PDH) in the testis were assessed as depicted in Fig. 7. Compared with

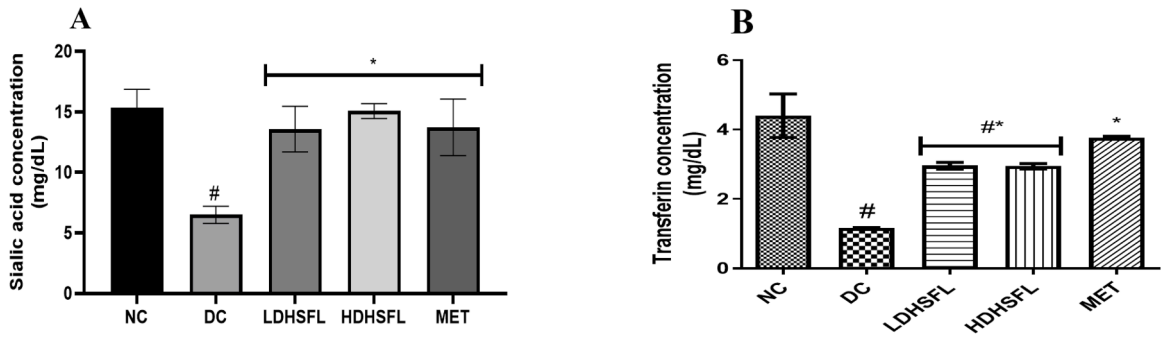

Fig. 5. Testis sialic acid and transferrin concentrations of flavonoid-rich extracts from *Hibiscus sabdariffa* leaf in streptozotocin-induced diabetic rats. Each value is a mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. DC. Legend: NC: Normal Control, DC: Diabetic Control, LDHSFL: Diabetic rats administered low dose (150 mg/kg body weight) of flavonoid-rich extract of *Hibiscus sabdariffa,* HDHSFL: Diabetic rats administered high dose (300 mg/kg body weight) of flavonoid-rich extract of *Hibiscus sabdariffa*, MET: Diabetic rats administered 200 mg/kg of metformin.

