The Journal of Anatomical Sciences
Email: journalofanatomicalsciences@gmail.com

J. Anat Sci 16(1)

Submitted: June 20th, 2025
Revised: September 10th, 2025
Accepted: September13th, 2025

Berberine attenuates blood glucose, improves sperm functions and reduces oxidative stress in STZ-induced diabetic rats

Oludare G.O.[1]*, Kannike S.O[2], Badejogbin O.C[3], Adesuyi J.O[1], Agboke V.A[1]

[1]Department of Physiology, College of Medicine of the University of Lagos, Lagos; [2]Department of Physiology, College of Medicine, Lagos State University, Lagos; [3]Department of Physiology, School of Basic Medical Sciences, Babcock University, Ilishan-Remo, Ogun, Nigeria.

*C**orresponding Author**: Email: goludare@unilag.edu.ng; Tel**:** +2347035363115
ORCID Number: 0000-0003-3301-3660

ABSTRACT

Managing diabetes and its complications, including infertility, necessitates crucial approaches such as dietary and lifestyle modifications. Research has shown that diabetic men face a heightened risk of reproductive dysfunction compared to their non-diabetic counterparts. This study investigated the effects of berberine on reproductive function in male rats induced to become diabetic with streptozotocin (STZ). Twenty-four Sprague-Dawley male rats were randomly assigned to four groups (n=6 per group): Group 1 (Control), Group 2 (Diabetic) received a single dose of STZ (60 mg/kg, intraperitoneally), Group 3 (Diabetic + Berberine) received STZ and berberine (200 mg/kg, orally), and Group 4 (Berberine only) received berberine (200 mg/kg, orally) daily. Diabetes was confirmed by fasting blood glucose (FBG) >200 mg/dL, and berberine was administered for 4 weeks. FBG was monitored weekly. At the end of the experiment, blood was collected by cardiac puncture for biochemical assays (lipid profile, oxidative stress, testosterone level), the testes were harvested for histological analysis, and the caudal epididymis was assessed for sperm analysis. Results indicated that STZ-induced diabetes significantly impaired sperm count, motility, morphology, and testosterone levels ($p<0.05$), along with disrupted lipid profiles and elevated oxidative stress markers. Histological evaluation revealed testicular vascular congestion in diabetic rats. Berberine supplementation markedly improved sperm parameters, testosterone levels, lipid profiles, and oxidative stress indices in diabetic rats. Although FBG levels in the berberine-treated diabetic group decreased over time, they did not fully normalize. In conclusion, berberine ameliorates reproductive dysfunction, reduces oxidative stress, improves lipid metabolism, and exerts a glucose-lowering effect in diabetic male rats.

Keywords: berberine, infertility, diabetes, antioxidant, testosterone.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic and hormonal condition characterized by the inability of the body to produce sufficient insulin or properly utilize it, resulting in persistently elevated blood glucose levels[1]. Diabetes mellitus has been primarily classified into Type 1 DM, Type 2 DM, and gestational diabetes. Type 1 DM typically occurs due to the autoimmune destruction of the pancreatic beta cells, leading to insulin deficiency. Conversely, Type 2 DM is marked by a gradual decline in insulin production combined with insulin resistance, while gestational diabetes arises during pregnancy in individuals who didn't previously have diabetes[2]. The World Health Organization (WHO) data indicates that diabetes is a rapidly growing disease of global concern, currently affecting about 422 million people worldwide[3]. Diabetes is linked to high rates of morbidity and premature mortality, contributing to approximately 1.5 million deaths annually[3]. Beyond the well-known complications, diabetes is increasingly documented as a contributing factor to male infertility. Data have shown that men with diabetes face an increased risk of infertility compared to non-diabetic individuals [4,5]. Research in human and animal models has demonstrated that diabetes adversely affects male reproductive health by disrupting the hypothalamic–pituitary–gonadal (HPG) axis, impairing testicular function, interfering with spermatogenesis, and leading to erectile and ejaculatory dysfunctions, all of which contribute to male infertility[4,6].

Male infertility associated with diabetes mellitus (DM) can stem from both congenital and acquired factors that disrupt reproductive function at various levels: pre-testicular, testicular, and post-testicular[4]. At the pre-testicular stage, DM is linked to hypogonadism, which may result from central disruptions, such as altered gonadotropin-releasing hormone (GnRH) secretion or elevated leptin levels, particularly in individuals who are overweight or obese, as well as peripheral issues like compromised Leydig cell activity. These factors contribute to decreased levels of gonadotropins and testosterone in circulation[7]. Within the testes, DM exacerbates oxidative stress by increasing reactive oxygen species (ROS) in seminal plasma, which can trigger lipid peroxidation, mitochondrial dysfunction, sperm DNA damage, and the accumulation of advanced glycation end-products (AGEs), ultimately impairing spermatogenesis[8,9]. Post-testicular complications may involve reduced sperm quality and disturbances in semen emission, often due to a higher incidence of accessory gland infections in diabetic men. These infections, when combined with the inflammatory effects of DM, can negatively affect semen parameters and contribute to sexual dysfunctions such as erectile and ejaculatory issues[10].

