Dose-Dependent Modulation of Albumin Binding Protein and Seminiferous Tubule Integrity by Quercetin in Streptozotocin-Induced Diabetic Rats

Supported by tetfund
TERTIARY EDUCATION TRUST FUND

Ajayi Stephen Olawale [1],Tongle Nanle John[1]
[1]Department of Anatomy, Federal University Wukari
Corresponding author: olawalestephen49@gmail.com

Received: February 14, 2025, Accepted: April 28, 2025

Abstract: Male infertility, a significant global health challenge, that is often exacerbated by testicular toxicity linked to diabetes, particularly Type 2 diabetes mellitus (T2DM). Streptozotocin (STZ)-induced diabetes in rats serves as a model for studying diabetes-related testicular damage. However, this study investigates the dose-dependent effects of quercetin, a naturally occurring flavonoid, on albumin-binding protein (ABP) levels and seminiferous tubule integrity in STZ-induced diabetic rats. Forty eight (48) Wistar rats were grouped into seven groups with six(6) rats per group and they were fed with high-fat diet (HFD) for 60days with 5days intra peritoneal STZ injection which was later treated with administration of quercetin at doses of 50, 75, and 100 mg/kg for 28days.It was shown that androgen binding protein (ABP) levels were significantly elevated in the HFD and STZ groups compared to the control, HFD+STZ group displayed the highest increase (3.75 ± 0.26 U/mg proteins). Quercetin administration at 50 mg/kg and higher effectively reduced ABP levels, the HFD+STZ+100 mg/kg quercetin group also showed decreased levels comparable to the control (2.32 ± 0.59 U/mg proteins). Testicular cell counts revealed significant preservation of spermatids in the 75 mg/kg quercetin group (130.25 ± 5.58) compared to untreated diabetic groups. Histological analysis also showed improved testicular architecture in quercetin-treated rats, with fewer pathological abnormalities. The findings suggest that quercetin poses a dose-dependent protective effect against STZ-induced testicular toxicity, offering a potential therapeutic agent for improving male reproductive function through gradual improvement in the signaling pathway which improve the testosterone production and testicular histoarchitecture which is the hallmark for improving male fertility in diabetic conditions.

Keywords: Quercetin, Albumin-binding protein, Streptozotocin, Testicular toxicity, Male infertility, Dose-dependent effects.

Introduction

Male infertility is a significant global health challenge, affecting approximately 30 to 50 million men worldwide and contributing to nearly half of all infertility cases (WHO, 2022). While male infertility is often overshadowed by female reproductive issues, recent evidence suggests that it plays a crucial role in childlessness and failed conception attempts (Okonofua *et al*., 2022). The underlying causes of male infertility are multifaceted, encompassing genetic, lifestyle, and environmental factors. However, a growing body of literature has identified testicular toxicity as a major contributor to impaired spermatogenesis and male reproductive dysfunction (Rodsprasert *et al*., 2022).

Testicular toxicity, also known as testicular failure or hypogonadism, which is characterized by the inability of the testes to produce viable sperm and sufficient testosterone levels (Mendiola *et al*., 2019). A significant body of evidence links testicular toxicity to environmental toxins, oxidative stress, and metabolic disorders, particularly Type 2 diabetes mellitus (T2DM) (Dierickx *et al*., 2021). T2DM is a chronic metabolic disorder characterized by insulin resistance, hyperglycemia, and systemic inflammation. Epidemiological studies have established a strong correlation between diabetes and male infertility, demonstrating that diabetic men exhibit reduced sperm count, motility, and increased DNA fragmentation (Maresch *et al*., 2017; Omolaoye *et al*., 2020; Zheng *et al*., 2023). Additionally, diabetic testicular dysfunction is associated with increased oxidative stress, chronic inflammation, disruption of the blood–testis barrier, and altered hormonal signaling, all of which compromise reproductive health (Onung *et al*., 2023; Nna *et al*., 2017).

Streptozotocin (STZ) is a widely used chemical agent for inducing diabetes in experimental models, mimicking the pathophysiological effects of T2DM, including testicular toxicity (Pinti *et al*., 2019). STZ, a nitrosourea derivative, selectively destroys pancreatic beta cells, leading to insulin deficiency and hyperglycemia (Ajayi *et al*., 2021). Beyond its diabetogenic effects, STZ is a potent alkylating agent that induces DNA damage, oxidative stress, and apoptosis in multiple organs, including the testes (Zhang *et al*., 2020; Srinivasan *et al*., 2018). Animal studies have demonstrated that STZ-induced diabetes results in tubular atrophy, germ cell apoptosis, and disruption of Sertoli and Leydig cell functions, ultimately impairing spermatogenesis (Shokri *et al*., 2023). Given the widespread prevalence of diabetes and its detrimental effects on male fertility, identifying effective therapeutic interventions to mitigate STZ-induced testicular damage is crucial.