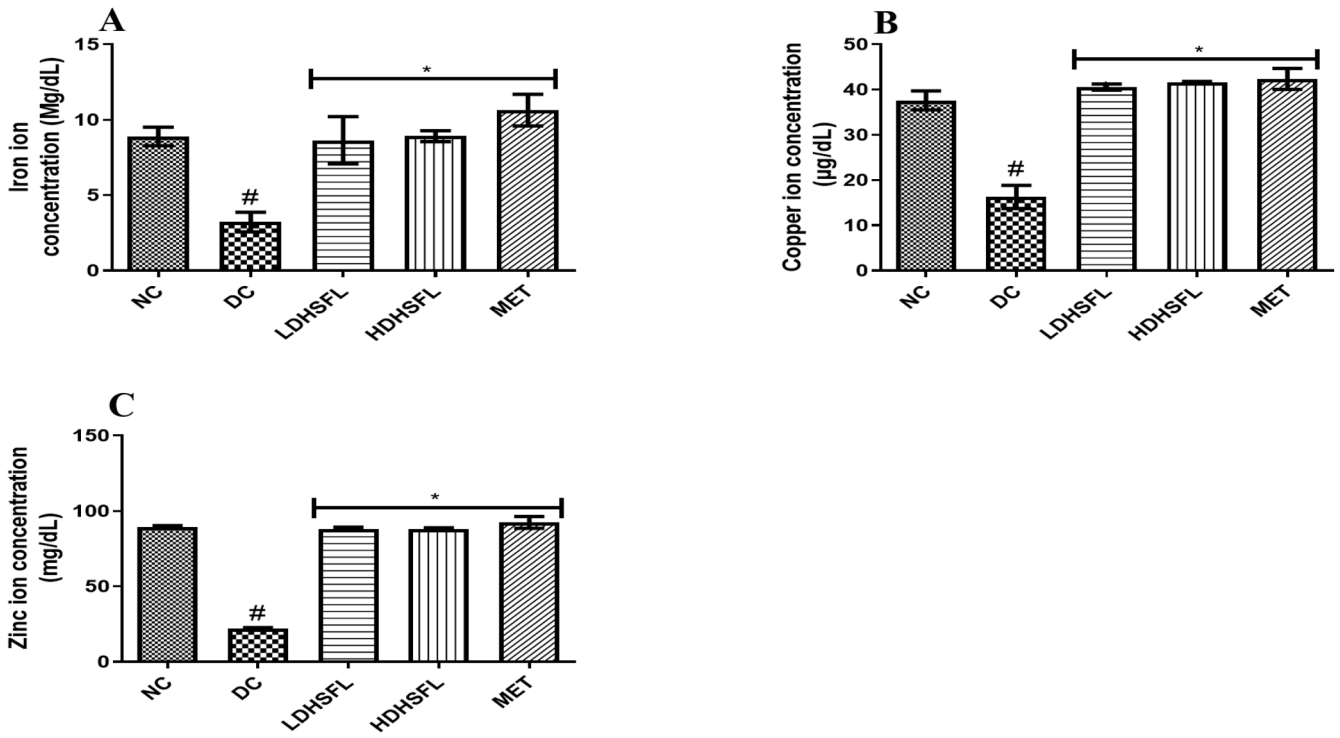

Fig. 6. Some testis ion concentrations of flavonoid-rich extracts from *Hibiscus sabdariffa* leaf in streptozotocin-induced diabetic rats. Each value is a mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. DC. Legend: NC: Normal Control, DC: Diabetic Control, LDHSFL: Diabetic rats administered low dose (150 mg/kg body weight) of flavonoid-rich extract of *Hibiscus sabdariffa,* HDHSFL: Diabetic rats administered high dose (300 mg/kg body weight) of flavonoid-rich extract of *Hibiscus sabdariffa*, MET: Diabetic rats administered 200 mg/kg of metformin.

the DC group, the LDHSFL and HDHSFL groups presented a significant ($p < 0.05$) decrease in glucose levels and a significant ($p < 0.05$) increase in G6PDH activity, suggesting a potential improvement in glucose metabolism in the testis after *H. sabdariffa* administration.

3.8. Effect of H. sabdariffa flavonoid-rich extract on testis cholesterol level and hysteroid dehydrogenase activity in STZ-induced diabetic rats

As shown in Fig. 8, cholesterol levels and the activities of 3β-hydroxysteroid dehydrogenase (3BHSD) and 17β-hydroxysteroid dehydrogenase (17BHSD) in the testis were evaluated. There was a significant ($p < 0.05$) decrease in the activities of 3BHSDH and 17BHSDH as well as a sharp increase ($p < 0.05$) in cholesterol levels in untreated diabetic rats compared with those in normal control rats. Conversely, both the LDHSFL and HDHSFL groups presented significant ($p < 0.05$) differences in cholesterol metabolism and sex hormone biosynthesis compared with the DC group, indicating the potential influence of *H. sabdariffa* leaf extracts on cholesterol metabolism and sex hormone biosynthesis.

4. Discussion

This study revealed the potential therapeutic effects of flavonoid-rich extracts from *Hibiscus sabdariffa* leaves in alleviating the testicular dysfunction induced by streptozotocin in diabetic rats. The multifaceted analysis encompassing redox biomarkers, reproductive hormone levels, gene expression, histoarchitecture, glycoprotein metabolism, ion concentrations, glucose metabolism, and cholesterol-related parameters provides a holistic understanding of the impact of *H. sabdariffa* on the diabetic testicular environment. Testicular tissue toxicity is a major