Clinical infertility is commonly defined as the failure of a coupexple to conceive after one year of regular, unprotected intercourse, with male infertility (MIF) contributing to roughly 30–50% of these cases[11]. In men, infertility can result from various causes, including impaired testicular function, reduced sperm quality, hormonal disturbances, metabolic syndrome, and lifestyle-related factors such as smoking, obesity, or exposure to ionizing radiation[12,13]. Approximately 50% of men diagnosed with infertility show abnormal semen profiles, characterized by low sperm count, decreased motility, and atypical morphology[14]. Despite progress in reproductive medicine, many couples continue to face challenges in achieving pregnancy, highlighting the pressing need for novel therapeutic strategies.

Berberine, a naturally occurring isoquinoline alkaloid derived from Rhizoma coptidis, has been used for centuries in traditional Chinese medicine. More recently, it has garnered attention for its wide-ranging biological and pharmacological effects, including anticancer, antiviral, and antibacterial properties[15,16]. Berberine (BBR) also exhibits hypoglycemic effects that are particularly effective under hyperglycemic conditions[17]. Insulin acts as a secretagogue by modulating KCNH6 potassium channels—dampening their activity through

accelerated closure, which prolongs depolarization and enhances insulin secretion in response to elevated glucose[18]. Additionally, berberine demonstrates insulin-independent glucose-lowering actions by impairing mitochondrial function, promoting glycolysis, and activating AMP-activated protein kinase (AMPK) signalling[19]. These versatile actions imply a potential beneficial role in managing male infertility associated with diabetes. Based on this evidence, the current study explores whether the hypoglycemic properties of berberine can positively influence reproductive outcomes in male rats with diabetes.

MATERIALS AND METHODS

Study design: Ethical approval for this study was obtained from the College of Medicine, University of Lagos, Nigeria, under approval number: CMUL/ACUREC/03/23/1161. A total of twenty-four (24) male Sprague-Dawley rats, weighing between 140 and 160 g, were sourced from the institution's animal care facility. They were housed in clean cages within standard laboratory conditions. The rats were fed standard rat chow (Livestock Feeds) and given water *ad libitum.* Their bedding was regularly changed to prevent infection and promote overall well-being. The animals were acclimatized for two weeks before the commencement of the study. The animals were randomly divided into four groups of six rats each. Group 1 (Control) received 10 ml/kg of distilled water. Group 2 (Diabetes only) is induced with diabetes through a single intraperitoneal injection of streptozotocin (STZ) at a dose of 60 mg/kg body weight. Group 3 (Diabetes + Berberine) comprised of rats treated with both STZ (60 mg/kg body weight) and berberine administered orally at a dose of 200 mg/kg body weight daily for 28 days following diabetes induction. Group 4 (Berberine only) received berberine alone at a dose of 200 mg/kg body weight orally for 28 days.

Chemicals

Streptozotocin and urethane (used as an anesthetic agent) in this study are products of Sigma-Aldrich Chemie GmbH**,** Taufkirchen, Germany. Berberine Hydrochloride from (*Babaris aristata*) bark was obtained from Puritans Pride, New York, United States.

Induction of diabetes

Rats fasted for 12 hours were induced to diabetes by a single intraperitoneal injection of STZ (60 mg/kg body weight). An ice-cold 0.1 mol/L citrate buffer (pH 4.5) was freshly prepared to dissolve STZ and injected within a few minutes of preparation to prevent degradation. After 48 hours of administration, blood glucose levels were measured in the rats, and those with levels exceeding 200 mg/dL were considered diabetic. Blood glucose was measured using an Accu-Chek glucometer and compatible test strips.

Blood and sample collection

Rats were anesthetized with urethane chloralose intraperitoneally (5 ml/kg of body weight). Rats were dissected on a dissecting board and the lower thoracic cavity exposed for the collection of blood samples via cardiac puncture into plain bottles. The liver samples were isolated and stored in phosphate buffer to maintain the cellular activities. The epididymis of each rat was removed, sliced into normal saline, and thoroughly mixed before smearing the sperm suspension on a glass slide. Each slide was well labelled for each group.

