International Journal of Medical Toxicology & Forensic Medicine
2026, Volume 16, E53622

Research Paper

Effects of Three Oral Orlistat Doses on Testicular Oxidative Stress, Histology, and Semen Quality in Male Wistar Rats Fed a High-Fat Diet: A Randomized Animal Experiment

Hussein Kamil Awad[1]*, Hiba Majid Rasool[2], Sura M. Alkadhimy[3], Noor Mahdi Fathil[4], Bilal Husam Jasim[5], Ashraf Ayyal Mutar Alrashedi[1], Asmaa M. Neamah[6], Emam Atiyah Ibadi[7], Mohammed A. Albarki[8]

1. *Unit of Environment and Prevention of Pollution, College of Science, University of Al-Qadisiyah, Diwaniyah, Iraq.*
2. *Department of Basic Sciences, College of Dentistry, Al-Iraqia University, Baghdad, Iraq.*
3. *Department of Biology, College of Science, University of Karbala, Karbala, Iraq.*
4. *Department of Biology, College of Science for Women, University of Baghdad, Baghdad, Iraq.*
5. *Biotechnology Department, College of Applied Sciences, University of Technology–Iraq, Baghdad, Iraq.*
6. *Department of Environment, College of Science, University of Al-Qadisiyah, Diwaniyah, Iraq.*
7. *Department of Environment, College of Science, University of Al-Qadisiyah, Diwaniyah, Iraq.*
8. *College of Pharmacy, Al-Mustaqbal University, Babylon, Iraqq.*

Citation Kamil Awad H, Majid Rasool H, M. Alkadhimy S, Mahdi Fathil N, Husam Jasim B, Mutar Alrashedi AA, et al. Effects of Three Oral Orlistat Doses on Testicular Oxidative Stress, Histology, and Semen Quality in Male Wistar Rats Fed a High-Fat Diet: A Randomized Animal Experiment. International Journal of Medical Toxicology and Forensic Medicine. 2026; 16:E53622.

https://doi.org/10.22037/ijmtfm.v16.53622

Article info:

Received: 29 September, 2026

Accepted: 14 October, 2026

Published: 07 November, 2026

Keywords:

Orlistat, Diet, High-Fat, Sperm Motility, Oxidative Stress, Testosterone, Rats, Wistar

ABSTRACT

Background: High-fat diet (HFD) exposure can alter testicular redox balance and male reproductive function. This study compared the effects of three oral orlistat doses on epididymal sperm characteristics, testicular biochemical indices, plasma testosterone, and testicular histology in HFD-fed rats.

Methods: Twenty-five male Wistar rats were randomized to five groups (n = 5 each): standard-diet control, HFD control, or HFD plus orlistat at 360, 480, or 600 mg/kg/day for 60 days. We assessed sperm motility, viability, and morphology; testicular lipid peroxidation, oxidant status, and antioxidant measures; plasma testosterone; and qualitative hematoxylin-and-eosin histology.

Results: The high-fat diet control exhibited elevated TBARS, sperm abnormalities, and testosterone compared to standard diet controls, whereas orlistat treatment reduced sperm motility and viability, particularly at higher doses; it lowered TBARS and MDA levels, modulated antioxidant markers such as GST and GSH at the highest dose, and caused focal tubular disorganization at 600 mg/kg/day while preserving tubular structure at moderate doses.

Conclusion: In this exploratory animal experiment, lower reported lipid-peroxidation measures co-occurred with lower sperm motility and viability at higher orlistat doses. The divergent biochemical and reproductive patterns support endpoint-specific interpretation rather than a uniformly beneficial response or an optimal therapeutic dose.

*** Corresponding Author:**
Hussein Kamil Awad, PhD
Unit of Environment and Prevention of Pollution, College of Science, University of Al-Qadisiyah, Diwaniyah, Iraq.
E-mail:

Copyright *hussein.Kamel@qu.edu.iq* © 2026 The Author(s);
This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0: https://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Obesity is associated with impaired male reproductive function through endocrine disturbance, inflammation, altered scrotal thermoregulation, and oxidative stress. Excess reactive species can damage the polyunsaturated lipids of sperm membranes and interfere with spermatogenesis, sperm function, and steroidogenesis. Epidemiological and experimental studies have consequently linked excess adiposity with altered semen quality and testicular injury [1–3]. However, individual reproductive endpoints need not change in parallel, and the response to a high-fat diet depends on the experimental context.