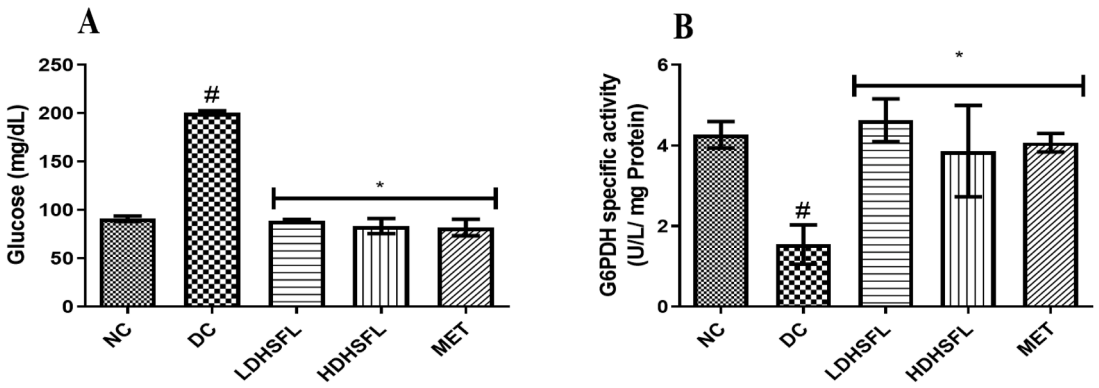

Fig. 7. Testis glucose level and glucose-6-phosphate dehydrogenase activity of flavonoid-rich extracts from *Hibiscus sabdariffa* leaf in streptozotocin-induced diabetic rats. Each value is a mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. DC. Legend: NC: Normal Control, DC: Diabetic Control, LDHSFL: Diabetic rats administered low dose (150 mg/kg body weight) of flavonoid-rich extract of *Hibiscus sabdariffa,* HDHSFL: Diabetic rats administered high dose (300 mg/kg body weight) of flavonoid-rich extract of *Hibiscus sabdariffa*, MET: Diabetic rats administered 200 mg/kg of metformin and G6PDH: glucose-6-phosphate dehydrogenase.