Quercetin, a naturally occurring flavonoid abundant in plants such as *Ocimum basilicum* (basil), has emerged as a promising candidate for protecting against diabetes-induced testicular toxicity (Nam *et al*., 2016). Known for its potent antioxidant, anti-inflammatory, and anti-diabetic properties, quercetin has been extensively studied for its ability to scavenge reactive oxygen species (ROS), chelate metal ions, and inhibit lipid peroxidation (Batiha *et al*., 2020). Mechanistically, quercetin exerts its protective effects by upregulating endogenous antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GPx) Zhang *et al*., 2020 by inhibiting nuclear factor kappa

B (NF-κB) signaling, a key mediator of inflammation in diabetic conditions (Murakami *et al.*, 2008; Li *et al*., 2016). Recent studies have demonstrated that quercetin supplementation restores spermatogenic activity, enhances sperm parameters, and mitigates testicular apoptosis in diabetic models (Dhanabalan *et al*., 2018; Adedara *et al*., 2019).

Despite the growing body of research on quercetin's therapeutic potential, the precise mechanisms underlying its dose-dependent effects on albumin-binding protein expression and seminiferous tubule integrity remain poorly understood. Albumin-binding proteins play a critical role in modulating hormone transport, bioavailability, and testicular function, yet their alterations in diabetic testicular pathology have not been extensively explored (Yuan *et al*., 2016). Furthermore, while quercetin has demonstrated protective effects in various diabetic complications, its impact on the structural integrity of seminiferous tubules, a key determinant of spermatogenesis, remains to be fully elucidated.

This study, therefore, aims to investigate the dose-dependent effects of quercetin on albumin-binding protein regulation and seminiferous tubule morphology in STZ-induced diabetic rats. By elucidating these mechanisms, the findings could provide valuable insights into the potential therapeutic role of quercetin in mitigating diabetes-associated male infertility and testicular dysfunction.

Material and Methods

Animal Source and Handling

A total of fifty-four (54) adult male Wistar rats, weighing between 150 and 200 g, were procured from the Animal House, College of Health Sciences, Benue State University, Makurdi, Nigeria. The animals were housed in polyacrylic cages under controlled laboratory conditions, with a temperature of 28 ± 1°C, a 12-hour light/dark cycle, and humidity maintained at 45-50%. Prior to the commencement of the experiment, the rats were allowed to acclimatize for two weeks.

Chemicals and Reagents

The following chemicals and reagents were used in the study: Streptozotocin (Sigma-Aldrich, USA), fructose (Kem Light Laboratories, India), Simas Margarine (PT Salim Ivomas, Indonesia), and normal diet feed (Grand Cereals Ltd, Nigeria). Additional reagents included a Mission Cholesterol Meter (ACON Lab., USA), On-call Plus glucometer, liver enzyme assay kits (Biovision, USA), rat insulin and C-peptide ELISA kits (Mercodia AB, Sweden; WKEA Med Supplies, China), and a nuclear extract kit (Active Motif, USA). All reagents were of analytical grade.

Collection and Preparation of Ocimum basilicum Leaves

Fresh *Ocimum basilicum* leaves were obtained from Wurkum Market, Makurdi, Nigeria. The plant material was authenticated by botanists at the Herbarium Unit, Department of Biological Sciences, Benue State University. The leaves were air-dried, pulverized, and stored in airtight containers for subsequent use.

Extract Preparation

The air-dried leaves were sorted, washed, and further air-dried for seven days before being ground into a fine powder. A 100 g portion of the powder was sequentially extracted with 900 mL of hexane, ethyl acetate, and ethanol for 48 hours each. The resultant filtrates were concentrated under reduced pressure at 40°C using a rotary evaporator. The final extract was reconstituted in distilled water to prepare doses of 100 and 200 mg/kg body weight..

Isolation and Identification of Quercetin

Quercetin was isolated by extracting the plant material with ethanol. The extract was then centrifuged at 15,000 rpm for 3 minutes. The resulting supernatant underwent purification through protein precipitation followed by solid-phase extraction using a Sep-Pak C18 cartridge (Waters, USA). The cartridge was pre-conditioned with methanol and distilled water before being used to elute the analytes with 60% methanol.

Induction of Diabetes and Treatment

Diabetes was induced in overnight-fasted rats by intraperitoneal injection of freshly prepared streptozotocin (30 mg/kg in 100 mM citrate buffer, pH 4.5) for five consecutive days. Fasting blood glucose levels were measured 10 days after the last streptozotocin injection, and rats with blood glucose levels exceeding 250 mg/dL were classified as diabetic. Treatment administration was performed orally for 28 days.

Experimental Design

The animals were weighed and grouped based on their average weight. Those with closed weight ranged were grouped together in the same cage into nine (9) different groups of six (6) animals per groups. Their weight were measured every week for the duration of ten weeks of the experiments using weigh balance of the Department Anatomy, College of Health Science, Benue State University, Makurdi as shown in table below:

Table 1: Animal Grouping and Administration Protocol

S/N	Groups	Substance and Route of Administration	Dosage of Substance Administered	Duration of Administration	No. of Animals
1	Control	Distilled Water	*Ad libitum*	80 days	6
2	HFD	HFD freely	HFD (freely)	60 days	6
3	STZ	STZ (intraperitoneal)	STZ (30 mg/kg)	STZ(5 consecutive days)	6
4	HFD+ STZ	HFD freely and STZ (intraperitoneal)	HFD (freely) and STZ (30 mg/kg)	HFD (60 days) and STZ (5 consecutive days)	6
5	HFD+Quer	HFD freely and plus Quercetin orally	HFD (freely) and Quercetin (50mg)	HFD (60 days) and Quercetin (28 days)	6
6	STZ+Quer	STZ (intraperitoneal) and Quercetin orally.	STZ (30 mg/kg) and Quercetin (50mg)	STZ (5 consecutive days) and quercetin (28 days)	6
7	HFD+ STZ+Quer	HFD freely, STZ (intraperitoneal) and quercetin orally.	HFD (freely), STZ (30 mg/kg) and Quercetin (75mg)	HFD (60 days), STZ (5 consecutive days) and Quercetin (28 days)	6
8	HFD+STZ+Que r	HFD freely, STZ (intraperitoneal) and quercetin orally.	HFD (freely), STZ (30 mg/kg) and Quercetin (100mg)	HFD (60 days), STZ (5 consecutive days) and Quercetin (28 days)	6