Sperm analysis and measurement of testosterone level

Sperm analysis was done using the microscope (Olympus CX 21, Beijing, China) at 400X magnification. Briefly, the hemocytometer was loaded with 10 µl of diluted sperm, allowed to stand for 5 minutes before counting was done. The Eosin and Nigrosin stain was used for sperm morphology as described by Melissa[20]. The motility of the sperm cells was assessed by placing 10 µl of sperm suspension on a slide for microscopic evaluation and expressed in percentage as described by Zemjanis[21]. Lastly,

the concentration of testosterone was assayed in serum samples collected from the rats using enzyme-linked immunosorbent assay (ELISA) kits from Elabscience (Wuhan, China).

Lipid profile analysis

The serum lipid profile, including total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL), was quantified using a fully automated chemistry analyzer (Mindray BS-120, Shenzhen, China). The analysis was performed via spectrophotometric methods employing commercially available reagent kits with the provided protocol by ERBA Diagnostics (Transasia Bio-Medicals Ltd., Mannheim, Germany).

Estimation of malondialdehyde

The testes were excised and immediately rinsed in ice-cold normal saline. The tissues were weighed and homogenized in ice-cold 0.1 M phosphate buffer (pH 7.4) at a ratio of 1:10 (w/v), using a Teflon-glass homogenizer. The resulting homogenate was centrifuged at 10,000 × g for 15 minutes at 4°C. The supernatant was carefully collected and used to estimate oxidative parameters. Malondialdehyde (MDA) was determined in the homogenized liver samples as described by Uchiyama and Mihara[22].

Determination of antioxidant enzymes

The protocol of Sum and Zigman[23] was employed to determine the activity of superoxide dismutase (SOD) in the testicular homogenate. Catalase (CAT) activities were determined by measuring the exponential disappearance of H_2O_2 at 240 nm and expressed in units/mg of protein[24]. Reduced glutathione levels (GSH) were determined by employing the methods of Gunzler[25]. Briefly, 10% TCA was added to the testicular homogenate, and the samples were centrifuged. Ellman's reagent was added to 1.0 ml of the supernatant in 100 ml of 0.1% sodium nitrate and 3.0 ml of phosphate buffer (0.2 M, pH 8.0), and the absorbance was read at 412 nm using PG Instruments T70 UV/VIS (Lutterworth, UK).

Histopathological analysis

The collected testes were fixed in 10% buffered formaldehyde solution for at least 24 h before histopathological study. Samples were then embedded in paraffin wax, and five-micron sections were prepared with a rotary microtome. The thin sections were stained with hematoxylin and eosin (H&E), mounted on glass slides with Canada balsam (Sigma, USA), and observed for pathological changes.

Statistical analysis

The GraphPad Prism Software (7.0) was employed for statistical analysis. The values are expressed as mean ± standard error of mean (SEM). One-way analysis of variance ANOVA followed by Student's Newmann-Keuls post-hoc test was used to determine the level of significant differences among the groups at $P<0.05$.

RESULTS

Berberine administration and fasting blood glucose

Figure 1 illustrates the weekly glucose levels in diabetic rats administered berberine. It was observed that the diabetic untreated group maintained significantly elevated fasting blood glucose levels throughout the study when compared to both the control and berberine-only groups ($p < 0.05$). In the diabetic rats supplemented with berberine, fasting blood glucose levels were reported to decline steadily during weeks 2 and 3, with values stabilizing by week 4. This reduction was found to be statistically significant in comparison to the diabetic untreated group ($p < 0.05$).

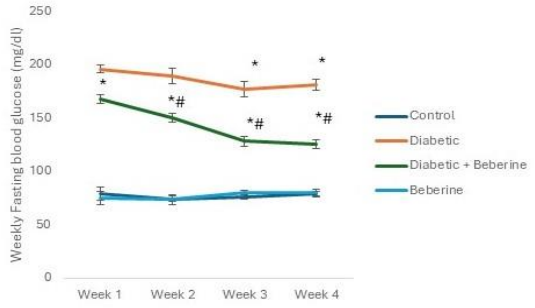

Figure 1: Fasting blood glucose levels of rats administered STZ and berberine
* signifies a significant difference from control, and # signifies a significant difference from the Diabetic group

Berberine Administration and Its Impact on Sperm Parameters and Testosterone Levels

Table 1 presents the impact of berberine on sperm parameters and testosterone levels. The table shows that sperm count, morphology, viability, and motility were significantly decreased in the diabetic group when compared to the control group ($p < 0.05$). However, supplementation of diabetic rats with berberine led to a notable improvement in these sperm parameters relative to the untreated diabetic group. Additionally, testosterone levels were significantly lower in the diabetic rats compared to the control ($p < 0.05$). Interestingly, berberine administration alone resulted in a significant increase in testosterone levels when compared to both the diabetic and the berberine-supplemented diabetic groups.