Orlistat is an intestinal lipase inhibitor that reduces dietary fat absorption and is used for weight management [4]. Reduced dietary lipid availability may modify HFD-associated metabolic and reproductive responses. Orlistat also inhibits fatty acid synthase in cellular experimental systems [5], but this mechanism is not assumed to account for reproductive changes following oral administration. Rodent studies have reported improvements in testicular oxidative and inflammatory indices with orlistat, but their doses, dietary protocols, and treatment schedules vary [3, 6]. This variation makes it important to assess sperm, endocrine, biochemical, and structural endpoints separately.

The present randomized animal experiment compared three oral orlistat dose levels in male Wistar rats fed an HFD for 60 days. The objectives were to characterize epididymal sperm motility, viability, and morphology; testicular lipid peroxidation and antioxidant measures; plasma testosterone; and qualitative testicular histology. The study evaluated whether these outcomes showed concordant or divergent patterns across dose groups; it was not intended to establish a clinical dosing recommendation.

Materials and Methods

Study design and animals

Twenty-five male albino Wistar rats, aged 2 months and weighing 190 ± 5 g, were obtained from the Animal House of the College of Science, University of Al-Qadisiyah. After 2 weeks of acclimatization, animals were housed in standard polypropylene cages (5 rats per cage) at 22–25 °C under a 12-hour light/dark cycle, with free access to water and diet. Rats were randomly allocated using a simple random procedure to five groups (n = 5 per group): standard-diet control (Control A), HFD control (Control B), and HFD plus oral orlistat at 360, 480, or 600 mg/kg/day (OS-360, OS-480, and OS-600, respectively). Saline or treatment was administered by oral gavage once daily for 60 days.

The five-animal group size was selected based on previous HFD and reproductive-toxicity experimental designs. The study was exploratory. Investigators blinded to group allocation conducted semen assessments and histological examinations. Body weights were recorded during monitoring. All 25 animals were included in the final summaries; no premature deaths or adverse clinical events were reported.

Diet and treatment

The HFD was prepared by mixing standard powdered chow with 30% melted animal abdominal fat by weight, adapting a previously described dietary formulation [7]. The mixture was formed into pellets and air-dried before administration. Orlistat was obtained as a commercial pharmaceutical formulation (Xenical®, Hoffmann-La Roche, Basel, Switzerland; batch ORL-2023-04). Capsule contents were dispersed in distilled water to form a suspension and administered by gavage at a total volume of 1 mL per rat per day. The protocol compared 360, 480, and 600 mg/kg/day as exploratory high-dose exposures over 60 days. These doses are experimental exposure levels and are not presented as clinically equivalent doses.

Sample collection

At the end of the 60-day exposure, animals were anesthetized with isoflurane and euthanized. Blood was collected from the vena cava into heparinized tubes and centrifuged at 860 × g for 20 minutes. Plasma was stored at −80°C until analysis. Testes were excised, weighed, and rinsed with saline. Tissue was either frozen at −80°C for biochemical analysis or fixed in 10% neutral buffered formalin for histological examination.

Sperm assessment

The cauda epididymis was minced in phosphate-buffered saline (pH 7.4) at 37°C. At least 200 spermatozoa per rat were examined at 400× magnification. Motility was expressed as the percentage exhibiting forward progression [8]. Viability was assessed in eosin–nigrosin-stained smears by classifying 200 spermatozoa as unstained (viable) or eosin-positive (nonviable) [9]. Morphology was evaluated in 200 spermatozoa at 1000× oil-immersion magnification, and head, neck/midpiece, and tail abnormalities were recorded separately [8]. The rat, rather than the individual spermatozoon, was the unit used for the reported group summaries.

