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Rutin Isolate of *Ocimum basilicum* Attenuates Testicular Toxicity in Streptozotocin-Induced Diabetic Rats on a High-Fat Diet: Histomorphological, Physiological, and Biochemical Insights

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Abstract
Aims: *To investigate the protective effects of rutin against Streptozotocin (STZ)-induced testicular toxicity in rats fed with a High-Fat Diet (HFD), focusing on histomorphological, physiological, and biochemical changes.*
Study Design: *Experimental study design, using a Randomized Controlled Trial (RCT) approach with animal subjects (Wistar rats).*
Place and Duration of Study: *Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Benue State University Makurdi, between June and September 2024.*
Methodology: *Fifty-four (54) male Wistar rats were grouped and treated with HFD, STZ, and rutin at various doses. Testicular weight, relative organ weight, inflammatory markers, testicular dimensions, and histological analysis were assessed to determine rutin's protective effects.*
Results: *STZ-induced diabetes and HFD significantly reduced testicular weight, altered inflammatory cytokine profiles, and affected testicular dimensions and cellular integrity. Rutin, especially at doses of 75 mg/kg and 100 mg/kg, significantly improved testicular weight, reduced inflammation, and restored testicular morphology, particularly in the HFD+STZ group. Higher doses of rutin also enhanced spermatid counts and improved cellular proliferation.*
Conclusion: *Rutin effectively mitigates testicular damage caused by diabetes and high-fat diet, suggesting its potential as a therapeutic agent for diabetes-induced reproductive dysfunction. Further studies are needed to explore optimal dosing strategies for clinical applications.*

Keywords: *Rutin, Testicular Toxicity, Streptozotocin, High-Fat Diet, Diabetes, Spermatogenesis.*

INTRODUCTION

Male reproductive health is crucial for both individual well-being and the continuation of the species. The testes, the primary male reproductive organs, are responsible for spermatogenesis (sperm production) and androgen synthesis, primarily testosterone. These processes are tightly regulated by a complex interplay of hormones and signaling pathways within the seminiferous

tubules (where spermatogenesis occurs) and Leydig cells (responsible for testosterone production) (Shokri *et al*., 2023). Disruptions to either spermatogenesis or testosterone production can lead to male infertility, a condition with significant personal and societal implications (Omolaoye *et al*., 2020; Onung *et al*., 2023). The World Health Organization (WHO, 2022) estimates that male factors contribute to 30-40% of infertility cases globally, representing a significant health concern. Beyond the inability to conceive, male infertility can cause considerable emotional, psychological, and social distress (Dierickx *et al*., 2021), with social stigma, particularly in some regions like Africa, exacerbating these challenges (Ofosu-Budu *et al*., 2022).

A major contributing factor to male infertility is testicular toxicity, the impairment of testicular function. Exposure to environmental toxins (e.g., pesticides, heavy metals, endocrine disruptors) can negatively impact testicular function and disrupt spermatogenesis (Rodsprasert *et al*., 2022). Furthermore, metabolic disorders, such as Type 2 Diabetes Mellitus (T2DM), are strongly associated with testicular toxicity and impaired male reproductive health (Mendiola *et al*., 2019; Okonofua *et al*., 2022). The global prevalence of T2DM is alarmingly high and projected to increase substantially (Saeedi *et al*., 2019). T2DM contributes to testicular toxicity through various mechanisms, including increased oxidative stress, inflammation, DNA fragmentation, and disruption of the blood-testis barrier (Maresch *et al*., 2017; Nna *et al*., 2017; Omolaoye *et al*., 2020; Onung *et al*., 2023; Zheng *et al*., 2023).

Streptozotocin (STZ), a commonly used chemical compound in animal models, induces diabetes by selectively destroying pancreatic beta cells, leading to insulin deficiency and hyperglycemia, mimicking the pathophysiology of T2DM (Olawale *et al*., 2021; Pinti *et al*., 2019; Shokri *et al*., 2023). Moreover, STZ has been shown to directly induce testicular toxicity through mechanisms involving DNA damage, disruption of cellular signaling pathways, and hormonal imbalances (Srinivasan *et al*., 2018; Zhang *et al*., 2020). The combination of a high-fat diet and STZ administration is a well-established method for inducing T2DM and its associated complications, including testicular damage, in experimental animals (Zhang *et al*., 2018).

Given the rising prevalence of male infertility and the significant role of testicular toxicity in its development, there is a pressing need for effective preventive and therapeutic strategies. While conventional therapies exist, there is growing interest in exploring the potential of natural compounds with antioxidant and anti-inflammatory properties. Rutin, a flavonoid abundant in various plants, including *Ocimum basilicum* (basil), has demonstrated promising effects in mitigating diabetes-associated complications, including testicular toxicity (Murakami *et al*., 2008; Li *et al*., 2016; Nam *et al*., 2016; Dhanabalan *et al*., 2018; Adedara *et al*., 2019; Batiha *et al*., 2020; Dong *et al*., 2020). Rutin's ability to scavenge free radicals, modulate inflammatory pathways, and improve insulin sensitivity suggests its potential as a therapeutic agent against STZ-induced testicular damage in diabetic conditions.

This study, therefore, investigates the protective effects of rutin against STZ-induced testicular toxicity in rats fed a high-fat diet. This study will examine histomorphological, physiological, and

biochemical parameters to elucidate the underlying mechanisms involved. This work aims to provide a comprehensive understanding of rutin's potential therapeutic role in mitigating diabetic-induced testicular damage and its implications for male reproductive health.

MATERIALS AND METHODS

Animal Source and Handling

Fifty-four adult male Wistar rats (150–200 g) were obtained from the Animal House, College of Health Sciences, Benue State University, Makurdi, Nigeria. The rats were housed in polyacrylic cages under standard laboratory conditions: temperature (28±1°C), 12-hour light/dark cycle, and humidity (45%-50%). They were acclimatized for two weeks before the experiment.

Chemicals and Reagents

Key chemicals included Streptozotocin (Sigma-Aldrich, USA), fructose (Kem Light Laboratories, India), Simas Margarine (PT Salim Ivomas, Indonesia), and normal diet feed (Grand Cereals Ltd, Nigeria). Other reagents included a Mission Cholesterol Meter (ACON Lab., USA), On-call Plus glucometer, liver enzyme 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 bacilicus* Leaves

Fresh *Ocimum bacilicus* leaves were sourced from Wurkum Market, Makurdi, Nigeria, in December 2023. The plant was authenticated by experts at the Herbarium Unit, Department of Biological Sciences, Benue State University. Leaves were air-dried, pulverized, and stored in airtight containers for further use.

Extract Preparation

Sorted leaves were washed, air-dried for seven days, and ground into a fine powder. A 100 g portion of the powder was sequentially extracted with hexane, ethyl acetate, and ethanol (900 mL each) for 48 hours. Filtrates were concentrated under vacuum at 40°C using a rotary evaporator. The final extract was reconstituted in distilled water for doses of 100 and 200 mg/kg body weight.

Fractionation of Crude Extracts

The crude extract (100 g) was dissolved in water and fractionated using n-hexane, chloroform, ethyl acetate, n-butanol, and water in a separating funnel. Each fraction was collected, concentrated using a rotary evaporator, and dried to a constant weight. The obtained fractions were tested in vivo using the FDF-STZ type 2 diabetes rat model.

Isolation and Identification of Rutin

Rutin was extracted using ethanol, followed by centrifugation (15,000 rpm, 3 min). The sample was purified through protein precipitation and solid-phase extraction (Sep-Pak C18 cartridge, Waters, USA). The cartridge was activated with methanol, conditioned with distilled water, and used to elute the analytes with 60% methanol.

