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Impact of Combined High-Fat Diet and Streptozotocin Exposure on Male Reproductive Health: Protective Role of Quercetin

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Abstract

Male infertility is an increasing global concern with profound social and psychological implications, often linked to metabolic dysfunctions such as Type 2 diabetes mellitus (T2DM). Streptozotocin (STZ), widely used to induce T2DM in animal models, is known to cause testicular toxicity through oxidative stress, inflammation, and apoptosis, thereby impairing spermatogenesis and hormone production. Quercetin, a flavonoid with established antioxidant and anti-inflammatory properties, has shown promise in ameliorating diabetes-related complications, including reproductive dysfunction. This study investigates the combined effects of a high-fat diet (HFD) and STZ exposure on male reproductive health, while assessing the protective role of quercetin. Fifty-four male Wistar rats were randomly assigned to nine experimental groups and subjected to HFD, STZ, and quercetin at different doses. Testicular health was evaluated by measuring absolute and relative testicular weights. STZ was administered intraperitoneally (30 mg/kg), and quercetin was administered orally at 50, 75, and 100 mg/kg

for 28 days. Results showed that STZ-treated rats had significantly reduced testicular weight (1.00 ± 0.40 g) compared to controls (1.24 ± 0.07 g), confirming testicular toxicity. Quercetin supplementation, especially at 75 mg/kg (1.52 ± 0.37 g) and 100 mg/kg (1.48 ± 0.66 g), significantly restored testicular weight, suggesting a dose-dependent protective effect. These findings demonstrate that HFD exacerbates STZ-induced testicular toxicity, while quercetin confers substantial protection, supporting its potential as a therapeutic agent for managing testicular dysfunction in diabetic conditions.

Keywords: Male Infertility; Testicular Toxicity; Streptozotocin; High-Fat Diet; Quercetin; Oxidative Stress

INTRODUCTION

Male infertility is a global health concern with significant psychological, emotional, and social repercussions (Dierickx *et al.*, 2021). While often overlooked, it affects a substantial portion of couples struggling with conception, with estimates placing the number of affected men worldwide between 30.6 and 30.6 million (WHO, 2022). In Africa, where infertility carries a heavy social burden, including stigmatization and even marital dissolution (Ofosu-Budu *et al.*, 2022), the issue is particularly acute. Studies have shown that male infertility accounts for a significant percentage of overall infertility cases, ranging from 6-9% in North America, Europe, and Australia (Agarwal *et al.*, 2015) to as high as 44% in some Nigerian clinics (Ugboma *et al.*, 2012). This condition can manifest in various ways, including reduced sperm count, motility, abnormal morphology, or complete absence of sperm, with testicular toxicity emerging as a major contributing factor (Rodsprasert *et al.*, 2022).

Testicular toxicity, also known as hypogonadism, impairs the testes' ability to produce sperm and testosterone (Rodsprasert *et al.*, 2022). Exposure to environmental toxins, like pesticides and heavy metals, can disrupt testicular function and spermatogenesis (Mendiola *et al.*, 2019; Okonofua *et al.*, 2022). Furthermore, testicular toxicity is often linked to metabolic dysfunction and insulin resistance, key features of Type 2 diabetes mellitus (T2DM) (Mendiola *et al.*, 2019; Okonofua *et al.*, 2022). T2DM, the most prevalent form of diabetes, affects millions globally, with projections indicating a rise to 643 million by 2030 and 783 million by 2045 (Saeedi *et al.*, 2019). This condition, characterized by insulin resistance and impaired insulin secretion (Ajani & Ibrahim, 2020), has been shown to

negatively impact male reproductive health (Maresch *et al.*, 2017; Omolaoye *et al.*, 2020; Zheng *et al.*, 2023). The mechanisms by which T2DM contributes to testicular damage are complex, involving oxidative stress, inflammation, DNA fragmentation, and disruption of the blood-testis barrier (Nna *et al.*, 2017; Onung *et al.*, 2023).

Streptozotocin (STZ), a chemical commonly used to induce diabetes in animal models, mimics the pathophysiology of T2DM (Pinti *et al.*, 2019). STZ induces T2D in animal models when multiple low doses are administered after feeding the animals with high fat diet for some weeks (Zhang *et al.*, 2018). STZ, a potent alkylating agent, damages DNA and disrupts cellular signaling pathways, leading to testicular oxidative stress, inflammation, and apoptosis, ultimately impairing spermatogenesis and fertility (Srinivasan *et al.*, 2018; Zhang *et al.*, 2020; Olawale *et al.*, 2021; Shokri *et al.*, 2023). Given the increasing prevalence of both T2DM and environmental toxin exposure, understanding their combined impact on male reproductive health is crucial.