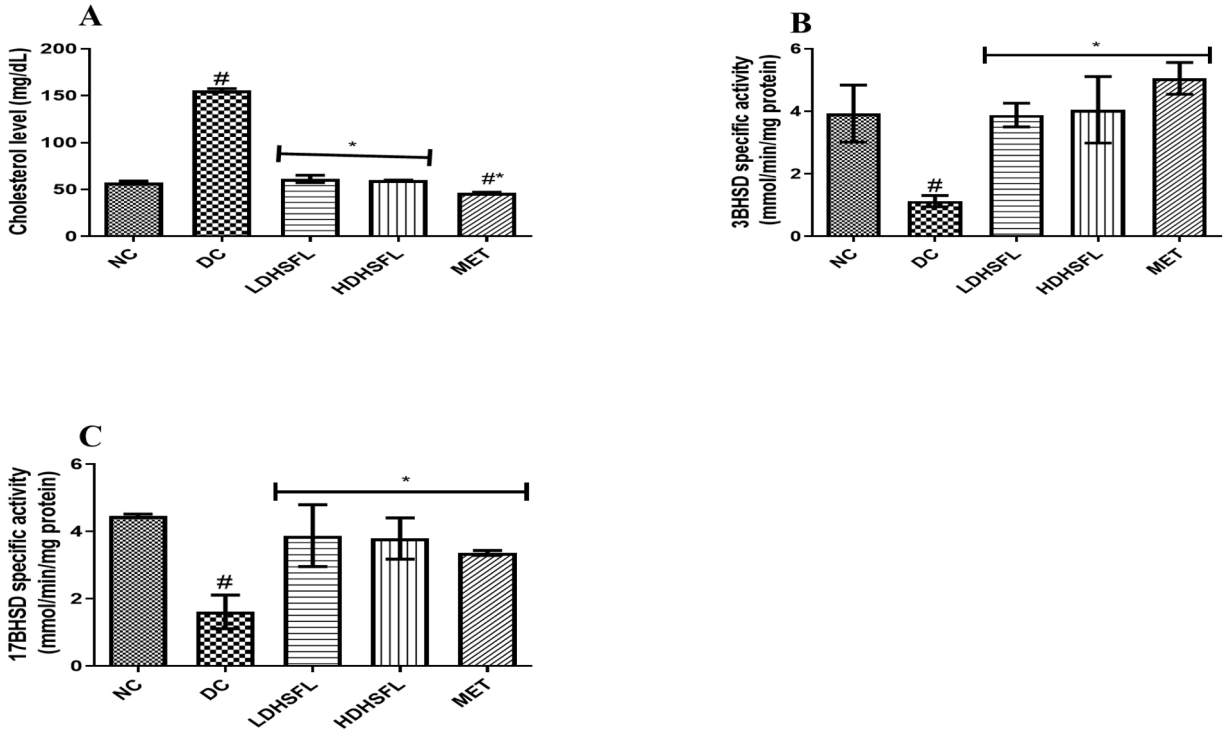

Fig. 8. Testis cholesterol level and hydroxysteroid dehydrogenase activities of flavonoid-rich extracts from *Hibiscus sabdariffa* leaf in streptozotocin-induced diabetic rats. Each value is a mean of eight determinations ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. DC. Legend: NC: Normal Control, DC: Diabetic Control, LDHSFL: Diabetic rats administered low dose (150 mg/kg body weight) of flavonoid-rich extract of *Hibiscus sabdariffa,* HDHSFL: Diabetic rats administered high dose (300 mg/kg body weight) of flavonoid-rich extract of *Hibiscus sabdariffa*, MET: Diabetic rats administered 200 mg/kg of metformin, 3BHSD: 3β-Hydroxysteroid dehydrogenase and 17BHSD: 17β-Hydroxysteroid dehydrogenases.

global health concern, particularly in developing countries, and can lead to male infertility (Adegoke et al., 2020; Sharma et al., 2021). Various factors, including oxidative attacks from conditions such as diabetes, contribute to this toxicity. Diabetes mellitus is associated with increased oxidative stress and apoptotic cell death in organs, including the testis (Asadi et al., 2017; Shoorei et al., 2019). Diabetic rats induced by streptozotocin exhibit elevated oxidative stress, glucose, and cholesterol levels, coupled with reduced sex hormone levels, which is consistent with the findings of previous studies. These effects are likely due to excessive oxidative stress and apoptosis in testicular tissues, contributing to significant testicular dysfunction (Khosravi et al., 2019; Majidi et al., 2021).

This study, in line with previous studies (Famurewa et al., 2019; Banwo et al., 2022), investigated the antioxidant potential of flavonoid-rich extracts from *H. sabdariffa* leaves in mitigating oxidative stress in the testicular organ, as shown in Fig. 1. Testicular vulnerability to free radical attacks, particularly polyunsaturated fatty acid (PUFA) attacks, is associated with male reproductive system dysfunction (Agarwal et al., 2020). The present study revealed a significant ($p < 0.05$) reduction in oxidative stress biomarkers (CAT, SOD, GPx, and GST) accompanied by a significant ($p < 0.05$) increase in malondialdehyde (MDA) levels, indicating the impact of free radical overload. The observed reduction in malondialdehyde (MDA) levels in the testes of rats treated with *H. sabdariffa* leaf extracts suggests potential attenuation of lipid peroxidation, indicating reduced oxidative stress (Mezni et al., 2020). Glutathione (GSH) functions as an effective antioxidant,

contributing to the cellular defense against reactive oxygen species. Antioxidant enzymes, including CAT and SOD, play crucial roles in protecting cells from oxidative stress. This study revealed a dose-dependent increase in the levels/activities of GSH, CAT, SOD, GPx, and GST in diabetic rats treated with *H. sabdariffa* leaf flavonoid-rich extracts. These findings suggest that the extracts have significant anti-free radical effects and play a role in oxidative defense within testicular tissues (Pérez-Torres et al., 2019). These findings collectively underscore the antioxidant properties of *H. sabdariffa*, which are crucial for mitigating the oxidative damage associated with diabetes, in accordance with previous research (Hajihasani et al., 2020).