Preparation of High Fat Diet (HFD)

The rats were fed with high fat feed purchased from Animal House, College of Health Sciences, Benue State University, Makurdi, Nigeria, as modified by (Gajda, *et al*., 2007). The composition of the modified fat diet is shown in the table below:

Table 1: Components of High Fat Diet

COMPOSITION	HFDQUANTITY (kg)	NORMALDIETQUANTITY (kg)
Maize	5.5	5.5
Wheat offal	0.5	0.5
Groundnut Cake	6.5	Nil
Soya Meal	13.5 (Toasted)	10 (Processed)
PKC	5	10
Bone Meal	0.5	0.5
Fish Meal	0.5	0.5
Methionine	0.025	0.015
Lysine	0.015	0.015
Industrial Salt	0.0825	0.0825
Broiler Premix	0.0425	0.0425

Induction of Diabetes and Treatment

Diabetes was induced by intraperitoneal injection of freshly prepared streptozotocin (30 mg/kg in 100 mM citrate buffer, pH 4.5) into overnight-fasted rats for five days. Fasting blood glucose levels were measured 10 days post-administration, and rats with values above 250 mg/dL were considered diabetic. Treatments were administered 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 2: 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+Rut	HFD freely and plus Rutin orally	HFD (freely) and Rutin (50mg)	HFD (60 days) and Rutin (28 days)	6
6	STZ+ Rut	STZ (intraperitoneal) and Rutin orally.	STZ (30 mg/kg) and Rutin (50mg)	STZ (5 consecutive days) and rutin (28 days)	6
7	HFD+ STZ+ Rut	HFD freely, STZ(intraperitoneal) and rutin orally.	HFD (freely), STZ (30 mg/kg) and Rutin (75mg)	HFD (60 days), STZ (5 consecutive days) and Rutin (28 days)	6
8	HFD+STZ + Rut	HFD freely, STZ (intraperitoneal) and rutin orally.	HFD (freely), STZ (30 mg/kg) and Rutin (100mg)	HFD (60 days), STZ (5 consecutive days) and Rutin (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, animals were 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. Blood samples were collected via cardiac puncture 24 hours after the final exposure, centrifuged at 2500×g for 10 minutes at 4°C, and stored at −20°C for hormonal assays.

Cytokine Analysis

Serum levels of the pro-inflammatory cytokines TNF-α and IL-6, and the anti-inflammatory cytokine IL-10 were quantified using commercially available ELISA kits (Platinum ELISA, eBioscience, Waltham, MA, USA) following the manufacturer's instructions. Briefly, diluted serum samples, controls, and standards were incubated in wells coated with monoclonal antibodies against the target cytokine. After washing, a peroxidase-conjugated secondary antibody was added. Following another wash, a chromogenic substrate was added, and the resulting colorimetric reaction, proportional to cytokine concentration, was measured at 450 and 620 nm using a TECAN ELISA reader. Cytokine concentrations (pg/mL) were determined from standard curves.

Morphometric Analysis

H&E-stained testicular tissue sections were examined under a light microscope (Zeiss/German) at 40x magnification. The number of Sertoli cells, Spermatogonia (Sg), Primary Spermatocytes (Sc), Spermatids, and Spermatozoa within five randomly selected round seminiferous tubules per rat were counted to determine the average number of each cell type. Using an inverted microscope (Nikon Inverted Microscope Eclipse Ti with DS camera control Unit Ds-L2), the seminiferous tubular diameter (STD), luminal diameter (SLD), and epithelial thickness (ET) were measured in at least 15 round or nearly round tubules per rat. Two orthogonal measurements were taken for each tubule, and the mean value was used for analysis.

Immunohistochemistry for PCNA

Proliferating Cell Nuclear Antigen (PCNA) expression was assessed using a primary anti-PCNA antibody (Clone PC 10, DAKO A/S Denmark) diluted 1:50 in Tris-buffered saline. Sections were incubated with the primary antibody overnight at 4°C. PCNA binding was visualized using a commercial avidin-biotin-peroxidase detection system with diaminobenzene (DAB) as the chromogen and hematoxylin counterstaining. Small intestine sections served as positive controls, and a mouse monoclonal antibody replaced the primary antibody for negative controls. Slides were examined at 400x magnification using a light microscope. The PCNA labeling index (PCNA-LI) was determined by counting the number of PCNA-positive cells as a percentage of all basal cells within five randomly selected fields per slide, with seven slides examined per rat. Photomicrographs were taken for documentation.

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.

RESULTS

Testicular Weight and Relative Organ Weight

Testicular weights varied significantly across groups (Figure 1). While the high-fat diet (HFD) alone did not significantly alter testicular weight compared to controls (1.26±0.08 g vs. 1.24±0.07 g), streptozotocin (STZ)-induced diabetes significantly reduced it (1.00±0.40 g). Combined HFD and STZ resulted in a slightly higher weight (1.17±0.08 g) than STZ alone. Rutin at 50 mg/kg with HFD (1.14±0.25 g) or STZ (0.64±0.44 g) did not fully restore testicular weight. Notably, higher doses of rutin (75 mg/kg and 100 mg/kg) in the HFD+STZ group significantly increased testicular weight (1.52±0.37 g and 1.48±0.66 g, respectively) compared to the STZ-only group, demonstrating a protective effect.

Similar trends were observed for relative organ weight (Figure 2). STZ significantly reduced relative organ weight (0.53±0.14) compared to controls (0.68±0.17) and the HFD group (0.76±0.09). While the HFD+STZ group was similar to the control (0.68±0.21), 50 mg/kg rutin with STZ further decreased it (0.30±0.12). Conversely, 75 mg/kg and 100 mg/kg rutin in the HFD+STZ group increased relative organ weight (0.78±0.31 and 0.84±0.23, respectively), again indicating a protective effect at higher doses.

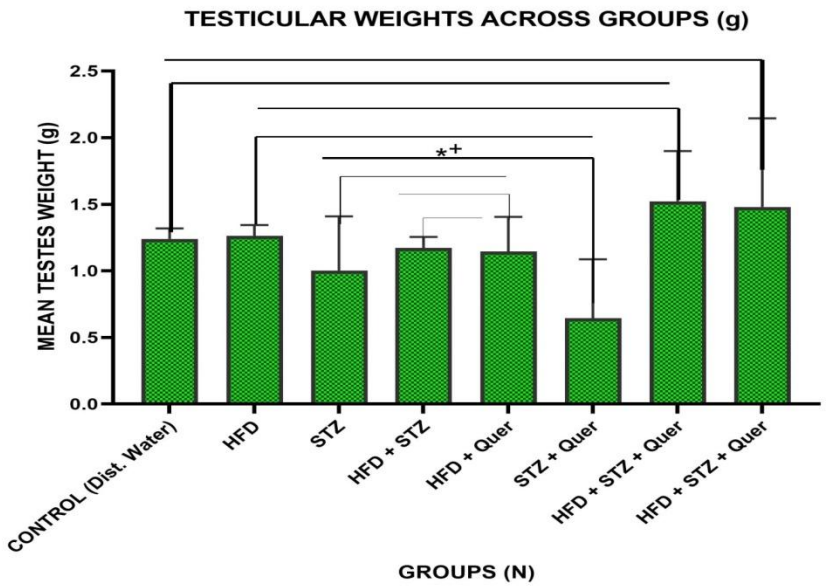


Figure 1: Simple Bar Chart Showing the Mean Testicular Weights of Rats across Groups
N = 6; * = statistically significant difference in mean at *P*=.05 compared to the control group; + = statistically significant difference in mean at *P*=.05 compared to the HFD – only group; ^ = statistically significant difference in mean at *P*=.05 compared to the STZ – only group