STZ= Streptozotocin,HFD= High Fat Diet; Concentration of STZ administered per rat = 30mg/kg mass =30/1000mg /kg= 0.03mg/kg body mass for x (g) of animal:0.03(x)mg of STZ

Animal Sacrifice and Sample Collection

At the end of the experiment, blood samples were collected via cardiac puncture 24 hours after the final exposure, and the blood samples were centrifuged at 2500×g for 10 minutes at 4°C, and stored at −20°C for hormonal assays animals were thereafter sacrificed via cervical dislocation. The abdominal cavity was opened, and the testes were carefully excised, weighed, and fixed in 10% formal saline to prevent enzymatic degradation and facilitate histological processing. The tissues were dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin wax. Serial sections (3–4 μm) were obtained using a rotary microtome, stained with hematoxylin and eosin, and examined under a light microscope. Photomicrographs of the desired sections were captured for further analysis.

Determination of Androgen Binding Protein (ABP)

Testicular ABP levels were determined by first preparing the cytosolic fraction from testicular homogenates. Briefly, the homogenate was subjected to centrifugation at 100,000 × g for 1 hour at 4°C to obtain the cytosolic fraction. The ABP was then adsorbed onto DEAE-Sephacel, and the DEAE-Sephacel resin was washed with Tris-EDTA (TE) buffer. The ABP was eluted from the DEAE-Sephacel using 1.0 M KCl. The KCl extract containing the ABP was subsequently applied to an androgen affinity column consisting of 6-(5α-androstan-17β-ol-17-yl) hexanoic acid, which was linked to Sepharose CL-4B following the method of Mickelson and Petra, as modified by Maresch *et al*. (2017). The affinity column was washed with Tris-Dimethyl sulfoxide (TDMK) buffer (50 mM Tris-HCl, pH 7.5, 10% dimethyl sulfoxide, 2 M KCl). The ABP was then eluted from the column with TDMKD buffer (TDMK containing 60 μg/ml 5α-dihydrotestosterone and 20% dimethyl sulfoxide). The eluate was concentrated and diafiltered using an Amicon stirred cell equipped with a YM-10 membrane. The affinity purified ABP preparations were subjected to photolysis for 60 minutes and processed as previously described (Maresch *et al*., 2017).

Morphometric Analysis

Germ cell quantification at stages VII and VIII of the seminiferous epithelial cycle was performed on hematoxylin and eosin (H&E)-stained slides. The slides were examined under a light microscope (Zeiss, Germany) using a x40 objective lens. Various germ cells, including sustentacular (Sertoli) cells, spermatogonia (Sg), primary spermatocytes (Sc), spermatids, and spermatozoa, present at these stages were counted. To ensure accuracy, five distinct round seminiferous tubules from each rat were examined, and the average number of each cell type was calculated for each animal.

Histological Tissue Processing and Sectioning

Tissues fixed in bouin;s fluid and were processed through graded ethanol, xylene, and paraffin embedding. Serial sections (3 μm) were obtained using a rotary microtome, floated in a water bath at 55°C, mounted on frosted-end slides, and placed on a hot plate for 40 minutes. Sections were then deparaffinized, rehydrated, air-dried, and stored

for staining with hematoxylin and eosin for histological examination.

Statistical Analysis

Data were expressed as MEAN±SD (n = 5) and analyzed using one-way ANOVA (Snedecor & Cochran, 1980). Post-hoc tests were conducted where necessary to determine significant differences between groups.

Ethical Approval

All experimental procedures were approved by the Ethics Committee of the Faculty of Basic Medical Sciences, Benue State University, Makurdi (Protocol No. CREC/THS/011), and conducted following institutional guidelines.

Results

Albumin Binding Protein

The levels of Albumin Binding Protein (ABP) across different experimental groups were compared using a one-way ANOVA, as shown in Figure 2. The control group showed a baseline ABP level of 2.35 ± 0.17 U/mg proteins.

Groups treated with High Fat Diet (HFD) (3.22±0.12) and Streptozotocin (STZ) (3.42±0.27) showed significantly elevated ABP levels compared to the control (P<0.05), indicating that both HFD and STZ independently increase ABP levels.

The HFD+STZ group (3.75±0.26) showed a further increase in ABP levels, which was significantly higher than both the HFD-only and STZ-only groups (P<0.05). This suggests a synergistic effect of HFD and STZ on ABP elevation.