The restoration of reproductive hormone levels in diabetic rats treated with *H. sabdariffa* leaf extracts (Fig. 2) suggests a potential regulatory effect on the endocrine system (Oyewopo et al., 2020). The improvement in hormonal balance, as evidenced by the increased levels of these biomarkers, underscores the potential of *Hibiscus sabdariffa* in ameliorating the hormonal disruptions associated with diabetes (Kasim et al., 2021). The testes play crucial roles in male reproduction, including spermatogenesis and steroidogenesis, processes tightly regulated by hypothalamic–pituitary gonadotropins such as LH and FSH (Wistuba et al., 2023). Testosterone, a key sex hormone and anabolic steroid, is vital for the development of male reproductive tissues and the expression of secondary sexual traits. Studies have associated testosterone therapy with mitigating insulin resistance and diabetic testicular dysfunction (Al-Darawsha, 2023). Male testicular steroidogenesis is regulated primarily by LH, which stimulates Leydig cells to produce and release androgens and involves factors/enzymes such as 17α-hydroxylase, 3β-HSD, and 17β-HSD. FSH also contributes by influencing Leydig cell function through Sertoli cell stimulation (Zhou et al., 2019; Barbagallo, et al., 2020). The data from this study, as shown in Fig. 2, indicate that, compared with those from diabetic controls, flavonoid-rich extracts from *H. sabdariffa* leaves significantly ($p < 0.05$) increased LH, FSH, and testosterone levels, potentially by increasing gonadotropin secretion from the hypothalamic–pituitary axis, improving the rhythm of androgen biosynthesis, and increasing steroidogenesis biosynthetic enzyme activities. These findings align with previous results (Suleiman et al., 2015; Budin et al., 2018), despite differences in the experimental animals used.

In this study, STZ administration, in accordance with previous reports (Bai and An, 2015; Zhu et al., 2019), led to drastic and substantial ($p < 0.05$) downregulation of phosphodiesterase-5 (PDE-5) and inducible nitric oxide synthase (iNOS) gene expression (Fig. 3) compared with that in the normal group. PDE-5 and iNOS are pivotal players in normal testicular function and are implicated in diabetes-induced testicular damage. PDE-5, which is an enzyme that hydrolyzes cyclic guanosine monophosphate (cGMP), regulates smooth muscle tone and vasodilation in the testes, which are crucial for erectile function. Dysregulation of PDE-5 can contribute to erectile dysfunction, and inhibiting PDE-5 is a common therapeutic approach (Auffenberg et al., 2016; Bennett, 2018). In diabetes, alterations in PDE-5 activity may impact vascular function and contribute to testicular dysfunction. iNOS, an enzyme responsible for nitric oxide (NO) production, plays a role in spermatogenesis and sperm function (Kumar et al., 2021). Diabetes-induced dysregulation of iNOS can lead to oxidative stress, which negatively affects sperm quality. The interplay between PDE-5 and iNOS involves the regulation of vascular tone and NO-cGMP signaling, maintaining the balance essential for normal vascular function and the erectile response. Dysregulation of PDE-5 and iNOS is implicated in conditions such as erectile dysfunction, which affects vascular function and penile erection in individuals with diabetes (Richa et al., 2017; Samidurai et al., 2023). The significant ($p < 0.05$) upregulation of PDE-5 and iNOS gene expression in the testis upon administration of *H. sabdariffa* leaf extracts indicates alleviation and reversal of the deleterious effects caused by STZ administration and thus suggests potential modulation of these genes. The observed restorative trend in PDE-5 and iNOS gene expression aligns with the previously discussed physiological roles of these enzymes in testicular function (Ademosun et al., 2018), emphasizing the potential regulatory effects of *H. sabdariffa* on these crucial pathways.