Though there are various therapeutic strategies for the treatment of type 2 diabetes (Yuan *et al.*, 2016; Ekweogu *et al.*, 2019).However, natural products, particularly those with antioxidant properties, are gaining attention due its cost effective particularly for those in under developed countries and its availability.*Ocimum basilicum* (basil), a widely used culinary herb, rich in bioactive compounds, including quercetin (Mostafa, 2021; Ugbogu *et al.*, 2021). Quercetin, a flavonoid known for its potent antioxidant and anti-inflammatory effects (Boots *et al.*, 2008), has demonstrated promise in mitigating diabetes-related complications (Dong *et al.*, 2020). Its ability to scavenge free radicals, chelate metal ions, and modulate inflammatory pathways (Murakami *et al.*, 2008; Li *et al.*, 2016; Batiha *et al.*, 2020) suggests a potential protective role against STZ-induced testicular damage. Furthermore, studies have shown that quercetin can improve spermatogenic activity, sperm parameters, and reduce testicular apoptosis in diabetic models (Dhanabalan *et al.*, 2018; Adedara *et al.*, 2019). Therefore, this study investigates the impact of combined high-fat diet and STZ exposure on male reproductive health and explores the potential protective effects of quercetin against this combined insult.

MATERIALS AND METHODS

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.

Methods

Study Loccation, Sample Collection and Identification

This study was carried out in the botanical farm of the Department of Biological Sciences at latitude 7.4871°N and longitude 4.2522°E and animal house, College of Health Sciences, Benue State University, Makurdi. Ocimum basilicus leaves were selected based on their traditional use in diabetes treatments.They were collected from a natural population in botanical farm, Benue State University, Makurdi during the early raining season, April, 2023. The fresh leaves were handpicked using sterile gloves and scissors, place in clean paper bags and immediately transported to the laboratory. The leaves were identified and authenticated by group of lecturers at the Department of Biological Sciences, Rev. Fr. Moses Orshio Adasu University, Makurdi and the leaves of Ocimum basilicus were shaded dried, pulvalized and extracted using Soxhlet extractor of 70% ethanol and was concentrated and stored at 4°C

Phytochemical Analysis of the Fractions

The presence or absence of some specific phytochemicals was examined in the OB leaf fractions using standard phytochemical screening procedures described by Trease & Evans (2002)

Isolation and Identification of Quercetin

Quercetin 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.

Determination of Lethal Dose of Rutin-LD_{50}

Ocimum gratissimum (commonly known as African basil or scent leaf) is a medicinal plant rich in phytochemicals, including rutin, a flavonoid with antioxidant, anti-

inflammatory, and vasoprotective properties. Before using plant extracts therapeutically, it is essential to assess their toxicity, typically via LD_{50} .The extraction of Rutin from Ocimum gratissimum leaves typically follows the solvent extraction method, based on the solubility of rutin in polar solvents.Collection and Drying: Fresh O. gratissimum leaves are collected, washed, air-dried under shade, and pulverized then the powdered leaves are soaked or subjected to Soxhlet extraction with 70% ethanol was the major solvent which was the filtered and concentrated under reduced pressure using rotatory evaporator. Quercetin was further isolated using column chromatography

Determination of LD_{50} (Median Lethal Dose)

LD_{50} represents the dose at which 50% of test animals (usually rodents) die upon administration.According to OECD423 (OECD,2001) guidelines a single dose of extract was administered orally at varying concentration 1000, 1500,2000, 3000 and 5000mg/kg to five Wistar rats in a group and were monitored for period of seven – fourteen days and the animals were continuously observed for variations in respiration, palpitations, behavior, toxicity, and other indicators of morbidity and/or mortality beginning at 24 hours. (Lorke1983)

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	HFD QUANTITY (kg)	NORMAL DIET QUANTITY (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

COMPOSITION	HFD QUANTITY (kg)	NORMAL DIET QUANTITY (kg)
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

Fifty four (54) 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

S/N	Groups	Substance and Route of Administration	Dosage of Substance Administered	Duration of Administration	No. of Animals
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+Quer	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

Blood Sample Collection for determining fasting blood Glucose level

Gently restrain the rat, Clean the tail tip with 70% ethanol, then make a small prick at the tail tip using a sterile lancet, gently starin the tail tip to obtain a drop of blood, drop the blood on the glucometer strip finally record the reading in mmol/L

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.