Quercetin administration at varying doses (50 mg/kg, 75 mg/kg, and 100 mg/kg) effectively mitigated the elevated ABP levels in groups treated with HFD, STZ, or their combination. Notably:

HFD+50 mg/kg Quercetin (2.62±0.45), STZ+50 mg/kg Quercetin (2.65±0.31), and HFD+STZ+75 mg/kg Quercetin (2.45±0.28) exhibited significant reductions in ABP compared to their respective non-Quercetin-treated counterparts (P<0.05). The HFD+STZ+100 mg/kg Quercetin group showed ABP levels (2.32±0.59) comparable to the control group.

These results suggest the deleterious effects of HFD and STZ on ABP levels, with a compounded impact when combined. Quercetin effectively counteracts these effects, with a dose-dependent trend, demonstrating its potential as a protective agent against ABP alterations induced by HFD and STZ.

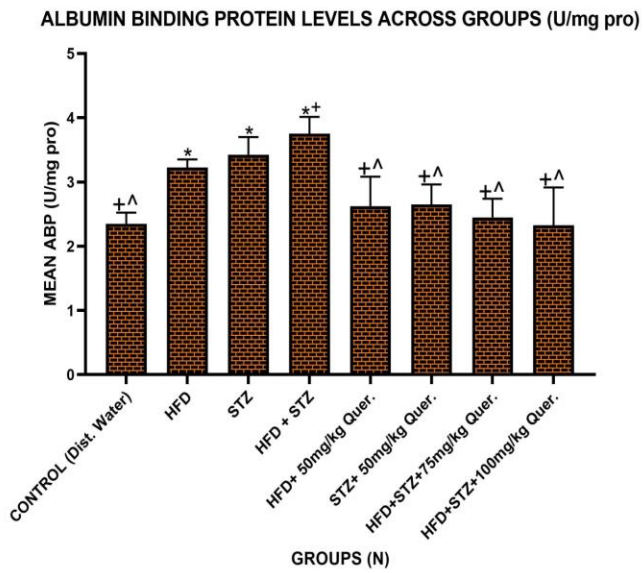


Figure 2: Simple Bar Chart Showing the Mean ABP Levels across Groups

*P<0.05 compared to the control group; ^{+}P<0.05 compared to the HFD – only group; ^P<0.05 compared to the STZ – only group.

Testicular Cells

The result presented in Figures 2 - 5 shows the mean testicular cell counts across different experimental groups. The results highlighted significant differences in specific cell types (spermatogonia, sustentacular cells, primary spermatocytes, and spermatids) when compared to the control and various experimental conditions on one – way ANOVA.

In the control group, spermatogonia (SPG) were observed at 37.65±0.77, with sustentacular cells at 12.50±0.70 and primary spermatocytes at 36.70±0.70. The high-fat diet (HFD) group showed a slight reduction in SPG (37.45±1.20) and an increase in sustentacular cells (13.50±0.70), with similar reductions in primary spermatocytes (35.20±4.52) and spermatids (129.40±19.65). Streptozotocin (STZ) exposure led to a significant decrease in SPG (31.20±1.55) and primary spermatocytes (30.40±1.41) compared to the control, with spermatids also significantly reduced (90.20±10.04).

The HFD + STZ group showed further declines in SPG (32.80±2.12), primary spermatocytes (27.40±1.41), and spermatids (85.90±0.70). Administration of quercetin at 50mg/kg in the HFD + STZ group did not significantly alter testicular cell counts. However, at 75mg/kg, quercetin administration led to a significant increase in spermatids (130.25±5.58) compared to the HFD + STZ group, although other cell types did not show significant differences.

These results suggest that, quercetin at 75mg/kg demonstrated a potential protective effect against the damage induced by HFD and STZ, especially in the spermatid population, suggesting its therapeutic potential for improving testicular health under these experimental conditions.

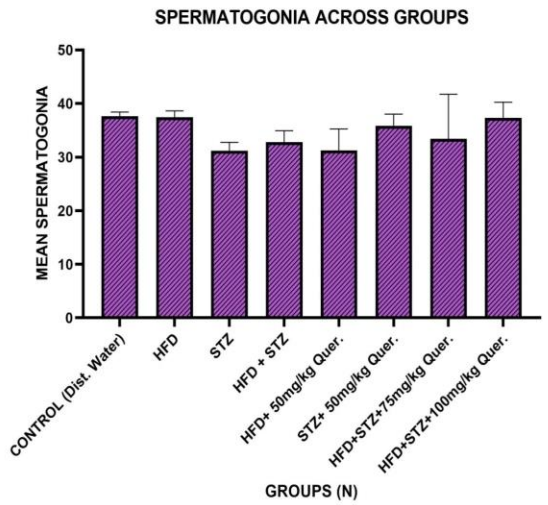


Figure 2: Simple Bar Chart Showing the Mean Spermatogonial Cells across Groups
*P<0.05 compared to the control group; ^{+}P<0.05 compared to the HFD – only group; ^P<0.05 compared to the STZ – only group