Biochemical measurements

Testicular tissue was homogenized in ice-cold phosphate-buffered saline (pH 7.4) and centrifuged at 10,000 × g for 15 minutes at 4 °C. The supernatant was used for biochemical assays. Lipid peroxidation was assessed by the thiobarbituric acid-reactive substances (TBARS) procedure of Ohkawa et al. [10], which expresses thiobarbituric acid reactivity as malondialdehyde (MDA) equivalents. Total oxidant status (TOS) was described using Erel's colorimetric method [11]. This assay measures composite oxidant status against a hydrogen peroxide calibration rather than directly measuring individual reactive oxygen species. Peroxiredoxin activity was measured using the fluorometric method of Mohsin et al. [12].

Glutathione S-transferase (GST) activity was assessed using reduced glutathione and 1-chloro-2,4-dinitrobenzene at 340 nm [13]. Reduced glutathione (GSH) was measured with Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid)) at 412 nm [14]. Glutathione peroxidase (GPx) was assessed by following GSH consumption in the presence of hydrogen peroxide [15]. Superoxide dismutase (SOD) activity was assessed by inhibition of pyrogallol autoxidation at 420 nm [16]. Biochemical values are reported on their assay scales without unit conversion.

Testosterone assay

Plasma testosterone was quantified using a commercial competitive testosterone enzyme-linked immunosorbent assay (DRG International, Inc., Springfield, NJ, USA; EIA-1559, lot 43K082), following the manufacturer's instructions. Testosterone concentrations are expressed in ng/mL. The manufacturer-specified analytical sensitivity was 0.083 ng/mL; this specification describes the kit rather than a study-specific validation result.

Histological examination

Formalin-fixed testicular tissue was dehydrated through graded alcohols, cleared in xylene, embedded in paraffin wax, sectioned at 5 µm, and stained with hematoxylin and eosin (H&E). Sections were examined using an Olympus BX51 light microscope (Olympus Corp., Tokyo, Japan), with nominal low- and high-power acquisition settings of 100× and 400×.

Histological examination was conducted in a randomized and blinded manner by an experienced pathologist unaware of group allocation. Findings are presented as qualitative observations of seminiferous-tubule organization, germinal epithelium, luminal spermatozoa, interstitial spacing, vacuolation, and focal disorganization. Representative images illustrate these observations without assigning quantitative morphometric or spermatogenic scores.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 20.0 (IBM Corp., USA). Quantitative outcomes are presented as mean ± SD for five rats per group. Between-group comparisons for the outcomes in Tables 1–4 used one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple pairwise comparisons. Statistical significance was defined as $P < 0.05$. Within a table column, values that do not share a superscript letter indicate a significant difference according to Tukey's test. Motility and viability are expressed in percentage points, and testosterone is expressed in ng/mL.

Results

Sperm characteristics

Control B had numerically higher motility and viability than Control A (95.3 ± 5.6% versus 90.1 ± 4.47%, and 75.6 ± 1.66% versus 73.8 ± 0.80%, respectively); the control groups shared superscript letters and did not differ significantly according to the reported Tukey comparisons. Motility was significantly lower in OS-360, OS-480, and OS-600 than in Control B (87.3 ± 6.4%, 85.6 ± 5.4%, and 84.5 ± 4.3%, respectively; $P < 0.05$). Viability was 72.6 ± 1.36%, 67.6 ± 2.18%, and 62.8 ± 2.88% across these groups and was significantly lower than Control B at 480 and 600 mg/kg/day. The differences from Control B were −8.0, −9.7, and −10.8 percentage points for motility and −3.0, −8.0, and −12.8 percentage points for viability. Head, neck, and tail abnormality percentages were significantly higher in Control B than in Control A. Neck abnormalities were significantly lower in OS-600 than in Control B, whereas head and tail abnormalities did not differ significantly between Control B and the orlistat groups (Table 1).