Histological examination of the testes of diabetic untreated rats, corroborated by the literature (Alasmari et al., 2018; Guimarães-Ervilha et al., 2021), revealed deadly modifications, comprising severely degenerated germ cells, clusters of sperm cells at the lumen, loss of germinal epithelium cells and loss of interstitial connective tissue and cells, as shown in Fig. 4. The preserved testicular histoarchitecture in the groups treated with *H. sabdariffa* leaf extracts, particularly the normal germinal epithelium cells and clustered sperm cells, contrasts strongly with the severe degeneration observed in the diabetic control group (Budin et al., 2018). The testes are central to male reproductive function and house Leydig cells and Sertoli cells, each of which play vital roles in testicular physiology. Leydig cells synthesize testosterone, which is crucial for male organ development, spermatogenesis, and secondary sexual traits. Regulated by luteinizing hormone (LH), Leydig cells maintain hormonal balance, influencing fertility and libido. Sertoli cells support spermatogenesis, creating a microenvironment regulated by follicle-stimulating hormone (FSH) (Santi et al., 2020; Oduwole et al., 2021). They form the blood–testis barrier, shielding developing sperm from the immune system. Dysfunction in Leydig or Sertoli cells can disrupt testosterone production and impair spermatogenesis, impacting male fertility (Sharma et al., 2021). Deciphering these roles highlights their importance in maintaining the intricate balance of testicular function. These histological improvements, as shown in Fig. 4, highlight the protective effects of *H. sabdariffa* against testicular structural damage induced by diabetes (Kasim et al., 2021).

STZ administration was shown to significantly ($p < 0.05$) decrease the levels of sialic acid, transferrin and specific ions (Zn, Cu and Fe) in the testes of untreated diabetic rats, as depicted in Figs. 5 and 6, respectively. Sialic acid, transferrin, and specific ions (Zn, Cu, and Fe) are integral to testicular function and are implicated in dysfunction (Kayode et al., 2020). Sialic acid, which is vital for cell interactions and glycosylation, is present in testicular tissues and impacts reproductive cell structure. Transferrin, a blood-borne iron transporter, regulates iron levels in the testes and is crucial for cellular processes, the imbalance of which can lead to oxidative stress and damage (Raspo et al., 2021). Zinc, copper, and iron, which are essential trace elements, maintain normal testicular function. Dysregulation may cause oxidative stress, which may in turn impact sperm quality (Mirnamniha et al., 2019). The significant ($p < 0.05$) increase in sialic acid and transferrin concentrations in the testes of rats treated with *H. sabdariffa* leaf flavonoid extracts suggests an increase in glycoprotein metabolism (Ajiboye et al., 2022). Additionally, alterations in testis ion concentrations suggest a potential regulatory effect of *H. sabdariffa* on specific ion homeostasis. These findings, in tandem with those of a previous study (Zambonino et al., 2023), suggest the intricate involvement of *H. sabdariffa* in metabolic pathways influencing glycoprotein synthesis and ion regulation within the testicular microenvironment.

Fig. 7 reveals that, upon STZ administration, there was a monumental alteration in glucose metabolism caused by the significant ($p < 0.05$) cumulative sharp increase in the level of testicular glucose as well as a marked ($p < 0.05$) decrease in glucose-6-phosphate dehydrogenase activity, which is not in contrast with the findings of a previous study (Rashid and Sil, 2015). Additionally, as shown in Fig. 8, STZ intoxication resulted in an abnormal level of testicular cholesterol as well as a sudden and significant ($p < 0.05$) decrease in the activities of steroidogenic enzymes such as 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenases. All of the above results did not differ from those of previous studies (Samova et al., 2018; Benko et al., 2022). Testicular glucose and glucose-6-phosphate dehydrogenase (G6PDH) are essential components for maintaining normal testicular function but can lead to dysfunction when dysregulated, particularly in conditions such as diabetes mellitus (Olaniyi et al., 2020). Glucose serves as a primary energy source for testicular cells, ensuring proper metabolic processes in germ cells and Sertoli cells. However, elevated glucose