Table 1: Sperm Parameters and Testosterone Levels Following Berberine Administration in Diabetic and Control Rats

	Control	Diabetic	Diabetic+ Berberine	Berberine
Sperm count (10^6)	78.1 ± 2.45	40.0 ± 3.14 *	67.33 ± 1.45 #	70.33 ± 3.53 #
Sperm Morphology (abnormal)	1.0 ±0.52	16.5 ± 1.10 *	3.0 ± 0.64 #	0.83 ± 0.31 #
Sperm motility (%)	82.16 ± 1.74	41.33 ± 1.83 *	80.33 ± 2.25 #	89.33 ± 1.69 #$
Sperm Viability	83.83 ± 1.21	46.33 ± 1.90 *	85.33 ± 1.45 #	91.83 ± 2.73 *#
Testosterone (nmol/L)	5.17 ± 0.15	3.51 ± 0.12 *	4.51 ± 0.26	6.01 ± 0.39#$

*signifies significant difference from control, # signifies significant difference from diabetes, $ signifies significant difference from diabetes + berberine $p<0.05$. Differences were considered significant when $P<0.05$.

Berberine and Its Influence on Lipid Metabolism

Table 2 presents the influence of berberine on lipid profile. High-density lipoprotein (HDL) levels were significantly reduced, while low-density lipoprotein (LDL) levels were significantly elevated in the diabetic rats compared to the control group. Additionally, the total cholesterol to HDL (TC/HDL) index was markedly increased in the diabetic group relative to the control ($p < 0.05$). However, supplementation of the diabetic rats with berberine was found to significantly lower both LDL levels and the TC/HDL index when compared to the untreated diabetic group ($p < 0.05$).

Table 2: Lipid Profile Parameters in Diabetic Rats Following Berberine Supplementation

	Control	Diabetic	Diabetic+ Berberine	Berberine
Total Cholesterol (mmol/L)	2.48 ± 0.07	2.83 ± 0.08	2.40 ± 0.16 #	2.45 ± 0.08 #
Triglycerides (mmol/L)	1.15 ± 0.03	1.33 ± 0.07	1.06 ± 0.07 #	1.01 ± 0.08 #
HDL (mmol/L)	1.11 ± 0.04	0.83 ± 0.07 *	0.98 ± 0.031	1.00 ± 0.05
LDL (mmol/L	1.05 ± 0.04	1.43 ± 0.03 *	0.93 ± 0.09 #	0.95 ± 0.11 #
TC/HDL	2.25 ± 0.15	3.54 ± 0.35 *	2.46 ± 0.20 #	2.47 ± 0.09 #

*signifies significant difference from control, # signifies significant difference from diabetes p<0.05. Differences were considered significant when P<0.05. HDL- High-Density Lipoprotein, LDL- Low-Density Lipoprotein, TC- Total Cholesterol.

Influence of Berberine on Oxidative Stress Markers

Table 3 presents the impact of berberine on oxidative stress markers. Malondialdehyde (MDA) levels, an indicator of lipid peroxidation, were elevated in diabetic rats, whereas the activities of antioxidant enzymes superoxide dismutase (SOD), catalase, and levels of reduced glutathione were significantly decreased compared to the control group ($p < 0.05$). The study further observed that supplementation of diabetic rats with berberine resulted in a reduction of MDA levels and an increase in SOD, catalase, and reduced glutathione levels relative to the untreated diabetic group ($p < 0.05$).

Table 3: Oxidative Stress Marker Levels Following Berberine Treatment

	Control	Diabetic	Diabetic+ Berberine	Berberine
Malondialdehyde (µmol/ml)	3.18 ± 0.28	6.88 ± 0.41 *	4.63 ± 0.25 #	3.44 ± 0.26 #
Superoxide dismutase (µmol/ml/min/mg pro)	0.98 ± 0.08	0.73 ± 0.03 *	0.92 ± 0.04	0.96 ± 0.06 #
Catalase (µmol/ml/min/mg pro)	5.49 ± 0.25	4.25 ± 0.08 *	5.64 ± 0.19 #	6.20 ± 0.53 #
Reduced glutathione (µmol/ml)	59.39 ± 2.03	48.84 ± 2.32 *	57.63 ± 1.03 #	58.20 ± 2.90 #

*Signifies significant difference from Control, # signifies significant difference from diabetes p<0.05. Differences were considered significant when P<0.05. MDA- Malondialdehyde, SOD- Superoxide dismutase,

Histological Changes in the Testes Following Berberine Treatment in Diabetic Rats

Histological observations of testicular tissue are presented in Figure 2. In the control group (Figure 2A), the testicular sections were reported to show seminiferous tubules lined by a complete spermatogenic series with numerous luminal spermatozoa, and no abnormalities were observed. In contrast, the diabetic group (Figure 2B) exhibited seminiferous tubules lined by spermatogenic cells, but with visible haemorrhages and congested blood vessels, indicating histopathological alterations. The testicular tissue of the diabetic rats supplemented with berberine demonstrated seminiferous tubules lined by spermatogenic series cells and containing numerous luminal spermatozoa, with no apparent abnormalities. Similarly, the testicular sections of the group treated with berberine alone showed normal histological architecture, including intact spermatogenic series and abundant luminal

spermatozoa. All sections were stained with hematoxylin and eosin (H&E) and observed at ×100 magnification.