Serum Hormonal Assay

Follicle-stimulating hormone (FSH) and testosterone levels were measured using enzyme immunoassay (EIA) kits based on WHO protocols. Serum samples were centrifuged at 1000 rpm for 10 minutes, aliquoted, and stored at −20°C to prevent degradation. Testosterone concentration was determined using a competitive binding EIA technique, where absorbance at 450 nm was measured spectrophotometrically.

Oxidative Stress and Antioxidant Enzyme Assays

- **Reduced Glutathione (GSH):** Estimated via the Ellman (1959) colorimetric method at 412 nm.
- **Glutathione Peroxidase (GPx):** Activity measured based on NADPH oxidation at 340 nm.
- **Catalase (CAT) Activity:** Assessed by measuring hydrogen peroxide decomposition at 240 nm.
- **Lipid Peroxidation (MDA):** Measured using thiobarbituric acid reactive substances (TBARS) assay at 535 nm.
- **Superoxide Dismutase (SOD) Activity:** Determined via inhibition of nitro-blue tetrazolium reduction at 560 nm.

Histological Tissue Processing and Sectioning

Tissues fixed in 10% formalin 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.

Ethical Considerations

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.

Statistical Analysis

Data were expressed as mean ± SD (n = 6) 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 Measurements

Significant variations in testicular weights were observed across the experimental groups (Figure 1). The control group exhibited a mean testicular weight of 1.24±0.07 g, representing normal physiological conditions. Rats fed only a high-fat diet (HFD) had a similar mean testicular weight (1.26±0.08 g), suggesting that HFD alone did not induce substantial changes. However, STZ-induced diabetic rats exhibited a significant reduction (1.00±0.40 g), demonstrating the testicular toxicity associated with streptozotocin exposure.

Rats treated with both HFD and STZ showed a slight improvement (1.17±0.08 g) compared to the STZ-only group, but the value remained lower than that of the control and HFD groups. Similarly, rats treated with HFD and quercetin (50 mg/kg) had a mean testicular weight of 1.14±0.25 g, indicating partial protective effects against oxidative stress. However, the STZ-treated group that received 50 mg/kg quercetin showed a significantly lower testicular weight (0.64±0.44 g, $p<0.05$) compared to the control and HFD groups, suggesting that low-dose quercetin did not sufficiently counteract STZ-induced damage.

Conversely, rats treated with HFD, STZ, and quercetin (75 mg/kg) exhibited a significant increase in testicular weight (1.52±0.37 g), surpassing the STZ-only and other treated groups, highlighting the protective role of quercetin at this dose. The HFD, STZ, and quercetin (100 mg/kg) group demonstrated a similar protective effect (1.48±0.66 g), comparable to the 75 mg/kg group. These findings suggest that quercetin, particularly at 75

mg/kg, significantly mitigates STZ-induced testicular toxicity in diabetic rats on a high-fat diet.

Estimated Relative Organ Weight

The estimated relative organ weight also varied significantly across groups (Figure 2). The control group had a mean relative organ weight of 0.68±0.17, serving as a reference value. The HFD group exhibited a slightly higher relative organ weight (0.76±0.09), suggesting minimal impact of a high-fat diet alone on testicular mass. However, the STZ-only group showed a marked reduction (0.53±0.14), indicating testicular atrophy likely due to STZ-induced metabolic dysfunction.

Interestingly, the HFD+STZ group had a relative organ weight of 0.68±0.21, similar to the control, suggesting that combined exposure did not exacerbate testicular weight loss beyond STZ alone. However, the STZ+50 mg/kg quercetin group exhibited a significantly lower relative organ weight (0.30±0.12, $p<0.05$), implying that low-dose quercetin may not effectively mitigate STZ-induced testicular atrophy and could even contribute to further weight reduction.

In contrast, higher doses of quercetin (75 mg/kg and 100 mg/kg) in HFD+STZ-treated rats resulted in increased relative organ weights (0.78±0.31 and 0.84±0.23, respectively), demonstrating a dose-dependent protective effect against metabolic-induced testicular damage.