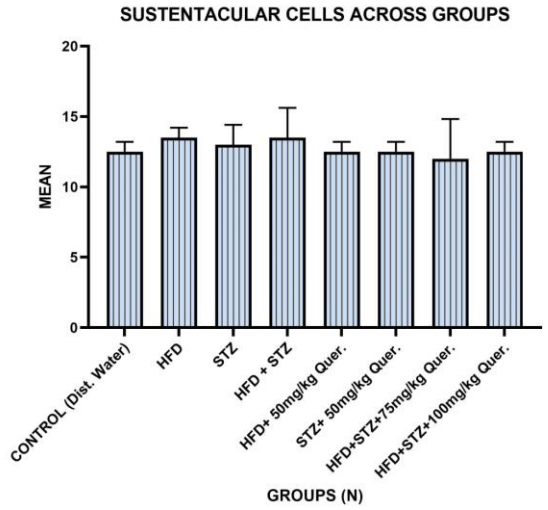


Figure 3: Simple Bar Chart Showing the Mean Sustentacular Cells across Groups
*P<0.05 compared to the control group; ^{+}P<0.05 compared to the HFD – only group; ^P<0.05 compared to the STZ – only group

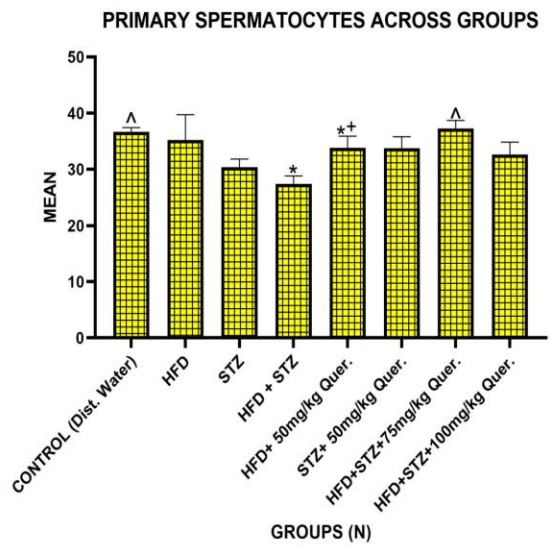


Figure 4: Simple Bar Chart Showing the Mean Primary Spermatocytes across Groups
*P<0.05 compared to the control group; ^{+}P<0.05 compared to the HFD – only group; ^P<0.05 compared to the STZ – only group

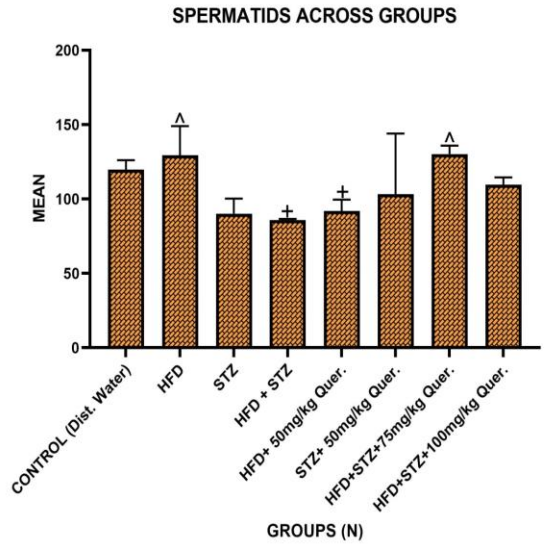
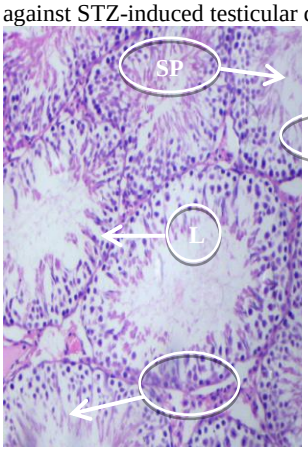


Figure 5: Simple Bar Chart Showing the Mean Spermatids across Groups
*P<0.05 compared to the control group; ^{+}P<0.05 compared to the HFD – only group; ^P<0.05 compared to the STZ – only group

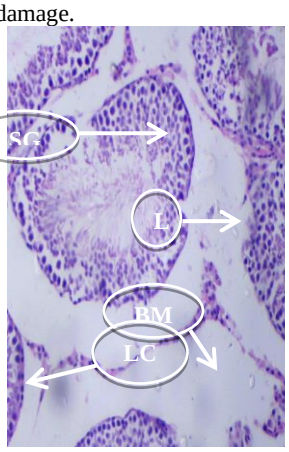
Histological Profile

Histological evaluation revealed normal testicular architecture in the control and quercetin-treated diabetic groups (50, 75, and 100 mg/kg), with well-preserved Leydig cells, Sertoli cells, spermatogonia, spermatozoa, and tubular diameters. However, in the untreated diabetic groups, notable histopathological abnormalities were observed, including disorganized Leydig and Sertoli cells,

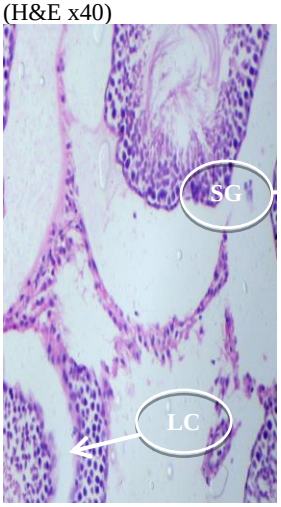
spermatogonia degeneration, and desquamation of epithelial cells. Additionally, the STZ group exhibited atrophic tubules with varying degrees of spermatogenic arrest. Quercetin-treated diabetic rats showed improved testicular histoarchitecture, with fewer tubules containing stagnant spermatogenic cells, suggesting a protective effect against STZ-induced testicular damage.