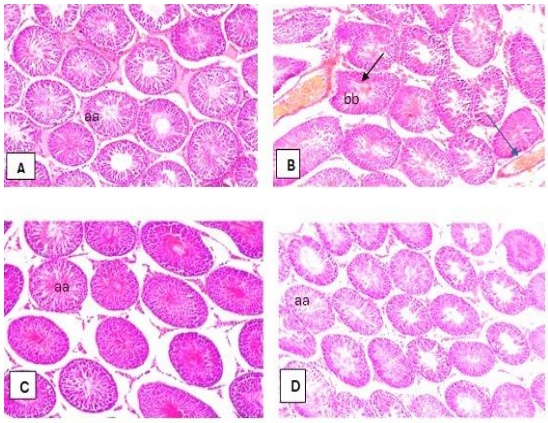

Figure 2: Photomicrograph of the testes. (H&E stain) x100. A = control, shows normal tubule lines by spermatogenic cells aa, B = Diabetic alone, shows seminiferous tubules with visible hemorrhages and congestion bb, C = Diabetic + Berberine, shows normal tubules with some congestion, and D = Berberine alone, shows normal tubules.

DISCUSSION

The growing burden of diabetes has intensified the search for therapeutic agents that can address both glycemic control and its associated complications. Berberine, an isoquinoline alkaloid often marketed as a herbal dietary supplement, has gained popularity for its potential to manage metabolic disorders, such as obesity, type 2 diabetes, and hyperlipidemia, particularly when combined with lifestyle modifications [17,26-29]. Given its glucose-lowering, lipid-modulating, and antioxidant properties, berberine may offer therapeutic benefits beyond glycemic control.

In this study, diabetic rats exhibited significantly elevated fasting blood glucose levels, which were notably reduced following berberine treatment. Interestingly, rats treated with berberine alone showed no significant difference in glucose levels compared to the non-diabetic control group, indicating that berberine does not induce hypoglycemia under normoglycemic conditions. This finding aligns with previous studies suggesting that berberine's glucose-lowering effects are predominantly evident in hyperglycemic states, thereby supporting its safety and selectivity as a therapeutic agent[17,30]. Berberine enhances insulin secretion by acting as an insulinotropic agent—binding to KCNH6 potassium channels, reducing their currents, and prolonging high-glucose-induced membrane depolarization. Its pharmacological profile positions berberine as a safer alternative to conventional oral hypoglycemic agents, which often carry a risk of hypoglycemia.

Meta-analyses have further confirmed the efficacy of berberine in managing type 2 diabetes mellitus, showing improvements in glycemic control and insulin sensitivity[19,31-33]. Beyond its insulinotropic effects, berberine modulates glucose and lipid metabolism through multiple signaling pathways, including the AMP-activated protein kinase, which can activate the p38 Mitogen-activated protein kinase, which contributes to the translocation of glucose transporter 4, which aids glucose uptake[34.] It also exhibits inhibitory effects on key enzymes involved in glucose regulation, such as dipeptidyl peptidase-4 (DPP-4) and protein tyrosine phosphatase 1B (PTP-1B), both of which are implicated in insulin resistance[35]. Although this study did not explore molecular mechanisms in depth, the observed reductions in blood glucose, along with enhanced antioxidant defenses and improved sperm parameters, underscore the potential of berberine as a multi-targeted therapeutic agent for diabetic complications.

This study corroborates previous findings that diabetes mellitus adversely affects male reproductive parameters. Key indicators such as reduced sperm count, decreased motility, and lower testosterone levels were observed in diabetic rats, highlighting the detrimental impact of hyperglycemia on male fertility. Additionally, an increased proportion of morphologically abnormal sperm further contributes to compromised fertility outcomes[6,36]. Berberine, a bioactive compound traditionally used in

Chinese medicine, has been recognized for its antioxidant, antidiabetic, and anti-inflammatory properties, which are particularly relevant in counteracting the reproductive dysfunctions associated with diabetes. The findings of this study demonstrate that berberine supplementation significantly improved sperm quality and elevated testosterone levels in diabetic rats, suggesting its potential as a fertility-enhancing agent in the context of diabetes-induced reproductive impairment. These functional improvements were supported by histological evidence, where berberine restored the normal architecture of seminiferous tubules that had been disrupted by diabetic pathology, aligning with the results of earlier studies[19,34].