Overall, these findings indicate that STZ significantly reduces testicular weight and relative organ weight, while higher doses of quercetin (75 mg/kg and 100 mg/kg) offer a protective effect, mitigating testicular atrophy in diabetic rats subjected to a high-fat diet.

Reproductive Hormones

Analysis of reproductive hormone levels across the experimental groups revealed significant variations (Figures 3 - 5).

Testicular Testosterone (T.Testosterone): The control group exhibited the highest mean level (3.67±1.53 ng/ml), while groups exposed to HFD, STZ, and HFD+STZ showed significantly reduced levels. The STZ-only and HFD+STZ groups had the lowest values. Quercetin treatment led to notable improvements, with the HFD+STZ+100 mg/kg group showing a significant recovery (2.42±0.53 ng/ml) compared to the untreated HFD+STZ group.

Serum Testosterone (S.Testosterone): The control group recorded the highest levels (2.02±0.57 ng/ml), with significant reductions observed in HFD, STZ, and HFD+STZ groups. Quercetin supplementation at 75 mg/kg and 100 mg/kg effectively restored serum testosterone levels, with the HFD+STZ+75 mg/kg group showing the highest recovery (2.55±0.12 ng/ml).

Luteinizing Hormone (LH): The control group had the highest LH levels (2.50±0.49 mIU/ml), while HFD, STZ, and HFD+STZ exposure resulted in significant declines. Quercetin moderately improved LH levels, particularly in the STZ+50 mg/kg (2.00±0.42 mIU/ml) and HFD+STZ+100 mg/kg (1.95±0.71 mIU/ml) groups.

These findings indicate that HFD and STZ exposure impair reproductive hormone profiles, with their combination leading to more severe disruptions. Quercetin demonstrated a dose-dependent protective effect, particularly at 75 mg/kg and 100 mg/kg, in mitigating these hormonal disturbances.

Table for Mean Reproductive Hormones Levels across Groups Compared on One – Way ANOVA

Group (N)	T.Testosterone (ng/ml)	S.Testosterone (ng/ml)	LH (mlU/ml)
Control (Dist. Water)	3.67±1.53$^{+\wedge}$	2.02±0.57$^{+\wedge}$	2.50±0.49$^{+\wedge}$
HFD	1.80±0.62*	1.36±0.72*	1.60±0.31*
STZ	1.22±0.28*	1.07±0.28*	1.02±0.29*
HFD+STZ	1.45±0.17*	1.00±0.18*	0.92±0.20^{*+}
HFD+ 50mg/kg QUE	2.00±0.59*	2.17±0.29$^{+\wedge}$	1.50±0.33*
STZ+ 50mg/kg QUE	1.30±0.27*	1.77±0.37$^{\wedge}$	2.00±0.42$^{\wedge}$
HFD+STZ+75mg/kg QUE	2.02±0.44*	2.55±0.12$^{+\wedge}$	1.77±0.34$^{*\wedge}$
HFD+STZ+100mg/kg QUE	2.42±0.53$^{*\wedge}$	2.05±0.47$^{+\wedge}$	1.95±0.71$^{\wedge}$

N = 6; T.Testosterone = Testicular Testosterone; S.Testosterone = Serum Testosterone; LH = Luteinizing Hormone; HFD = High Fat Diet; STZ = Streptozotocin; Quercetin = QUE; * = statistically significant difference in mean at $p<0.05$ compared to the control group; + = statistically significant difference in mean at $p<0.05$ compared to the HFD – only group; ^ = statistically significant difference in mean at $p<0.05$ compared to the STZ – only group

Oxidative Stress Markers

The impact of HFD, STZ, and quercetin on oxidative stress markers

Superoxide Dismutase (SOD): The control group exhibited the highest SOD activity (23.60±1.95 U/mg protein). HFD (17.22±1.66) and STZ (15.65±2.75) groups showed significant reductions ($p<0.05$), indicating increased oxidative stress. Quercetin treatment at 50 mg/kg (21.80±3.96) and 75 mg/kg (21.62±3.15) significantly enhanced SOD activity compared to the HFD-only group.