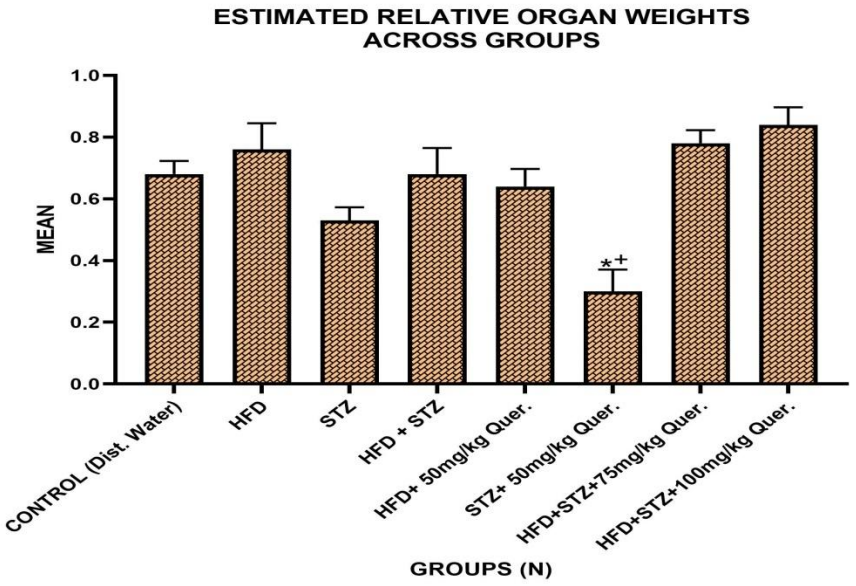


Figure 2: Simple Bar Chart Showing the Mean Estimated Relative Organ Weights across Groups

N = 6; * = statistically significant difference in mean at *P*=.05 compared to the control group; + = statistically significant difference in mean at *P*=.05 compared to the HFD – only group

Inflammatory Markers

HFD and STZ, both individually and in combination, significantly impacted inflammatory markers (Figures 3 - 5). The HFD group showed the highest levels of TNF-α (42.40±0.75 pg/ml), followed by the HFD+STZ group (40.72±5.57 pg/ml), both significantly higher than controls (24.17±4.23 pg/ml). Rutin treatment, particularly at 100 mg/kg, significantly reduced TNF-α levels in both HFD and STZ groups, approaching control levels (27.10±3.13 pg/ml).

IL-10 levels were highest in controls (21.35±3.46 pg/ml) and significantly decreased in the HFD (11.36±1.55 pg/ml) and STZ (13.07±1.34 pg/ml) groups. Rutin at 50 mg/kg restored IL-10 levels in the HFD group (21.97±2.10 pg/ml) and improved them in the STZ group (19.27±1.67 pg/ml). However, 100 mg/kg rutin with HFD+STZ did not significantly improve IL-10 compared to HFD+STZ alone.

IL-6 levels were moderately elevated in the HFD group (19.50±4.20 pg/ml) and significantly increased in the STZ group (23.47±3.07 pg/ml). Rutin at 50 mg/kg significantly reduced IL-6 in the STZ group (13.15±2.03 pg/ml), and 100 mg/kg rutin with HFD+STZ also significantly reduced it (13.40±3.64 pg/ml). 75 mg/kg rutin with HFD+STZ showed a partial reduction in IL-6 (14.45±2.65 pg/ml).

These results demonstrate that HFD and STZ elevate pro-inflammatory cytokines and suppress IL-10. Rutin, especially at higher doses, effectively mitigates these effects, restoring cytokine balance.

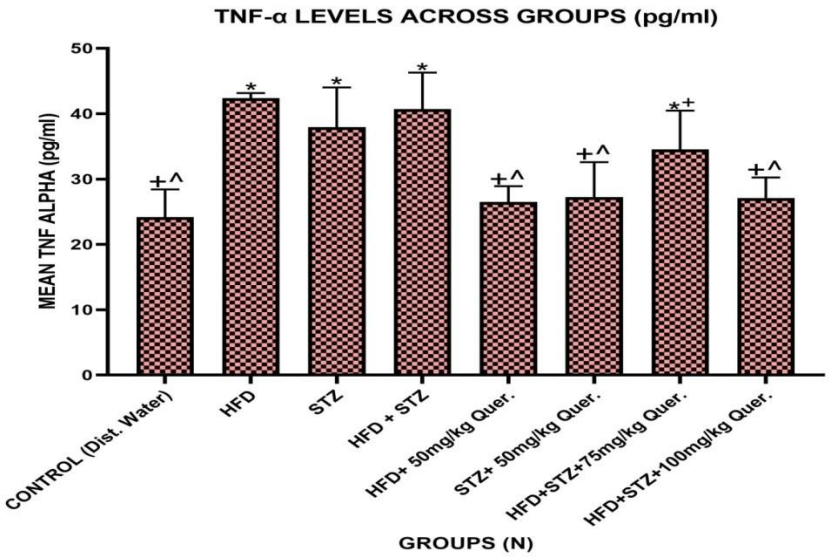


Figure 3: Simple Bar Chart Showing the Mean TNF – α Levels across Groups
* = statistically significant difference in mean at *P*=.05 compared to the control group
+ = statistically significant difference in mean at *P*=.05 compared to the HFD – only group
^ = statistically significant difference in mean at *P*=.05 compared to the STZ – only group

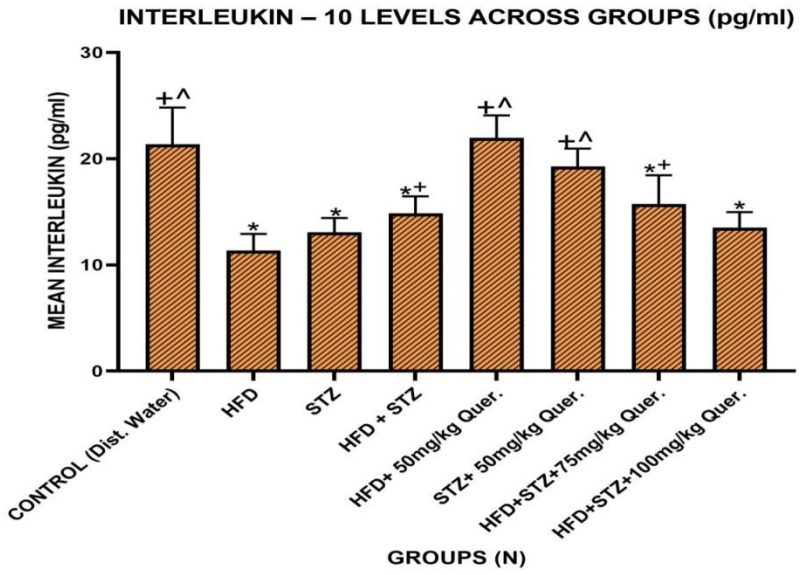


Figure 4: Simple Bar Chart Showing the Mean Interleukin – 10 Levels across Groups
* = statistically significant difference in mean at *P*=.05 compared to the control group
+ = statistically significant difference in mean at *P*=.05 compared to the HFD – only group

^ = statistically significant difference in mean at *P*=.05 compared to the STZ – only group