Oxidative stress and inflammation are key pathological mechanisms through which DM disrupts spermatogenesis and impairs testicular function, ultimately compromising male reproductive health[35]. The overproduction of ROS in diabetic conditions leads to cellular damage, including lipid peroxidation, DNA fragmentation, and germ cell apoptosis, all of which interfere with normal sperm development and function[36]. The current study supports these findings, demonstrating that DM induces significant oxidative stress in testicular tissue. Berberine, a naturally occurring isoquinoline alkaloid found in plants of the *Berberidaceae* family, has been widely studied for its potent antioxidant and anti-inflammatory properties. In this study, berberine supplementation led to a notable enhancement in the activity of key antioxidant enzymes, SOD, catalase, and reduced glutathione, accompanied by a reduction in MDA, a biomarker of lipid peroxidation. These effects highlight the potential of berberine in preserving testicular integrity and improving sperm quality under diabetic conditions[37,38]. The therapeutic benefits of berberine have been attributed to its modulation of several critical signaling pathways. It activates AMP-activated protein kinase (AMPK), a central regulator of cellular energy homeostasis, which in turn reduces mitochondrial ROS production and improves oxidative metabolism. Additionally, berberine has been shown to enhance the expression and activity of endogenous antioxidant enzymes, such as SOD, catalase, and glutathione peroxidase, reinforcing cellular defense mechanisms against oxidative injury[39]. Beyond its antioxidant activity, berberine also modulates inflammatory responses by inhibiting key pro-inflammatory signaling cascades. These include nuclear factor kappa B (NF-κB), mitogen-activated protein kinases (MAPKs), and nuclear factor erythroid 2 related factor 2 (Nrf2), pathways known to mediate inflammation and oxidative damage in diabetic complications. Evidence suggests that berberine's capacity to regulate these pathways plays a crucial role in mitigating DM-induced tissue damage, including reproductive organs[39,40].

This study also observed favorable alterations in lipid profiles among the berberine-supplemented diabetic rats, specifically showing increased HDL levels, decreased LDL levels, and a reduced TC/HDL ratio. These changes suggest a potential vascular protective effect of berberine, as it lowers cholesterol-related cardiovascular risk. The lipid-lowering properties of berberine are well-documented and are primarily attributed to its ability to enhance LDL receptor (LDLR) expression in hepatocytes. It does so by stabilizing LDLR mRNA and simultaneously inhibiting the expression of the proprotein convertase subtilisin/kexin type 9 (PCSK9), a protein that promotes LDLR degradation. This suppression occurs via accelerated degradation of hepatocyte nuclear factor 1α (HNF1α), leading to reduced PCSK9 transcription[41-43]. Animal studies and preliminary clinical trials have consistently demonstrated the lipid-lowering potential of berberine. Clinical research involving patients not receiving other lipid-lowering treatments has shown that berberine significantly reduces levels of LDL, triglycerides, and total cholesterol[45]. Additionally, when used in combination with conventional therapies such as statins, berberine appears to enhance the lipid-lowering effect. These results suggest that berberine may serve as an effective standalone or adjunct treatment

for dyslipidaemia[18,27,46]. However, larger and more rigorous randomized clinical trials are necessary to establish its therapeutic efficacy and safety profile.

Conclusion

Berberine supplementation significantly improved oxidative balance and reproductive function in diabetic male rats, with histological analysis confirming its protective effects on testicular structure. These findings suggest berberine can serve as an adjunct therapy for diabetes-related reproductive issues, though further human studies are needed.

Acknowledgement

Authors appreciate Mr. D Ota for the technical assistance rendered during the bench work and the biochemical assay.

Declaration of Conflicting Interest: The authors declared that they had no potential conflicts of interest.

Funding: The authors received no financial support for this project.

Authors' Contributions: OGO, KSO, BOC conceived the idea of the study. OGO, KSO, designed the study. KOS, AJO, and AVA carried out the study. OGO, KSO, and BOC analyzed and interpreted the data. OGO, KSO, and BOC drafted the manuscript. OGO, KSO, BOC, AJO, and AVA revised the manuscript and approved the final manuscript for publication.