Lipid-peroxidation and oxidant-status measures

TBARS was significantly higher in Control B than in Control A (3.40 ± 0.055 versus 1.34 ± 0.042; $P < 0.05$). Values were 2.90 ± 0.077, 2.60 ± 0.045, and 1.40 ± 0.060 in OS-360, OS-480, and OS-600; only OS-600 differed significantly from Control B according to the reported Tukey grouping. The separately reported MDA values were 36.98 ± 0.35 in Control A and 37.99 ± 0.40 in Control B, with no significant difference between the controls. MDA was significantly lower in all orlistat groups than in Control B (35.70 ± 0.39, 34.75 ± 0.36, and 34.80 ± 0.34, respectively; $P < 0.05$). They showed no significant between-group differences in the reported analysis (Table 2).

Table 1. Epididymal sperm characteristics in standard-diet, HFD-control, and HFD-plus-orlistat groups.

Group	Motility (%)	Viability (%)	Neck abnormality (%)	Tail abnormality (%)	Head abnormality (%)
Control A	$90.1^{ab} \pm 4.47$	$73.8^{ab} \pm 0.80$	$1.6^{b} \pm 1.3$	$7.1^{b} \pm 3.1$	$2.6^{b} \pm 1.1$
Control B	$95.3^{a} \pm 5.6$	$75.6^{a} \pm 1.66$	$3.5^{a} \pm 1.6$	$9.2^{a} \pm 4.1$	$6.7^{a} \pm 4.6$
OS-360	$87.3^{b} \pm 6.4$	$72.6^{ab} \pm 1.36$	$2.5^{ab} \pm 1.7$	$12.2^{a} \pm 5.6$	$8.8^{a} \pm 4.7$
OS-480	$85.6^{b} \pm 5.4$	$67.6^{b} \pm 2.18$	$2.4^{ab} \pm 1.5$	$10.3^{a} \pm 6.5$	$7.7^{a} \pm 4.4$
OS-600	$84.5^{b} \pm 4.3$	$62.8^{bc} \pm 2.88$	$2.3^{b} \pm 1.4$	$9.3^{a} \pm 6.6$	$4.3^{a} \pm 4.1$

International Journal of Medical Toxicology & Forensic Medicine

Data are mean ± SD (n = 5 rats per group). Control A, standard-diet control; Control B, HFD control; OS-360, OS-480, and OS-600, HFD plus orlistat at 360, 480, and 600 mg/kg/day, respectively. Within a column, values that do not share a superscript letter differ at $P<0.05$ by one-way ANOVA with Tukey's post hoc test.

Table 2. Reported testicular lipid peroxidation, oxidant-status, and peroxiredoxin measures.

Group	TBARS (reported mmol/mg)	MDA (reported nmol/g)	Oxidant-status index	Peroxiredoxin
Control A	$1.34^{b} \pm 0.042$	$36.98^{a} \pm 0.35$	$15.95^{a} \pm 0.10$	$3.90^{a} \pm 0.04$
Control B	$3.40^{a} \pm 0.055$	$37.99^{a} \pm 0.40$	$15.71^{a} \pm 0.02$	$3.74^{a} \pm 0.03$
OS-360	$2.90^{ab} \pm 0.077$	$35.70^{b} \pm 0.39$	$15.40^{a} \pm 0.40$	$3.44^{a} \pm 0.14$
OS-480	$2.60^{ab} \pm 0.045$	$34.75^{b} \pm 0.36$	$16.27^{a} \pm 0.04$	$3.37^{a} \pm 0.23$
OS-600	$1.40^{b} \pm 0.060$	$34.80^{bc} \pm 0.34$	$16.38^{a} \pm 0.40$	$3.65^{a} \pm 0.15$

International Journal of Medical Toxicology & Forensic Medicine

Data are mean ± SD (n = 5 rats per group). Within a column, values that do not share a superscript letter differ at $P<0.05$ by one-way ANOVA with Tukey's post hoc test.