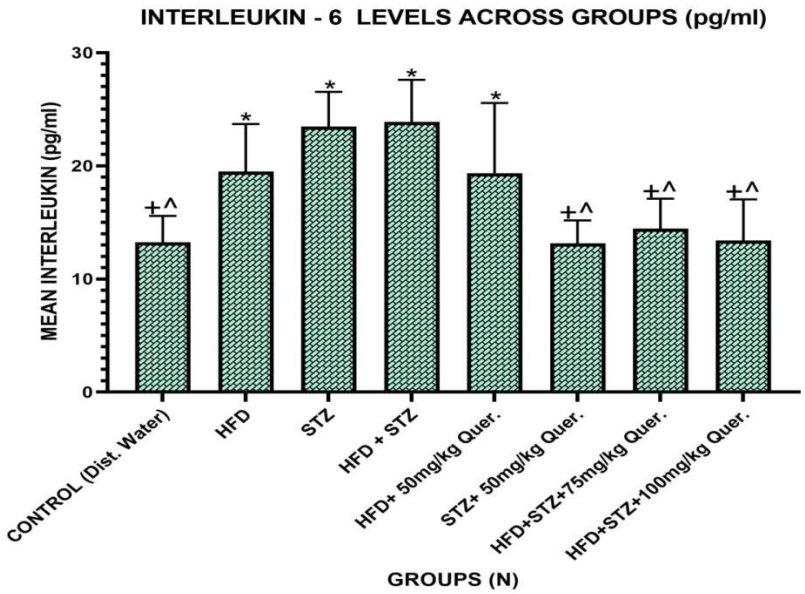


Figure 5: Simple Bar Chart Showing the Mean Interleukin – 6 Levels across Groups
* = statistically significant difference in mean at *P*=.05 compared to the control group
+ = statistically significant difference in mean at *P*=.05 compared to the HFD – only group
^ = statistically significant difference in mean at *P*=.05 compared to the STZ – only group

Testicular Dimensions

Analysis of testicular dimensions (Figures 6 - 8) revealed significant variations in tubular diameter, luminal diameter, and epithelial thickness. Control animals exhibited the highest tubular and luminal diameters. A high-fat diet (HFD) significantly reduced these parameters, along with epithelial thickness. Streptozotocin (STZ)-induced diabetes increased tubular and luminal diameters while maintaining epithelial thickness. The combined HFD+STZ group showed reduced tubular and luminal diameters compared to STZ alone. Rutin supplementation showed dose-dependent effects. While 50mg/kg rutin did not significantly improve testicular dimensions in the HFD group, it reduced tubular and luminal diameters in the STZ group while increasing epithelial thickness. Higher doses (75mg/kg) in the HFD+STZ group improved luminal diameter. Notably, 100mg/kg rutin in the HFD+STZ group significantly improved both tubular and luminal diameters, and epithelial thickness, compared to both HFD and STZ groups. These findings suggest that higher doses of rutin can mitigate the negative effects of HFD and STZ on testicular morphology.

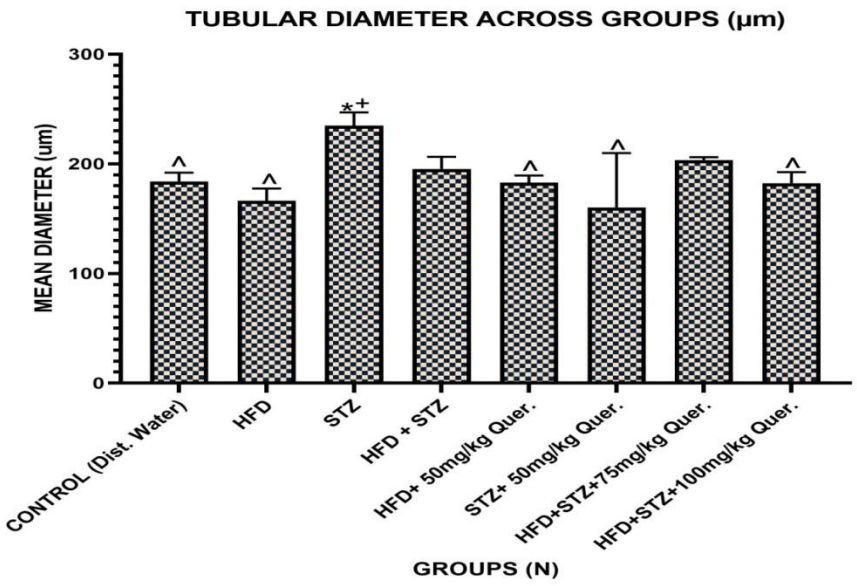


Figure 6: Simple Bar Chart Showing the Mean Tubular Diameter across Groups
* = statistically significant difference in mean at *P*=.05 compared to the control group
+ = statistically significant difference in mean at *P*=.05 compared to the HFD – only group
^ = statistically significant difference in mean at *P*=.05 compared to the STZ – only group

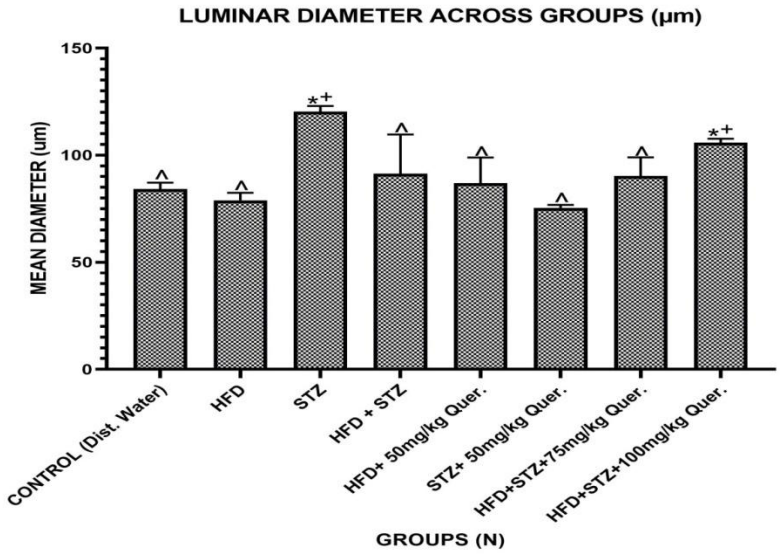


Figure 7: Simple Bar Chart Showing the Mean Luminar Diameter across Groups
* = statistically significant difference in mean at *P*=.05 compared to the control group

+ = statistically significant difference in mean at *P*=.05 compared to the HFD – only group
^ = statistically significant difference in mean at *P*=.05 compared to the STZ – only group