REFERENCES

1. Brutsaert FE. Diabetes mellitus and disorder of carbohydrate metabolism. Endocrinology Research, 2022; 90: 12-54.
2. Antar SA, Ashour NA, Sharaky M, Khattab M, Ashour NA, Zaid RT, *et.al.* Diabetes mellitus: Classification, mediators, and complications; A gate to identify potential targets for the development of new effective treatments. Biomedicine and Pharmacotherapy, 2023; 168:115734.
3. Khetarpal SP, 2023, November 14. World Diabetes Day: Access to Diabetes Care [Statement]. World Health Organization Regional Office for South-East Asia. Retrieved September 10, 2025, from the WHO website
4. Lotti F, Maggi M. Effects of diabetes mellitus on sperm quality and fertility outcomes: Clinical evidence. Andrology, 2023; 11(2):399-416.
5. Huang R, Chen J, Guo B, Jiang CF, Sun W. Diabetes-induced male infertility: potential mechanisms and treatment options. Molecular Medicine, 2024; 30: 11.
6. Badejogbin OC, Chijioke-Agu OE, Olubiyi MV, Agunloye MO. Pathogenesis of testicular dysfunction in diabetes: exploring the mechanism and therapeutic interventions. Journal of Assisted Reproduction and Genetics, 2025; 42: 367-379.
7. Condorelli RA, Vicari E, Calogero AE, La Vignera S. Male accessory gland inflammation prevalence in type 2 diabetic patients with symptoms possibly reflecting autonomic neuropathy. Asian Journal of Andrology, 2014; 16(5):761-6.
8. Neto FT, Bach PV, Najari BB, Li PS, Goldstein M. Spermatogenesis in humans and its affecting factors. Seminars in Cell and Developmental Biology. 2016; 59:10-26.
9. Dimitriadis F, Adonakis G, Kaponis A, Mamoulakis C, Takenaka A, Sofikitis N. Pre-testicular, testicular, and post-testicular causes of male infertility. In: Simoni M, Huhtaniemi I, (eds). Endocrinology of the Testis and Male Reproduction 2017, Cham: Springer; p.1-47.
10. Verma S. Obesity and diabetes Diabetes &Metabolic Syndrome Clinical Research and Reviews, 2016; 11:1-6.
11. Eisenberg ML, Esteves SC, Lamb DJ, Hotaling JM, Giwercman A, Hwang K, *et.al*. Male infertility. Nature Reviews Disease Primers, 2023; 9(1):49.
12. Mai Z, Yang D, Wang D, Zhang J, Zhou Q, Han B, *et al.* A narrative review of mitochondrial dysfunction and male infertility. Translational Andrology and Urology, 2024; 13(9):2134–45.

13. Spadella MA, Silva EJR, Chies AB, Almeida LA. Insights into antioxidant strategies to counteract radiation-induced male infertility. Antioxidant and Redox Signal. 2024; 40(13–15):776–801.
14. Rambhatla A, Shah R, Pinggera GM, Mostafa T, Atmoko W, Saleh R, *et.al*. Pharmacological therapies for male infertility. Pharmacol Rev. 2024 Oct 21: PHARMREV-AR-2023-001085.
15. Almatroodi SA, Alsahli MA, Rahmani AH. Berberine: an important emphasis on its anticancer effects through modulation of various cell signaling pathways. Molecules, 2022; 27(18):5889.
16. Wu M, Yang S, Wang S, Cao Y, Zhao R, Li X. Effect of berberine on atherosclerosis and gut microbiota modulation and their correlation in high-fat diet-fed ApoE-/- mice. Frontiers in Pharmacology, 2020; 11: 223.
17. Xie W, Su F, Wang G, Peng Z, Xu Y, Zhang Y. Glucose-lowering effect of berberine on type 2 diabetes: a systematic review and meta-analysis. Frontiers in Pharmacology 2022; 13:1015045.
18. Zhao MM, Lu J, Li S, Wang H, Cao X, *et.al*. Berberine is an insulin secretagogue targeting the KCNH6 potassium channel. Nature Communications, 2021; 12 (1), 5616.
19. Guo J, Chen H, Zhang X, Lou W, Zhang P, Qiu Y, *et.al*. The Effect of berberine on metabolic profiles in type 2 diabetic patients: A systematic review and meta-analysis of randomized controlled trials. Oxidative Medicine and Cellular Longevity, 2021:2074610.
20. Melissa R. Sperm Morphology: in pathophysiology of the reproductive system, glossary of hypertexts for biomedical sciences. 2004 [cited 2025 17-June].
21. Zemjanis R. Collection and evaluation of semen. Diagnostic and therapeutic technique in animal reproduction, 1970; 2:467-523.
22. Uchiyama M, Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Analytical Biochemistry, 1978; 86(1):271-8.
23. Sun M, Zigman S. An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. Analytical Biochemistry, 1978; 90(1):81-9.
24. Aebi H. Catalase in vitro. Methods Enzymol 1984; Elsevier pp.121-126
25. Gunzler WA. Glutathione peroxidase. CRC handbook of methods for oxygen radical research. 1985; 285-90.
26. Graziani V, Suprani T, Di Bartolo P, Marchetti F. Transi DEA study group. From pediatric to adult care: a survey on the transition process in type 1 diabetes mellitus and the diabetes services in Italy. Acta Diabetologica. 2024; 61(8):1069-1076.
27. Kong W, Wei J, Abidi P, Lin M, Inaba S, Li C. Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. Nature Medicine, 2024; 10(12):1344-1351.
28. Koppen LM, Whitaker A, Rosene A, Beckett RD. Efficacy of berberine alone and in combination for the treatment of hyperlipidemia: a systematic review. Evidence-Based Complementary and Alternative Medicine, 2017; 22(4):956-968.
29. Asbaghi O, Ghanbari N, Shekari M. The effect of berberine supplementation on obesity parameters, inflammation, and liver function enzymes: a systematic review and meta-analysis of randomized controlled trials. Clinical Nutrition ESPEN 2020;38: 43-49.
30. Zhao JV, Yeung WF, Chan YH, Vackova D, Leung JYY, Ip DKM, *et.al*. Effect of Berberine on Cardiovascular Disease Risk Factors: A Mechanistic Randomized Controlled Trial. Nutrients, 2021; 13(8):2550.
31. Dong H, Wang N, Zhao L, Lu F. Berberine in the treatment of type 2 diabetes mellitus: A systemic review and meta-