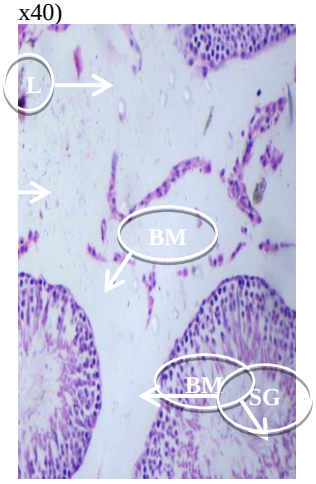
A: Photomicrograph of Testes from Group 1 (Control) Showing Spermatozoa (SP), Spermatogonia (SG), Lumen (L), Leydig Cells (LC), and Basement Membrane (BM) (H&E x40)



B: Photomicrograph of Testes from Group 2Showing Spermatozoa (SP), Spermatogonia (SG), Lumen (L), Leydig Cells (LC), and Basement Membrane (BM) (H&E x40)

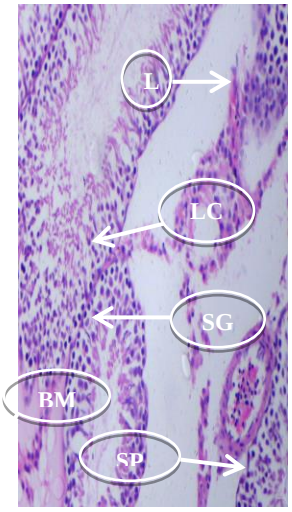


C: Photomicrograph of Testes from Group 3 Showing Spermatogonia (SG), Lumen (L), Leydig Cells (LC), and Basement Membrane (BM) (H&E x40)

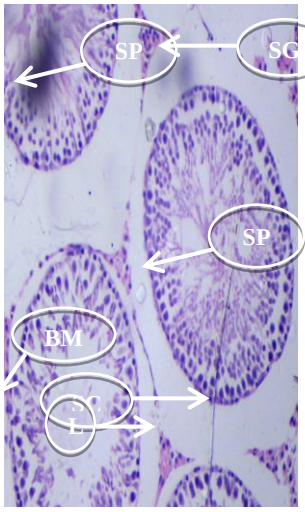


D: Photomicrograph of Testes from Group 4 Showing Spermatogonia (SG), Lumen (L), Sertoli Cells (SC), and Basement Membrane (BM) (H&E x40)

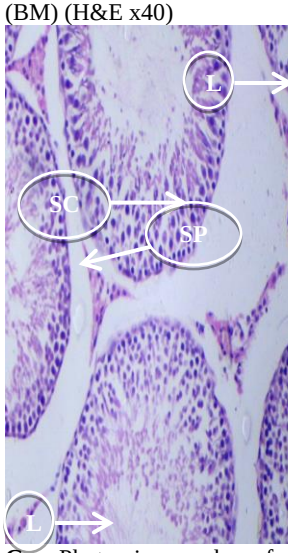
Figure 6 (A – D): Photomicrographs of Testes from Groups 1 - 4 Showing Spermatozoa (SP), Spermatogonia (SG), Lumen (L), Leydig Cells (LC), Sertoli Cells (SC) and Basement Membrane (BM) (H&E x40)



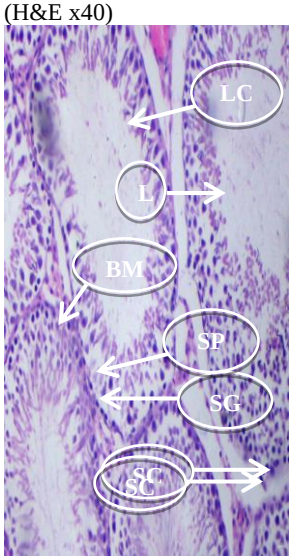
E: Photomicrograph of Testes from Group 5 Showing Spermatozoa (SP), Spermatogonia (SG), Lumen (L), Leydig Cells (LC), Sertoli Cells (SC) and Basement Membrane (BM) (H&E x40)



F: Photomicrograph of Testes from Group 6 Showing Spermatozoa (SP), Spermatogonia (SG), Lumen (L), Leydig Cells (LC), Sertoli Cells (SC) and Basement Membrane (BM) (H&E x40)



G: Photomicrograph of Testes from Group 7 Showing Spermatozoa (SP), Spermatogonia (SG), Lumen (L), Leydig Cells (LC), Sertoli Cells (SC) and Basement Membrane (BM) (H&E x40)



H: Photomicrograph of Testes from Group 8 Showing Spermatozoa (SP), Spermatogonia (SG), Lumen (L), Leydig Cells (LC), Sertoli Cells (SC) and Basement Membrane (BM) (H&E x40)

Figure 7 (E – H): Photomicrographs of Testes from Groups 5 - 8 Showing Spermatozoa (SP), Spermatogonia (SG), Lumen (L), Leydig Cells (LC), Sertoli Cells (SC) and Basement Membrane (BM) (H&E x40)

Discussion

The present study aimed to assess the dose-dependent modulation of albumin binding protein (ABP) levels and seminiferous tubule integrity in Streptozotocin (STZ)-induced diabetic rats, with quercetin serving as a potential protective agent. The results demonstrated that both high-fat diet (HFD) and STZ independently elevated ABP levels, with a compounded effect observed in the HFD+STZ group. Furthermore, quercetin administration significantly mitigated the increase in ABP levels, showing a dose-dependent protective effect. Additionally, quercetin administration resulted in marked improvements in testicular cell counts and histological architecture, particularly at the 75 mg/kg dose, further supporting its therapeutic potential.