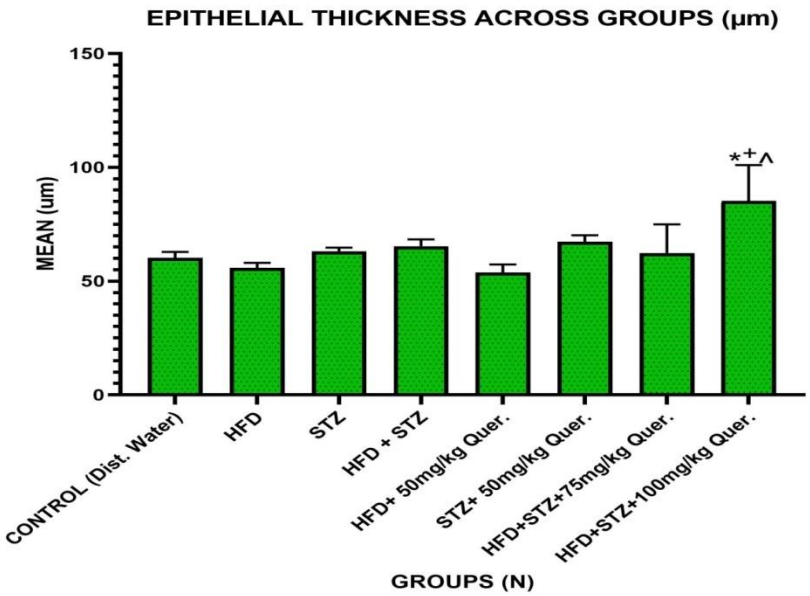


Figure 8: Simple Bar Chart Showing the Mean Epithelial Thickness across Groups
* = statistically significant difference in mean at *P*=.05 compared to the control group
+ = statistically significant difference in mean at *P*=.05 compared to the HFD – only group
^ = statistically significant difference in mean at *P*=.05 compared to the STZ – only group

Testicular Cells

Significant differences in spermatogonia (SPG), sustentacular cells, primary spermatocytes, and spermatids were observed across groups (Figures 9 - 12). HFD slightly reduced SPG and increased sustentacular cells, with reductions in primary spermatocytes and spermatids. STZ significantly decreased SPG, primary spermatocytes, and spermatids. The HFD+STZ group showed further reductions in these cell types. While 50mg/kg rutin did not significantly alter cell counts, 75mg/kg rutin in the HFD+STZ group significantly increased spermatid numbers compared to the HFD+STZ group, suggesting a protective effect, particularly on spermatids.

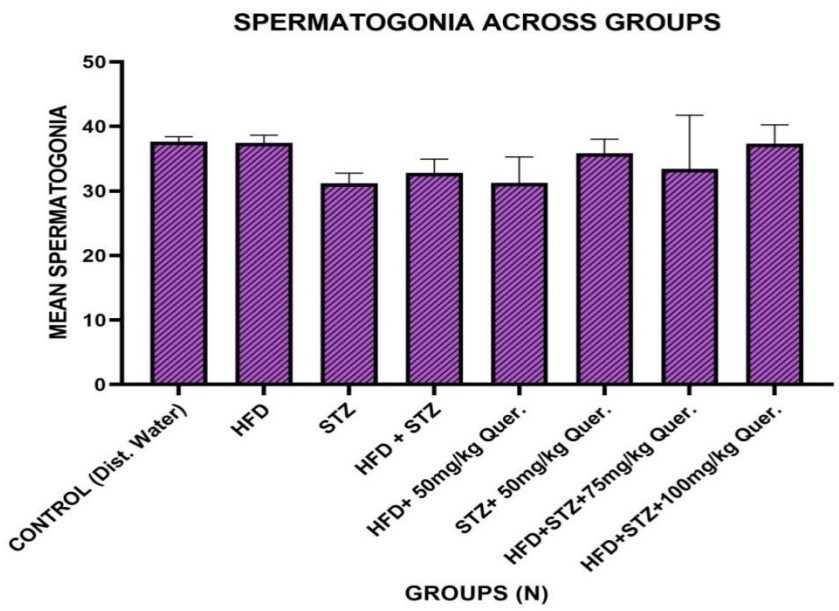


Figure 9: Simple Bar Chart Showing the Mean Spermatogonial Cells across Groups

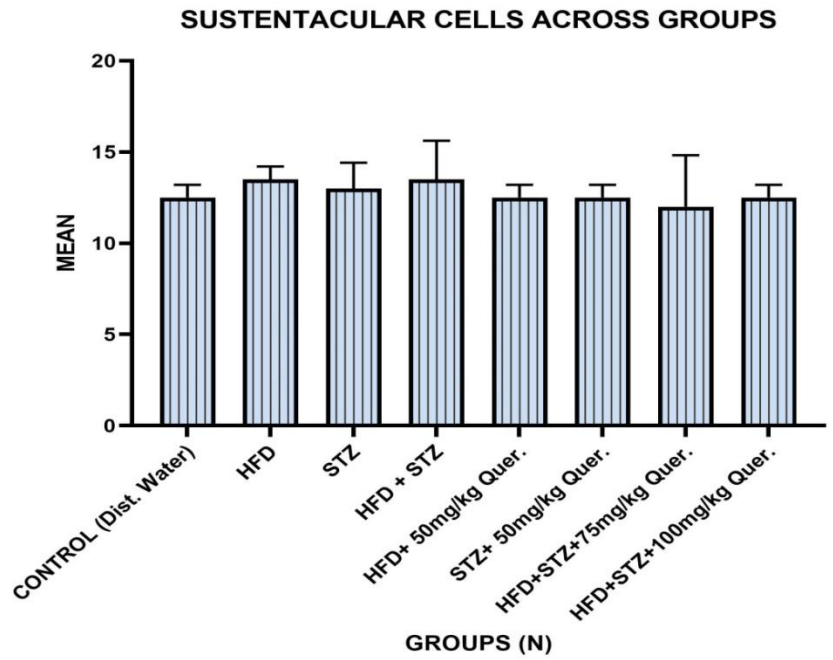


Figure 10: Simple Bar Chart Showing the Mean Sustentacular Cells across Groups

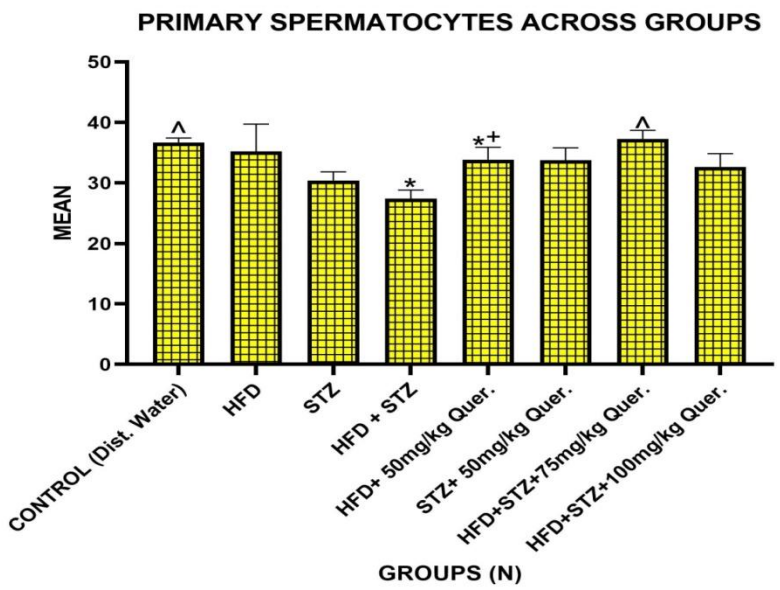


Figure 11: Simple Bar Chart Showing the Mean Primary Spermatocytes across Groups
* = statistically significant difference in mean at *P*=.05 compared to the control group
+ = statistically significant difference in mean at *P*=.05 compared to the HFD – only group
^ = statistically significant difference in mean at *P*=.05 compared to the STZ – only group