Malondialdehyde (MDA): The highest MDA levels were observed in the HFD+STZ group (6.90±10.07 nmol/mg protein), suggesting increased lipid peroxidation. Quercetin significantly reduced MDA levels at 50 mg/kg (1.30±0.37) and 75 mg/kg (0.85±0.34), indicating reduced oxidative damage.

Glutathione Peroxidase (GPx): GPx activity was markedly lower in the HFD (1.32±0.17) and HFD+STZ (1.30±0.18) groups compared to the control (2.25±0.31). Quercetin supplementation partially restored GPx activity, with the STZ+50 mg/kg group (2.15±0.25) nearing normal levels.

Glutathione (GSH): The control group maintained the highest GSH levels (24.55±3.29 U/mg protein), while significant reductions were seen in the HFD (13.50±3.40) and HFD+STZ (17.35±3.75) groups ($p<0.05$). Quercetin supplementation effectively restored GSH levels, particularly at 50 mg/kg (21.60 ±3.09) and STZ+50 mg/kg (23.10±2.25).

These results suggest that HFD and STZ exposure exacerbate oxidative stress by reducing antioxidant enzyme activity and increasing lipid peroxidation. Quercetin exhibited dose-dependent antioxidant properties, significantly improving oxidative stress markers, with the most pronounced effects observed at 50 mg/kg and 75 mg/kg.

Table for the Mean Oxidative Stress Markers across Groups Compared on One – Way ANOVA

Group (N)	SOD (U/mg pro)	MDA (nmol/mg pro)	GPx (U/mg pro)	GSH (U/mg pro)
Control (Normal saline)	23.60±1.95$^{+\wedge}$	0.80±0.21	2.25±0.31$^{+\wedge}$	24.55±3.29$^{+\wedge}$
HFD	17.22±1.66*	1.65±0.38	1.32±0.17*	13.50±3.40*
STZ	15.65±2.75*	1.77±0.69	1.52±0.12*	16.70±2.54*

Group (N)	SOD (U/mg pro)	MDA (nmol/mg pro)	GPx (U/mg pro)	GSH (U/mg pro)
HFD+STZ	13.62±1.17*	6.90±10.07*+	1.30±0.18*	17.35±3.75*
HFD+ 50mg/kg QER	21.80±3.96+^	1.30±0.37	1.70±0.27*	21.60±3.09+^
STZ+ 50mg/kg QER	17.22±1.79*	0.92±0.27	2.15±0.25+^	23.10±2.25+^
HFD+STZ+75mg/kg QER	21.62±3.15+^	0.85±0.34	1.60±0.16*	19.10±3.55*+
HFD+STZ+100mg/kg QER	17.82±3.37*	0.90±0.23	1.70±0.46*	19.82±2.83*+

N = 6; SOD = Superoxide Dismutase; MDA = Malondialdehyde; Glutathione Peroxidase; GSH = Glutathione; HFD = High Fat Diet; STZ = Streptozotocin; Quercetin = QER; * = statistically significant difference in mean at $p<0.05$ compared to the control group; + = statistically significant difference in mean at $p<0.05$ compared to the HFD – only group; ^ = statistically significant difference in mean at $p<0.05$ compared to the STZ – only group

Sperm Count

The control group exhibited the highest sperm count (62.10±3.88 $\times 10^6$/ml). Both the high-fat diet (HFD) and streptozotocin (STZ) groups showed significantly reduced sperm counts (24.67±22.50 $\times 10^6$/ml and 19.40±19.45 $\times 10^6$/ml, respectively) compared to the control ($p<0.05$), indicating adverse effects on male fertility. Quercetin supplementation at 50 mg/kg significantly improved sperm count in the HFD+50 mg/kg Quer. group (56.65±21.48 $\times 10^6$/ml, $p<0.05$), while the STZ+50 mg/kg Quer. group showed no significant change. In the combined HFD+STZ treatment, sperm count increased moderately with higher quercetin doses (41.15±11.31 $\times 10^6$/ml at 100 mg/kg) but did not reach statistical significance relative to the control. These findings suggest a dose-dependent protective role of quercetin, particularly in HFD-induced reproductive toxicity.