Antioxidant measures

GSH was significantly higher in Control B than in Control A, whereas GST, GPx, and SOD did not differ significantly between the control groups. GST and GSH were significantly lower in OS-600 than in Control B (12.11 ± 2.27 versus 22.82 ± 0.17, and 188.13 ± 5.57 versus 206.19 ± 4.98, respectively; $P < 0.05$). GST did not decline uniformly across the orlistat doses, with a higher mean in OS-480 than in OS-360. GPx values decreased numerically from 48.85 ± 4.55 to 45.79 ± 4.50 and 40.56 ± 2.13 across OS-360, OS-480, and OS-600. Corresponding SOD values were 8.30 ± 2.48, 8.20 ± 1.45, and 5.90 ± 1.45. Control B shared superscript letters with each treatment group for GPx and SOD, indicating no significant difference between Control B and the treatment groups in the reported Tukey comparisons (Table 3).

Plasma testosterone

Plasma testosterone was significantly higher in Control B than in Control A (2.50 ± 0.06 versus 1.43 ± 0.02 ng/mL; $P < 0.05$). Values were 1.80 ± 0.05, 1.60 ± 0.04, and 1.44 ± 0.03 ng/mL in OS-360, OS-480, and OS-600, respectively. The corresponding differences from Control B were −0.70, −0.90, and −1.06 ng/mL. Only OS-600 differed significantly from Control B according to the reported Tukey grouping, and its concentration was close to Control A, with which it shared a superscript letter (Table 4).

Histological observations

Control A showed generally regular seminiferous-tubule architecture, preserved germinal epithelium, and luminal spermatozoa (Figure 1).

Table 3. Reported testicular lipid peroxidation, oxidant-status, and peroxiredoxin measures.

Group	GST	GSH (reported mg/dL)	GPx (reported mmol/min/mL)	SOD (reported U/mg)
Control A	$20.07^{a} \pm 0.85$	$185.17^{b} \pm 2.98$	$51.89^{a} \pm 4.67$	$10.28^{a} \pm 1.45$
Control B	$22.82^{a} \pm 0.17$	$206.19^{a} \pm 4.98$	$50.93^{ab} \pm 5.70$	$11.35^{ab} \pm 3.50$
OS-360	$17.44^{ab} \pm 0.23$	$198.18^{ab} \pm 3.95$	$48.85^{b} \pm 4.55$	$8.30^{b} \pm 2.48$
OS-480	$19.79^{ab} \pm 2.50$	$190.13^{ab} \pm 3.88$	$45.79^{b} \pm 4.50$	$8.20^{b} \pm 1.45$
OS-600	$12.11^{b} \pm 2.27$	$188.13^{b} \pm 5.57$	$40.56^{b} \pm 2.13$	$5.90^{b} \pm 1.45$

International Journal of Medical Toxicology & Forensic Medicine

Data are mean ± SD (n = 5 rats per group). Within a column, values that do not share a superscript letter differ at $P<0.05$ by one-way ANOVA with Tukey's post hoc test.

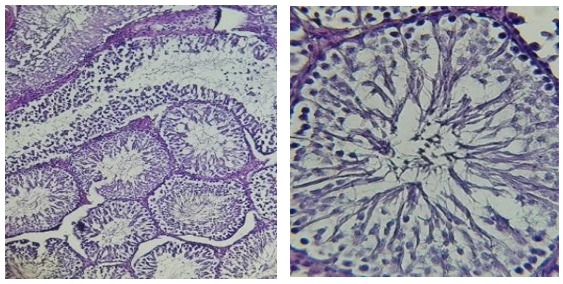
Table 4. Plasma testosterone concentrations.

Group	Testosterone (ng/mL)
Control A	$1.43^{b} \pm 0.02$
Control B	$2.50^{a} \pm 0.06$
OS-360	$1.80^{ab} \pm 0.05$
OS-480	$1.60^{a} \pm 0.04$
OS-600	$1.44^{b} \pm 0.03$

International Journal of
Medical Toxicology & Forensic Medicine

Data are mean ± SD (n = 5 rats per group). Testosterone is expressed in ng/mL. Group definitions are as in Table 1. Within a column, values that do not share a superscript letter differ at $P<0.05$ by one-way ANOVA with Tukey's post hoc test.