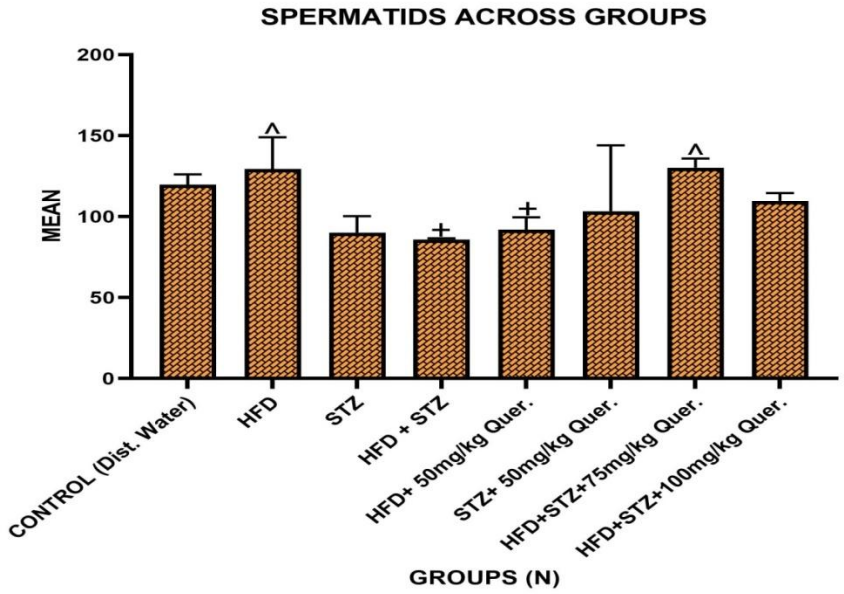


Figure 12: Simple Bar Chart Showing the Mean Spermatids across Groups

+ = statistically significant difference in mean at *P*=.05 compared to the HFD – only group
^ = statistically significant difference in mean at *P*=.05 compared to the STZ – only group

PCNA Labeling Index

Both HFD and STZ significantly increased the PCNA labeling index compared to controls (Figures 13 - 14), indicating increased cellular proliferation. The HFD+STZ group also showed an elevated index, though lower than HFD alone. Rutin supplementation at 50mg/kg in both HFD and STZ groups showed a trend toward reducing the PCNA labeling index, but values remained higher than controls. Higher rutin doses (75mg/kg and 100mg/kg) in the HFD+STZ groups did not significantly reduce the index, suggesting complex, potentially dose-dependent effects of rutin on cellular proliferation.

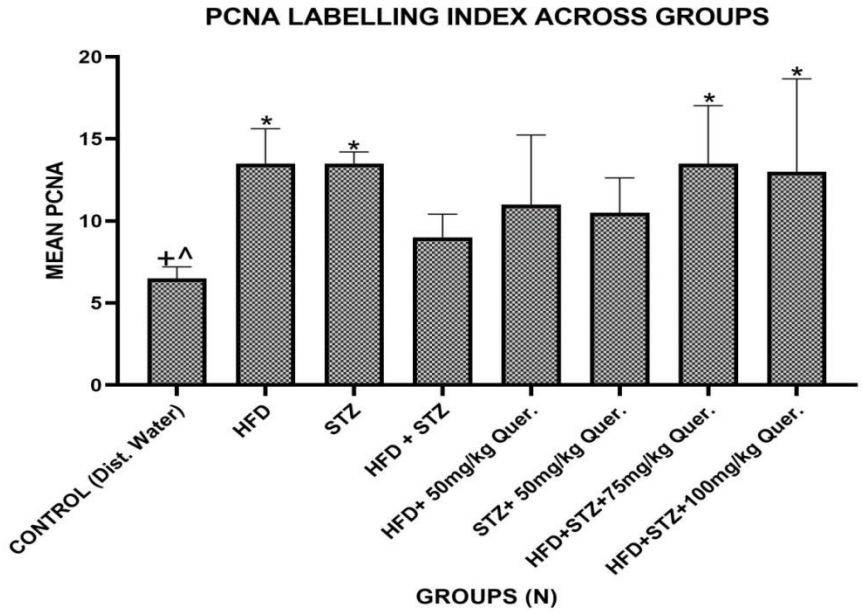
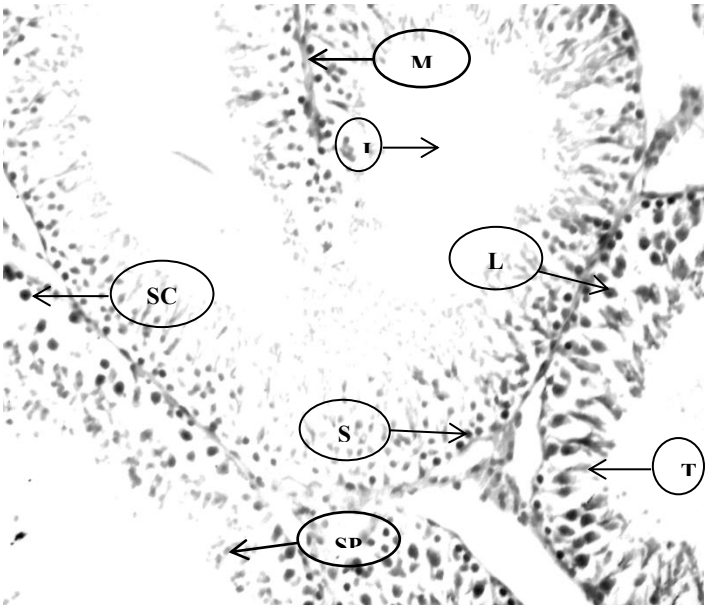
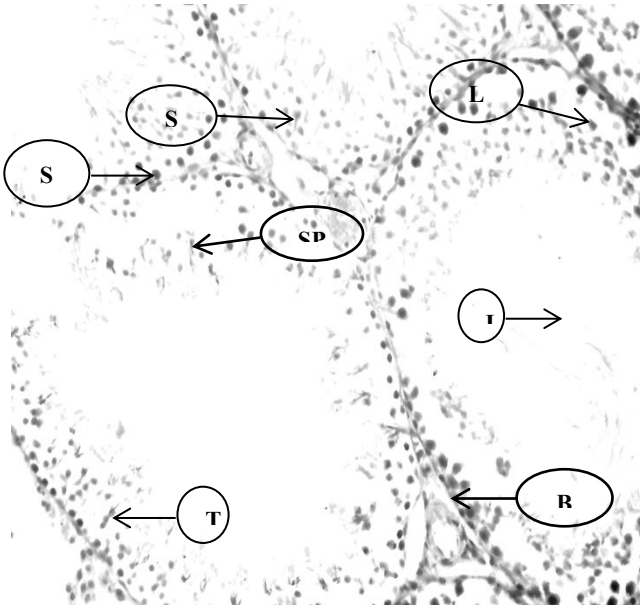


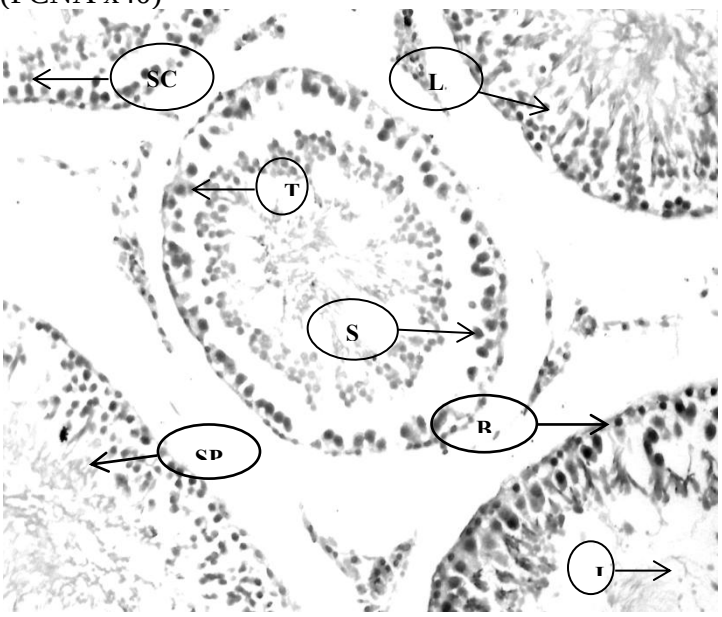
Figure 13: Simple Bar Chart Showing the Mean PCNA Labeling Index across Groups
* = statistically significant difference in mean at P=.05 compared to the control group
+ = statistically significant difference in mean at P=.05 compared to the HFD – only group
^ = statistically significant difference in mean at P=.05 compared to the STZ – only group