levels, as observed in individuals with diabetes, can induce oxidative stress and inflammation within the testes, impairing overall function and sperm quality. G6PDH, an enzyme crucial for maintaining the cellular redox balance, plays a protective role against oxidative damage in germ cells (Liu et al., 2017). Dysfunction in G6PDH activity may disrupt redox homeostasis, increasing the susceptibility of testicular cells to oxidative stress and contributing to male infertility (Dutta et al., 2021).

On the other hand, cholesterol serves as a precursor for the synthesis of steroid hormones, including testosterone, which is essential for normal testicular function. Dysregulation of cholesterol metabolism can impact steroidogenesis, leading to hormonal imbalances and testicular dysfunction (Shi et al., 2018). Enzymes such as 3-BHSD and 17-BHSD are involved in the biosynthesis and metabolism of steroid hormones in the testes. Alterations in the activity or expression of these enzymes can disrupt the production of testosterone and other androgens, affecting male reproductive health. Dysfunction of the 3BHSD and 17BHSD enzymes may result in decreased testosterone levels, impaired spermatogenesis, and male infertility (Benko et al., 2022). Therefore, comprehending the intertwined roles of cholesterol and steroidogenic enzymes in testicular function is imperative for identifying potential therapeutic targets and interventions to address testicular damage and dysfunction (Kanimozhi et al., 2018). The observed significant ($p < 0.05$) decrease in testis glucose levels and significant ($p < 0.05$) increase in G6PDH activity in rats treated with *H. sabdariffa* leaf flavonoid extracts suggest a potential improvement in glucose metabolism. Furthermore, significant changes in cholesterol levels and the activities of hydroxysteroid dehydrogenases suggest a regulatory effect on cholesterol metabolism and steroidogenic enzyme activities upon *H. sabdariffa* leaf flavonoid extract administration (Ghosh et al., 2021). These results point toward the broader metabolic influence of *H. sabdariffa* in the diabetic testicular milieu, as supported by Gad et al. (2021).

5. Conclusion

In conclusion, the intricate web of testicular physiology involves a multitude of factors, including cellular components, enzymes, and biochemical pathways, all of which are essential for maintaining normal reproductive function in males. From the pivotal roles of Leydig cells and Sertoli cells in hormone production and spermatogenesis to the importance of molecules such as sialic acid, transferrin, and specific ions in cellular homeostasis, every element contributes to the delicate balance required for optimal testicular function. Moreover, enzymes such as glucose-6-phosphate dehydrogenase and 3β-hydroxysteroid dehydrogenase, along with metabolic substrates such as glucose and cholesterol, play indispensable roles in energy metabolism and steroid hormone synthesis within the testes. However, disruptions in these processes, as observed in conditions such as diabetes, oxidative stress, and inflammation, can lead to testicular dysfunction, impaired spermatogenesis, and male infertility. This comprehensive study highlights the diverse therapeutic effects of flavonoid-rich extracts from *H. sabdariffa* leaves in mitigating the testicular dysfunction associated with diabetes. The observed improvements in redox balance, hormonal regulation, gene expression, histoarchitecture, glycoprotein metabolism, ion homeostasis, glucose metabolism, and cholesterol-related parameters collectively emphasize the potential of *Hibiscus sabdariffa* as a promising natural intervention for ameliorating diabetic complications in the male reproductive system. It is recommended that more mechanistic parameters be carried out to reascertain this claim.

Funding

This manuscript was funded by all the authors.

Institutional review board statement

All experimental protocols were approved by the FUOYE Faculty of Science Ethics Committee (ethics number FUOYEFSC 201122-REC2022/008).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Not applicable.

Data availability

This information is available upon special request from the corresponding author.

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