analysis. Evidence Based Complementary and Alternative. Medicine 2012; 591654.
32. Lan J, Zhao Y, Dong F, Yan Z, Zheng W, Fan J, *et.al*. Meta-analysis of the effect and safety of berberine in the treatment of type 2 diabetes mellitus, hyperlipidemia, and hypertension. Journal of Ethnopharmacology, 2015; 161: 69–81.
33. Liang Y, Xu X, Yin M, Zhang Y, Huang L, Chen R. Effects of berberine on blood glucose in patients with type 2 diabetes mellitus: A systematic literature review and a meta-analysis. Endocrine Journal. 2019; 66 (1): 51–63.
34. Chen L, Luo W, Chu H, Wang J, Dai X. cirDLPAG$_4$/HECTD$_1$ mediates ischemia-reperfusion injury in endothelial cells via ER stress. RNA Biology, 2020; 17 (2): 240-253.
35. Gallwitz B. "GLP-1 agonists and dipeptidyl-peptidase IV inhibitors". Handbook of Experimental Pharmacology, 2011; 203:53–74.
36. Song J, Gao X, Tang Z, Li H, Ruan Y, Liu Z, *et.al*. Protective effect of Berberine on reproductive function and spermatogenesis in diabetic rats via inhibition of ROS/JAK2/NFκB pathway. Andrology, 2020; 8(3):793-806.
37. Dutta S, Sengupta P, Slama P, Roychoudhury S. Oxidative stress, testicular inflammatory pathways, and male reproduction. International Journal of Molecular Sciences, 2021; 22(18), 10043.
38. Li, Z, Geng YN, Jiang JD, Kong WJ. Antioxidant and anti-inflammatory activities of berberine in the treatment of diabetes mellitus. Evid Based Complement Alternat Med, 2014: 289264
39. Saleh SR, Attia R, Ghareeb DA. The ameliorating effect of berberine-rich fraction against gossypol-induced testicular inflammation and oxidative stress. Oxidative Medicine and Cellular Longevity, 2018; 3:1056173.
40. Ma X, Chen Z, Wang L, Wang G, Wang Z, Dong X, *et.al*. The Pathogenesis of Diabetes Mellitus by Oxidative Stress and Inflammation: Its Inhibition by Berberine. Frontiers in Pharmacology 2018. 27:9:782.
41. García-Muñoz AM, Victoria-Montesinos D, Ballester P, Cerdá B, Zafrilla P. A descriptive review of the antioxidant effects and mechanisms of action of berberine and silymarin. Molecules, 2024; 29(19):4576.
42. Abidi P, Zhou Y, Jiang JD, Liu J. Extracellular signal-regulated kinase-dependent stabilisation of hepatic low-density lipoprotein receptor mRNA by herbal medicine berberine. Arteriosclerosis Thrombosis and Vascular Biology, 2005; 25:2170 2176.
43. Li Z, Jiang JD, Kong WJ. Berberine upregulates hepatic low-density lipoprotein receptor through Ras-independent but AMP-activated protein kinase-dependent Raf-1 activation. Biological and Pharmaceutical Bulletin 2014; 37:1766 1775.
44. Dong B, Li H, Singh AB, Cao A, Liu J. Inhibition of PCSK9 transcription by berberine involves down-regulation of hepatic HNF1α protein expression through the ubiquitin-proteasome degradation pathway. J Biol Chem, 2015; 290:4047–4058.
45. Kong WJ, Wei J, Zuo ZY, Wang Y, Song D, You X. A combination of simvastatin with berberine improves the lipid-lowering efficacy. Metabolism, 2008; 57: 1029–1037.
46. Koppen LM, Whitaker A, Rosene A, Beckett RD. Efficacy of berberine alone and in combination for the treatment of hyperlipidemia: a systematic review. Evidenced-Based Complementary Alternative Medicine, 2017; 22(4):956-968.