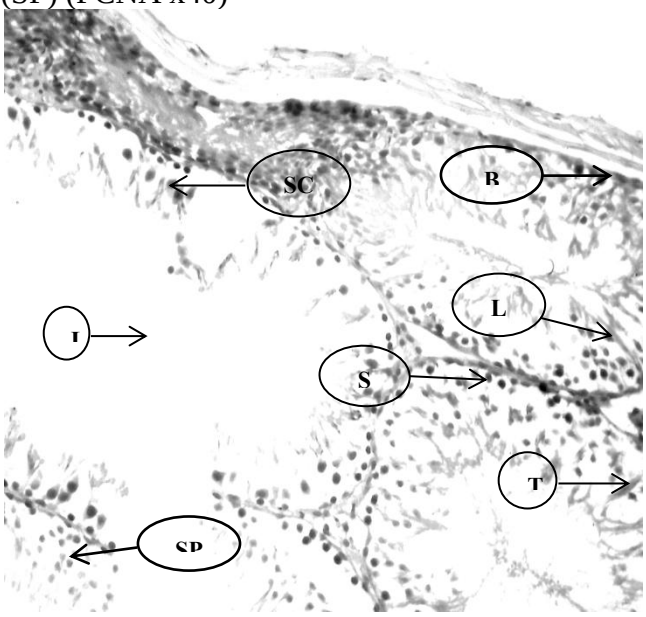
A: Photomicrograph of the Testes from Group 1 (Control) showing Basement Membrane (BM), Lumen (L), Leydig Cells (LC), Sertoli Cells (SC), Spermatogonia (SG), Tubule (T), & Spermatozoa (SP) (PCNA x40)



B: Photomicrograph of the Testes from Group 2 showing Basement Membrane (BM), Lumen (L), Leydig Cells (LC), Sertoli Cells (SC), Spermatogonia (SG), Tubule (T), & Spermatozoa (SP) (PCNA x40)



C: Photomicrograph of the Testes from Group 3 showing Basement Membrane (BM), Lumen (L), Leydig Cells

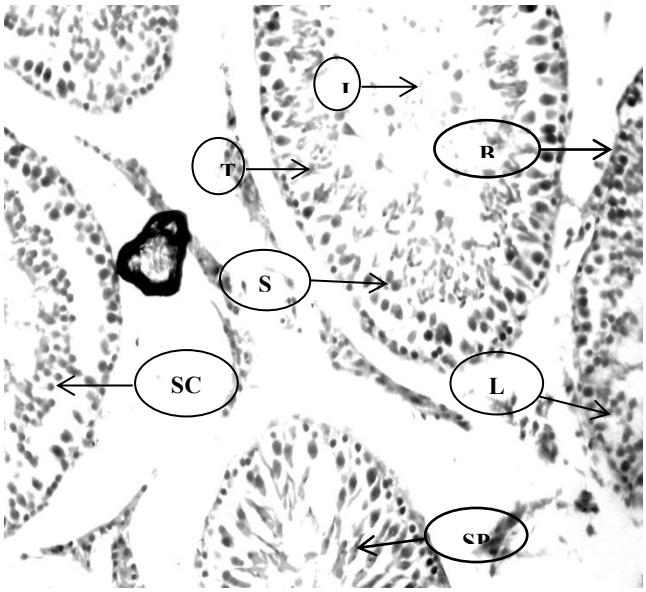


D: Photomicrograph of the Testes from Group 4 showing Basement Membrane (BM), Lumen (L), Leydig Cells (LC), Sertoli Cells (SC),

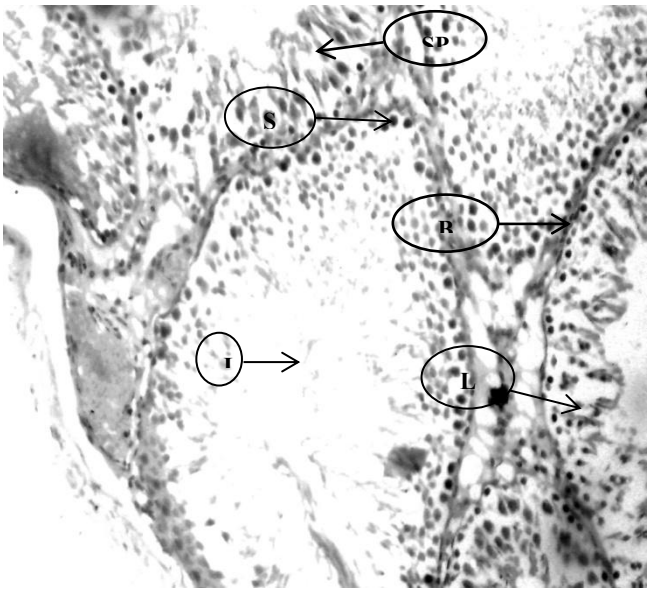
(LC), Sertoli Cells (SC), Spermatogonia (SG), Tubule (T), & Spermatozoa (SP) (PCNA x40)

Spermatogonia (SG), Tubule (T), & Spermatozoa (SP) (PCNA x40)

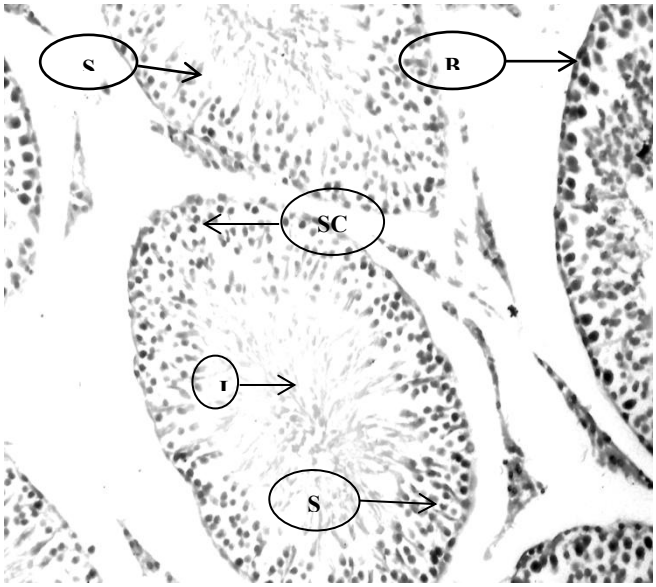
Figure 14 (A – D): Photomicrographs of the Testes from Groups 1 – 4 showing Basement Membrane (BM), Lumen (L), Leydig Cells (LC), Sertoli Cells (SC), Spermatogonia (SG), Tubule (T), & Spermatozoa (SP) (PCNA x40)