The results demonstrated that both HFD and STZ treatment independently elevated ABP levels, which could be indicative of altered metabolic or physiological conditions associated with these interventions. The synergistic effect observed in the HFD+STZ group, where ABP levels were significantly higher than those in the HFD-only and STZ-only groups, suggests that the combined impact of insulin resistance and high-fat diet exacerbates protein binding disturbances. This finding is consistent with previous studies that have shown that insulin resistance, such as that induced by STZ, can lead to alterations in protein metabolism and binding (Zhao *et al*., 2021). Additionally, the significant reduction in ABP levels with quercetin administration, especially at the higher doses (75 mg/kg and 100 mg/kg), supports the notion that quercetin possesses anti-inflammatory and antioxidative properties that counteract the detrimental effects of STZ and HFD on protein binding (Liu *et al*., 2022). These findings align with recent research showing that quercetin has a modulatory role in reducing protein binding abnormalities in various pathological states, including diabetes (Rajendran *et al*., 2020). The dose-dependent trend observed further highlights quercetin's potential as a therapeutic agent in managing metabolic disturbances associated with diabetes.

The study also revealed significant differences in the counts of specific testicular cells, with significant reductions in spermatogonia, primary spermatocytes, and spermatids in the STZ and HFD+STZ groups compared to the control. The HFD group, although showing slight reductions in spermatogonia and primary spermatocytes, did not exhibit as profound damage to testicular cell populations as the STZ group. This observation underscores the potent effect of STZ in inducing diabetic-like conditions that impair testicular function and spermatogenesis, as observed by the significant decrease in spermatid counts and other cell types (García *et al*., 2021). The further decline in testicular cells in the HFD+STZ group suggests that the combination of high-fat diet and diabetes exacerbates testicular damage. These findings are in line with recent studies that have demonstrated that STZ-induced diabetes leads to significant impairment in spermatogenesis, including reduced spermatogonia and primary spermatocytes, as well as spermatid depletion (Sadeghi *et al*., 2022).

Interestingly, quercetin treatment at the 75 mg/kg dose resulted in a significant recovery of spermatids, suggesting a protective effect, which is consistent with previous studies indicating that quercetin can improve spermatogenesis and mitigate testicular damage induced by metabolic disorders (Mansouri *et al*., 2020). While the lower doses of quercetin (50 mg/kg) did not significantly alter the testicular cell populations, the 75 mg/kg dose showed a notable restoration of spermatid counts, further suggesting a dose-dependent response. These results highlight the therapeutic potential of quercetin in improving testicular health, particularly in conditions exacerbated by diabetes and metabolic dysfunction.

Histopathological examination corroborated the findings from cell counts, showing that the control and quercetin-treated diabetic groups exhibited well-preserved testicular architecture, including intact Leydig cells, Sertoli cells, and well-structured seminiferous tubules. In contrast, untreated diabetic groups exhibited severe testicular damage, including atrophic tubules, disorganized Leydig and Sertoli cells, and degeneration of spermatogonia, which is consistent with the degenerative effects of diabetes on male reproductive health (Yin *et al*., 2020). The protective effects of quercetin were particularly evident in the diabetic rats, where quercetin treatment alleviated the testicular damage and improved tubule integrity, as evidenced by the presence of more organized spermatogenic cells and a reduction in stagnant tubules.

These findings align with previous studies on the protective effects of quercetin on reproductive health under diabetic conditions. For instance, in a study by El-Gindy *et al*. (2022), quercetin treatment significantly improved testicular morphology and sperm quality in diabetic rats, similar to our findings of improved histological architecture and reduced spermatogenic arrest. Furthermore, other studies have highlighted quercetin's role as an anti-inflammatory and antioxidant agent that can counteract oxidative stress-induced damage to reproductive tissues (Ali *et al*., 2021). These results collectively support the therapeutic potential of quercetin in mitigating STZ-induced testicular damage.

Conclusion

Investigating the use of natural products, particularly, a phytochemical suppliments to defend and guide blood – testis barrier and thereafter protect the testis tissue against testicular toxicity and diabetes. Quercetin an isolating agents from phytochemical screening of flavonoid from Ocimum basilicus is known for its anti-inflammatory, anti-oxidant, ant –diabetes properties which counter the type 2 diabetes effect and restore normal testicular histomorphology.

Conflict of Interest

The authors declare that there is no conflict of interest, and that all references sources have been duly cited.

Acknowledgements

We are grateful to the Department of Anatomy, Benue State University and the Department Anatomy, Federal University, Wukari, Nigeria for their immense contribution towards successful completion of this research studies.

References

Adedara, I. A., Owoeye, O., Adeyemo, M. O., & Farombi, E. O. (2019). Quercetin reverses

Ajayi Stephen,O lawale ,IIiya Ezekeil, Ejika Daniel Eze,,Idowu Oluwamuyiwa .Moses Dele Adams,Karimah Mohammed Rabiu and Prisca Ojochogu Ajeka. 2020). Comparative Histoarchitectural and Biomedical Studies of the Hippocampus Peroxisome Proliferative Activated Receptor Agonist Treated Insulin Resistant Rat, *American Journal of Biomedical Sci*.2020, 12(1): 37-54.