Sperm Morphology

The control group displayed the highest percentage of normal sperm morphology (75.65%), while the STZ group exhibited a significant reduction (49.40%, $p < 0.05$), indicating STZ's detrimental effect on sperm quality. The HFD group showed a slight decline (73.90%). The HFD+STZ group demonstrated moderate improvement (64.15%) but remained significantly lower than the control. Quercetin treatment improved sperm morphology in the HFD+50 mg/kg Quer. group (70.57%) and higher doses (75 mg/kg and 100 mg/kg) brought values closer to the control. However, STZ+50 mg/kg Quer. and

HFD+STZ+Quercetin groups (75 mg/kg and 100 mg/kg) exhibited lower sperm morphology percentages (47.30% and 45.45%, respectively), suggesting a potential adverse effect of quercetin when combined with STZ and HFD at higher doses.

Sperm Motility

Sperm motility was highest in the control group (73.70±7.63%), while the HFD and STZ groups exhibited significantly lower motility (31.42±25.19% and 31.47±1.74%, respectively, $p< 0.05$). Quercetin treatment at 100 mg/kg significantly improved motility (64.07±16.75%), whereas the 75 mg/kg dose (34.72±31.18%) showed no significant improvement. These results indicate that both HFD and STZ severely impair sperm motility, while quercetin exerts a dose-dependent protective effect, with the 100 mg/kg dose offering the most noticeable improvement.

Sperm Progressivity

The control group showed the highest sperm progressivity (2.00±0.00), while the HFD, STZ, and HFD+STZ groups had significantly reduced progressivity scores (1.50±0.57 and 1.00±0.00 for HFD+STZ, $p<0.05$). Quercetin treatment at all doses improved sperm progressivity in the HFD+STZ group, with the highest improvement observed at 100 mg/kg (1.75±0.50). However, no group reached the control levels, indicating that while quercetin offers some protection, it does not fully counteract the negative effects of HFD and STZ on sperm motility.

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.

DISCUSSION

The observed variations in testicular weight across the experimental groups highlight the detrimental effects of streptozotocin (STZ)-induced diabetes on male reproductive health, as well as the potential protective role of quercetin. The significant reduction in testicular weight in the STZ-only group aligns with previous findings indicating testicular atrophy due to diabetes-induced oxidative stress, inflammation, and apoptosis (Shen *et al.*, 2021). Similar studies have reported that STZ-induced diabetes significantly reduces testicular weight in rats due to impaired spermatogenesis and germ cell loss (Chen *et al.*, 2022).

Interestingly, the high-fat diet (HFD) group exhibited testicular weights comparable to the control, suggesting that diet alone did not exert substantial damage. However, combining HFD with STZ resulted in slightly improved testicular weights compared to STZ alone, which may be attributed to HFD-induced lipid accumulation counteracting some of the weight loss. This finding corroborates previous reports that HFD alone does not drastically impair testicular structure but exacerbates damage when combined with metabolic disturbances like diabetes (Wang *et al.*, 2023).

The protective effect of quercetin was evident, particularly at higher doses (75 mg/kg and 100 mg/kg), where significant improvements in testicular weight were observed. This supports existing literature indicating that quercetin mitigates testicular atrophy through its antioxidative and anti-inflammatory properties (Alharthi *et al.*, 2021). The lower testicular weight in the STZ+50 mg/kg quercetin group suggests that this dose was insufficient to counteract the STZ-induced toxicity, aligning with studies showing a dose-dependent effect of flavonoids in testicular protection (Luo *et al.*, 2022).

The relative testicular weight findings further substantiate the impact of STZ-induced diabetes on testicular atrophy. The significantly lower relative testicular weight in the STZ-only group indicates metabolic dysfunction and testicular tissue loss, which is consistent with findings by Rahman *et al.* (2023), who reported significant testicular shrinkage in diabetic rodents due to oxidative stress-mediated apoptosis.

The minimal effect of HFD alone supports the notion that while obesity-related metabolic changes can impact fertility, they do not necessarily lead to drastic testicular atrophy unless combined with other stressors like diabetes (Zhou *et al.*, 2024). The STZ+50 mg/kg quercetin group exhibited further reductions in relative organ weight, suggesting

that low-dose quercetin might not be sufficient to prevent STZ-induced testicular degeneration.

Conversely, quercetin at 75 mg/kg and 100 mg/kg significantly improved relative testicular weight, reinforcing its protective role against diabetes-induced oxidative stress. Previous studies have demonstrated that quercetin at higher doses enhances testicular antioxidant enzyme activity and preserves germ cell integrity in diabetic rats (Eid *et al.*, 2021). These results suggest that quercetin's efficacy in preserving testicular weight and function is dose-dependent, with optimal effects observed at 75 mg/kg and above.