The HFD-control sections showed focal tubular disorganization, interstitial widening, and vacuolation (Figure 2). OS-360 and OS-480 showed generally organized seminiferous tubules and luminal spermatozoa (Figures 3 and 4). OS-600 showed generally preserved tubules in broad fields with focal germ-cell disorganization in higher-detail fields (Figure 5). These descriptions refer to representative H&E images and do not imply a quantitative ranking of structural outcomes.

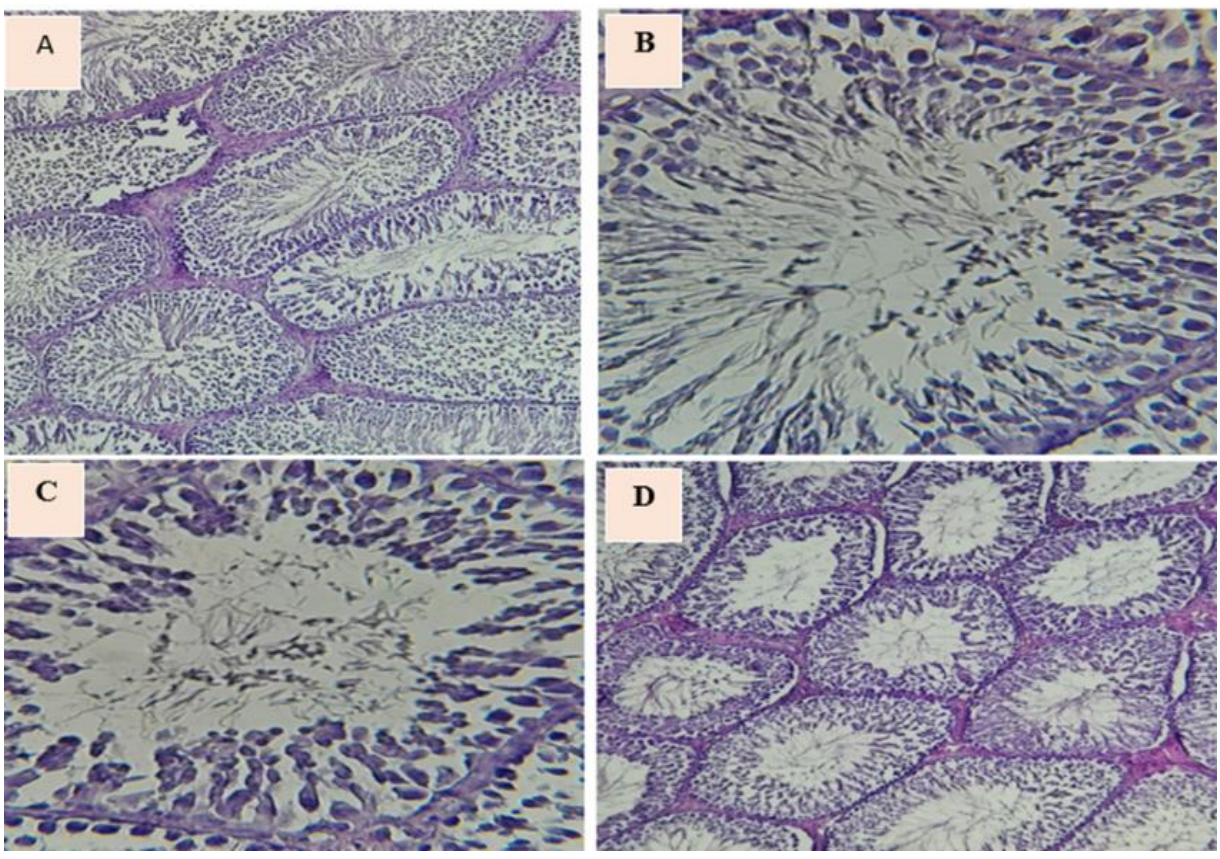


International Journal of
Medical Toxicology & Forensic Medicine

Figure 1. Representative H&E-stained testicular sections from Control A (standard-diet control). Left, broad-field view showing multiple seminiferous tubules; right, higher-detail view showing preserved germinal epithelium and luminal spermatozoa. Images illustrate qualitative architecture.