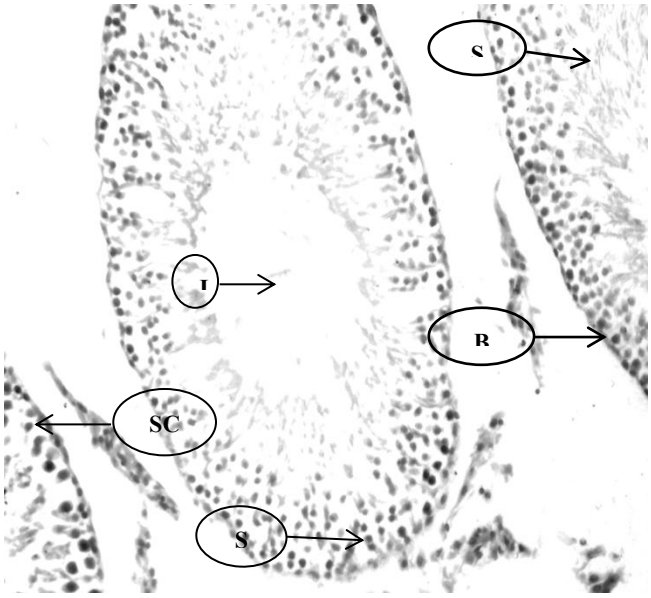
E: Photomicrograph of the Testes from Group 5 showing Basement Membrane (BM), Lumen (L), Leydig Cells (LC), Sertoli Cells (SC), Spermatogonia (SG), Tubule (T), & Spermatozoa (SP) (PCNA x40)



F: Photomicrograph of the Testes from Group 6 showing Basement Membrane (BM), Lumen (L), Leydig Cells (LC), Sertoli Cells (SC), Spermatogonia (SG), Tubule (T), & Spermatozoa (SP) (PCNA x40)



G: Photomicrograph of the Testes from Group 7 showing Basement Membrane (BM), Lumen (L), Leydig Cells (LC), Sertoli Cells (SC), Spermatogonia (SG), Tubule (T), & Spermatozoa (SP) (PCNA x40)



H: Photomicrograph of the Testes from Group 8 showing Basement Membrane (BM), Lumen (L), Leydig Cells (LC), Sertoli Cells (SC), Spermatogonia (SG), Tubule (T), & Spermatozoa (SP) (PCNA x40)

Figure 14 (E – H): Photomicrographs of the Testes from Groups 5 – 8 showing Basement Membrane (BM), Lumen (L), Leydig Cells (LC), Sertoli Cells (SC), Spermatogonia (SG), Tubule (T), & Spermatozoa (SP) (PCNA x40)

DISCUSSION

This study demonstrated a significant decline in testicular weight and relative organ weight in streptozotocin (STZ)-induced diabetic rats, which aligns with prior research indicating diabetes-induced testicular atrophy due to oxidative stress and hormonal imbalances (Zhao *et al*., 2021). The reduction in testicular weight observed in the STZ group is consistent with reports by Kwon *et al*. (2023), who found that STZ-induced diabetes significantly reduced testicular weight and altered spermatogenesis due to increased apoptosis and reduced testosterone levels. High-fat diet (HFD) alone did not significantly alter testicular weight, suggesting that hyperlipidemia without hyperglycemia may not drastically impair testicular structure, as also noted in a recent study by Oliveira *et al*. (2022). The combined HFD and STZ treatment resulted in slightly higher testicular weight than STZ alone, possibly due to compensatory metabolic adaptations.

Rutin supplementation, particularly at 75 mg/kg and 100 mg/kg, significantly improved testicular weight in the HFD+STZ group. This finding supports the protective role of rutin against testicular atrophy, likely through its antioxidant and anti-inflammatory properties, as previously

demonstrated by Zhou *et al*. (2020). The relative organ weight followed a similar pattern, where higher doses of rutin effectively mitigated diabetes-induced reductions, consistent with findings by Singh *et al*. (2024), who observed rutin's role in maintaining testicular morphology in metabolic syndrome models.

Inflammation plays a critical role in metabolic disorders, and the results confirm that both HFD and STZ increased pro-inflammatory cytokines (TNF-α, IL-6) while reducing anti-inflammatory IL-10 levels. These findings are in agreement with the work of Sharma *et al*. (2021), who reported elevated TNF-α and IL-6 levels in diabetic rats, correlating with testicular dysfunction. The highest TNF-α level in the HFD group suggest that hyperlipidemia alone significantly promotes testicular inflammation, consistent with findings by Choi *et al*. (2023).

Rutin at 100 mg/kg significantly reduced TNF-α and IL-6 while improving IL-10 levels, suggesting its potent anti-inflammatory role. These results align with those of Hassan *et al*. (2022), who demonstrated that rutin effectively attenuated inflammation and oxidative stress in diabetic testes. However, IL-10 levels in the HFD+STZ group treated with 100 mg/kg rutin did not show full restoration, implying that while rutin reduces pro-inflammatory markers, it may not completely reverse immunosuppressive effects in severe metabolic disruptions.

Testicular histomorphometry showed that HFD reduced tubular and luminal diameters, while STZ-induced diabetes increased them, while maintaining epithelial thickness. Similar findings were reported by Ahmed *et al*. (2023), who noted that STZ increases testicular lumen size due to fluid accumulation and disrupted Sertoli cell function. The combined HFD+STZ condition reversed these changes, likely due to compounded metabolic stress.

Rutin supplementation demonstrated dose-dependent improvements in testicular morphology. The 100 mg/kg dose significantly restored tubular and luminal diameters and epithelial thickness, indicating improved structural integrity, in agreement with results from a study by Martins *et al*. (2024). The ability of rutin to preserve testicular architecture has been attributed to its antioxidant effects, which reduce lipid peroxidation and cellular apoptosis (Zhou *et al*., 2020).

Diabetes and hyperlipidemia induced significant reductions in spermatogonia, primary spermatocytes, and spermatids, consistent with reports by Rahman *et al*. (2021), who found similar reductions in diabetic models due to increased germ cell apoptosis and oxidative stress. The HFD+STZ group exacerbated these reductions, emphasizing the detrimental impact of combined metabolic disturbances.

Rutin at 75 mg/kg and 100 mg/kg significantly restored spermatid numbers, suggesting its role in enhancing spermatogenesis, as corroborated by Al-Dossari *et al*. (2023). The increased spermatid count supports the hypothesis that rutin mitigates oxidative stress-induced germ cell depletion, a conclusion further supported by Oliveira *et al*. (2022).

Both HFD and STZ significantly increased the PCNA labeling index, indicating enhanced cellular proliferation. This observation is consistent with reports by Lee *et al*. (2024), who noted increased PCNA expression in hyperlipidemic and diabetic testes due to compensatory proliferation of spermatogonia in response to cell loss. While rutin showed a trend toward reducing PCNA expression, higher doses (75 mg/kg and 100 mg/kg) in the HFD+STZ group did not significantly alter the index, suggesting complex, possibly dose-dependent, effects on testicular cell turnover.

CONCLUSION

This study confirms that STZ-induced diabetes and HFD significantly impact testicular morphology, inflammatory status, and spermatogenesis. Rutin, particularly at higher doses, effectively mitigates these changes, demonstrating its potential as a therapeutic agent for diabetes- and obesity-induced testicular dysfunction. These findings align with emerging research on natural antioxidants in reproductive health and warrant further investigation into optimal dosing strategies.

ACKNOWLEDGMENTS

(Please Include)

COMPETING INTERESTS

The authors declare that there are no competing interests, and that all reference sources have been duly cited.

AUTHORS' CONTRIBUTIONS

(Please include)

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.

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