The alterations in reproductive hormone levels observed in this study further illustrate the adverse impact of STZ and HFD on male reproductive health. The significant reduction in testicular and serum testosterone levels in the STZ-only and HFD+STZ groups aligns with research indicating that diabetes-induced oxidative stress disrupts Leydig cell function, leading to impaired testosterone biosynthesis (Chen *et al.*, 2023). Previous studies have shown that STZ-induced diabetes decreases luteinizing hormone (LH) and testosterone levels due to hypothalamic-pituitary-gonadal (HPG) axis dysfunction (Shahid *et al.*, 2021).

Quercetin supplementation significantly restored testosterone levels, particularly at 75 mg/kg and 100 mg/kg, further supporting its role in preserving Leydig cell function and maintaining steroidogenesis. These findings are consistent with reports that quercetin enhances testicular antioxidant defense mechanisms and reduces oxidative stress-mediated suppression of testosterone production (Yang *et al.*, 2022). The moderate recovery of LH levels in quercetin-treated groups suggests that quercetin may have a regulatory effect on the HPG axis, as previously documented in studies on flavonoids and male fertility (Khan *et al.*, 2024).

The oxidative stress marker analysis confirms that STZ exposure and HFD significantly contribute to testicular oxidative damage. The observed decline in superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in the STZ and HFD+STZ groups is in agreement with previous findings that diabetes-induced oxidative stress depletes antioxidant enzyme reserves, leading to increased lipid peroxidation and cellular damage (Alam *et al.*, 2023).

Quercetin supplementation significantly improved SOD and GPx levels, particularly at 50 mg/kg and 75 mg/kg, indicating its potent antioxidant activity. These

results are supported by findings from Luo *et al.* (2022), which demonstrated that quercetin supplementation restores antioxidant enzyme function and reduces oxidative damage in diabetic testes. The reduction in malondialdehyde (MDA) levels in quercetin-treated groups further reinforces its role in mitigating lipid peroxidation and oxidative stress, consistent with recent studies demonstrating flavonoids' protective effects against testicular oxidative damage (Zhao *et al.*, 2023).

The increased glutathione (GSH) levels in quercetin-treated groups highlight its ability to enhance endogenous antioxidant defenses, which is in line with previous reports that quercetin upregulates glutathione synthesis and protects testicular tissue from oxidative injury (Eid *et al.*, 2021). The dose-dependent improvements in oxidative stress markers suggest that higher doses (75 mg/kg and 100 mg/kg) provide optimal protection, further corroborating previous findings on flavonoid-mediated testicular protection (Wang *et al.*, 2023).

The observed decrease in sperm count in the HFD and STZ groups compared to the control aligns with previous studies highlighting the adverse effects of metabolic stress and diabetes on male fertility. In particular, a high-fat diet has been shown to cause oxidative stress and inflammation, which can impair spermatogenesis and reduce sperm production (Tiwari *et al.*, 2021). Similarly, STZ-induced diabetes has been reported to disrupt testicular function by inducing oxidative stress and compromising the hypothalamic-pituitary-gonadal axis (Yin *et al.*, 2020). Quercetin supplementation, especially at 50 mg/kg, partially restored sperm count in the HFD+50 mg/kg quercetin group, suggesting its antioxidative properties as a potential mechanism for protecting against diet-induced reproductive toxicity. However, no significant improvement was observed in the STZ+50 mg/kg quercetin group, suggesting that the protective effects of quercetin might be limited under the more severe metabolic stress induced by STZ.

These findings are consistent with studies demonstrating the protective effects of quercetin against various forms of oxidative damage. For instance, in a study by Rahman *et al.* (2022), quercetin was found to enhance spermatogenesis in rats exposed to oxidative stress induced by lead and cadmium. Furthermore, quercetin's role in mitigating metabolic disruptions associated with obesity and diabetes has been reported by Wang *et al.* (2023), who noted improvements in sperm quality in diabetic rats treated with quercetin.

Sperm morphology was significantly compromised in the STZ group compared to the control. This reduction in normal sperm morphology can be attributed to STZ-induced oxidative stress, which damages spermatozoa and disrupts sperm head formation, a phenomenon observed in several diabetic models (Mishra *et al.*, 2021). The slight decrease in sperm morphology in the HFD group is in line with the findings of previous studies, where high-fat diets were shown to reduce sperm quality due to alterations in the lipid metabolism of the testicular cells (Santos *et al.*, 2020).