Ali, M., Yousuf, A., & Choudhury, A. S. (2021). Protective effects of quercetin on oxidative and sperm quality in diabetic rats. *Reproductive Toxicology*, *103*, 1-8.

Batiha, G. E.-S., Beshbishy, A. M., Ikram, M., Mulla, Z. S., El-Hack, M. E. A., Taha, A. E., binding abnormalities in diabetes. *Journal of Medicinal Chemistry*, *63*(19), 10699-10711.

Dhanabalan, S., Sekar, D., Muralidharan, B., & Palani, S. (2018). Quercetin modulates

Dierickx, K., Demyttenaere, K., &Verhaeghe, J. (2021). Psychological impact of male disturbances in diabetic models. *Pharmacology & Therapeutics*, *238*, 108106.

El-Gindy, A., Khalil, M., & Mostafa, S. (2022). Quercetin ameliorates testicular dysfunction extract of *Chromolaenaodorata* on reproductive parameters of alloxan-induced diabetic male rats.*Nigerian Journal of Biochemistry*, *26*(1), 1–11.

García, A., Martínez, A., & Hernández, J. (2021). Effects of streptozotocin-induced diabetes https://www.who.int/news-room/fact-sheets/detail/infertility induced testicular toxicity in rats.*Reproductive Toxicology*, *94*, 1–9.

Li, Y., Yao, J., Han, C., Yang, J., Chaudhry, M. T., Wang, S., & Yin, Y. (2016). Quercetin,

Liu, X., Wei, S., & Zhang, F. (2022). The role of quercetin in the regulation of metabolic male reproductive health: A mechanistic insight. *Journal of Diabetes Research*, *2023*, 123456.

Maresch, C. C., Stute, D. C., Alves, M. G., Oliveira, P. F., de Kretser, D. M., & Linn, T.

Mendiola, J., Torres-Estay, A., & Agarwal, A. (2019). Type 2 diabetes mellitus and male *Moringaoleifera* leaves on some indices of male reproductive functions in alloxan-induced diabetic rats. *Journal of Ethnopharmacology*, *246*, 112242.

Murakami, A., Ashida, H., & Terao, J. (2008). Multitargeted cancer prevention by quercetin.

Nam, J. O., Kim, H. J., & Lee, S. W. (2016). Quercetin improves insulin sensitivity in high-

Negida, A. (2020). The pharmacological activity of quercetin: New trends and future directions. *Biomedicine & Pharmacotherapy*, *128*, 110417.

Nna, V. U., Oguonu, T., &Uzoegwu, P. N. (2017).Effects of alloxan-induced diabetes on the

Okonofua, F. E., Imade, G. E., & Salami, T. A. (2022).Diabetes mellitus and male

Olawale, F. A., Akanbi, O. R., &Oyekunle, M. A. (2021). Ameliorative effects of ethanolic

Omolaoye, A. M., Adejumo, A. A., &Akintayo, O. A. (2020). Effects of aqueous extract of on spermatogenesis and testicular histology in rats. *Biology of Reproduction*, *105*(2), 329-338.

Onung, I. C., Uchendu, C. C., &Nwankwo, C. S. (2023).Ameliorative effect of ethanolic oxidative stress and inflammation-mediated reproductive toxicity induced by arsenic in rats. *Biological Trace Element Research*, *190*(1), 106–116.

Pinti, V. C., Manrique, C. P., &Gagliardino, J. J. (2019).Streptozotocin-induced diabetes in

Rajendran, R., Ramesh, S., & Kumar, V. (2020). Quercetin as a potent modulator of protein rats: A model for the study of pancreatic islet dysfunction. *Journal of Diabetes Investigation*, *10*(6), 1548–1558.

Rodsprasert, W., Daorai, A., &Kritas, S. K. (2022).Environmental toxicants and male

Sadeghi, H., Farsani, M. A., & Heidari, Z. (2022). Streptozotocin-induced diabetes impairs

Shokri, M., Hosseinzadeh, M., &Nazari, M. (2023). Protective effects of crocin against spermatogenesis and sperm quality in male rats. *Andrologia*, *54*(2), e14121. Mansouri, M., Shamsi, M. B., & Ghorbanian, M. (2020). The effects of quercetin on spermatogenesis and testicular histology in diabetic rats. *Toxicology Reports*, *7*, 828-834.

Srinivasan, S., Govindaraj, P., &Pari, L. (2018).Protective effect of resveratrol against streptozotocin-induced testicular toxicity in rats.*Toxicology and Applied Pharmacology*, *343*, 1–10.

World Health Organization. (2022). Infertility prevalence estimates, 1990–2021. WHO.

Yin, L., Li, X., & Huang, L. (2020). Streptozotocin-induced diabetes results in testicular

Yuan, Y., Sun, H., & Huang, Q. (2016). Quercetin restores testosterone synthesis and Leydig

Zhang, X., Wang, Y., & Liu, W. (2020).Protective effect of lycopene against streptozotocin-

Zhao, Y., Wang, S., & Zhang, L. (2021). Insulin resistance increases protein binding

Zheng, W., Zhang, X., Wang, Y., & Wu, Y. (2023). The detrimental impact of diabetes on