The moderate improvement observed in the HFD+STZ+quercetin groups is promising, though not fully restoring sperm morphology to control levels. Interestingly, the higher doses of quercetin in the STZ+quercetin and HFD+STZ+quercetin groups resulted in further reductions in sperm morphology, which suggests that quercetin may exert a dose-dependent effect. Higher doses may potentially exacerbate oxidative stress or disrupt normal sperm development under the combined metabolic disturbances of HFD and STZ. These findings align with studies such as that by Al-Masri *et al.* (2021), who observed that high doses of quercetin in diabetic rats led to dose-dependent changes in sperm morphology, potentially due to its interactions with the endocrine system or its effects on gene expression.

Sperm motility was significantly reduced in the HFD and STZ groups, which corroborates findings from similar studies. It has been well-established that both obesity and diabetes impair sperm motility by altering mitochondrial function and increasing reactive oxygen species (ROS) production (Zhao *et al.*, 2020). The significant improvement in motility observed at the 100 mg/kg quercetin dose provides further evidence for quercetin's protective role as an antioxidant, which has been previously shown to enhance mitochondrial activity and sperm motility (Li *et al.*, 2021). However, the absence of significant improvement at the 75 mg/kg dose suggests a threshold effect of quercetin, where higher doses may be required to achieve a noticeable benefit in motility.

These results support those of Lee *et al.* (2022), who demonstrated that quercetin treatment restored sperm motility in diabetic rats by reducing oxidative stress and enhancing antioxidant defenses in testicular tissues. Similarly, quercetin's beneficial effects on sperm motility have been attributed to its ability to modulate mitochondrial function and protect against oxidative damage (Poojari *et al.*, 2023).

The sperm progressivity scores were also reduced in the HFD, STZ, and HFD+STZ groups compared to the control group. Although quercetin improved sperm progressivity across all doses in the HFD+STZ group, no group reached the control levels. The lack of complete restoration suggests that quercetin's protective role, while significant, may not fully counteract the combined effects of HFD and STZ on sperm motility and function. A similar pattern of partial restoration of sperm progressivity was observed in the study by Khalid *et al.* (2020), where quercetin treatment improved sperm motility and progressivity in diabetic rats, although the full protective effect was not observed.

Histological examination of the testicular tissues further corroborated the observed functional outcomes. In the control and quercetin-treated groups, the testicular architecture was well-preserved, with normal Leydig, Sertoli, and spermatogenic cells. However, significant histopathological abnormalities were observed in the STZ group, including atrophic tubules and disorganized cellular structures, which are characteristic of STZ-induced diabetic testicular damage (Mekhemar *et al.*, 2020). Quercetin treatment improved these histological features, consistent with its antioxidative and anti-inflammatory properties, and fewer tubules showed stagnant spermatogenic cells, indicating a potential protective effect. This aligns with previous studies (El-Sayed *et al.*, 2021), which demonstrated that quercetin administration improved testicular histology in diabetic rats by reducing inflammation and oxidative damage.

CONCLUSION

The study demonstrates that STZ-induced diabetes significantly impairs male reproductive health, as evidenced by reduced testicular weight, hormonal disturbances, and compromised sperm parameters. A high-fat diet alone did not cause severe reproductive damage but exacerbated the effects of diabetes. Quercetin supplementation, particularly at higher doses, effectively mitigated testicular atrophy, oxidative stress, and hormonal disruptions, suggesting its potential as a protective agent against diabetes-induced reproductive toxicity. These findings highlight quercetin's dose-dependent protective role, though higher doses may not fully restore sperm morphology and motility.

Contribution to knowledge

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-inflmmatory, anti-oxidant, anti–diabetes properties which counter the type 2 diabetes effect and restore normal testicular histmorphology

Recommendation

Further studies on the effects of Rutin on the process of steroidogenesis and spermatogenesis in health and disease are needed. More also direct evidence of the mechanisms on Quercetin's impact specifically in the testis of diabetic individuals still needs further investigation.

Conflict of Interest

The research was conducted in the absence of any commercial, Institution or financial relationships that could be construed as a potential conflict of interest.

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