Discussion

This randomized animal experiment identified divergent reproductive, biochemical, and endocrine responses across three oral orlistat dose groups in HFD-fed Wistar rats. The reported Tukey comparisons indicated lower sperm motility in each orlistat group and lower viability at 480 and 600 mg/kg/day than in the HFD control. MDA was lower in all treatment groups, whereas TBARS differed from the HFD control



International Journal of
Medical Toxicology & Forensic Medicine

Figure 2. Representative H&E-stained testicular sections from Control B (HFD control). (A, D) Broad-field views of seminiferous tubules and interstitial tissue. (B, C) Higher-detail views of individual tubules. Focal tubular disorganization, interstitial widening, and vacuolation are described qualitatively.

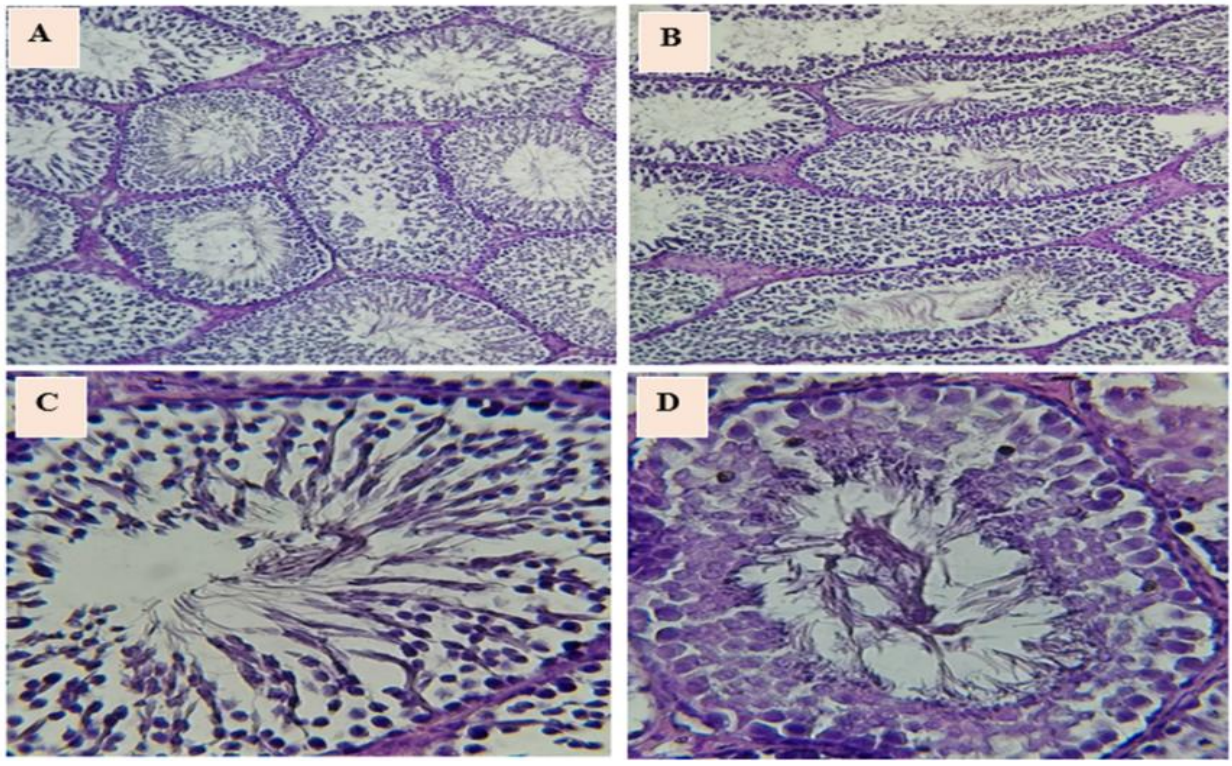


Figure 3. Representative H&E-stained testicular sections from OS-360 (HFD plus orlistat, 360 mg/kg/day). (A, B) Broad-field views showing generally organized seminiferous tubules. (C, D) Higher-detail views of germinal epithelium and luminal spermatozoa. Images illustrate qualitative architecture.

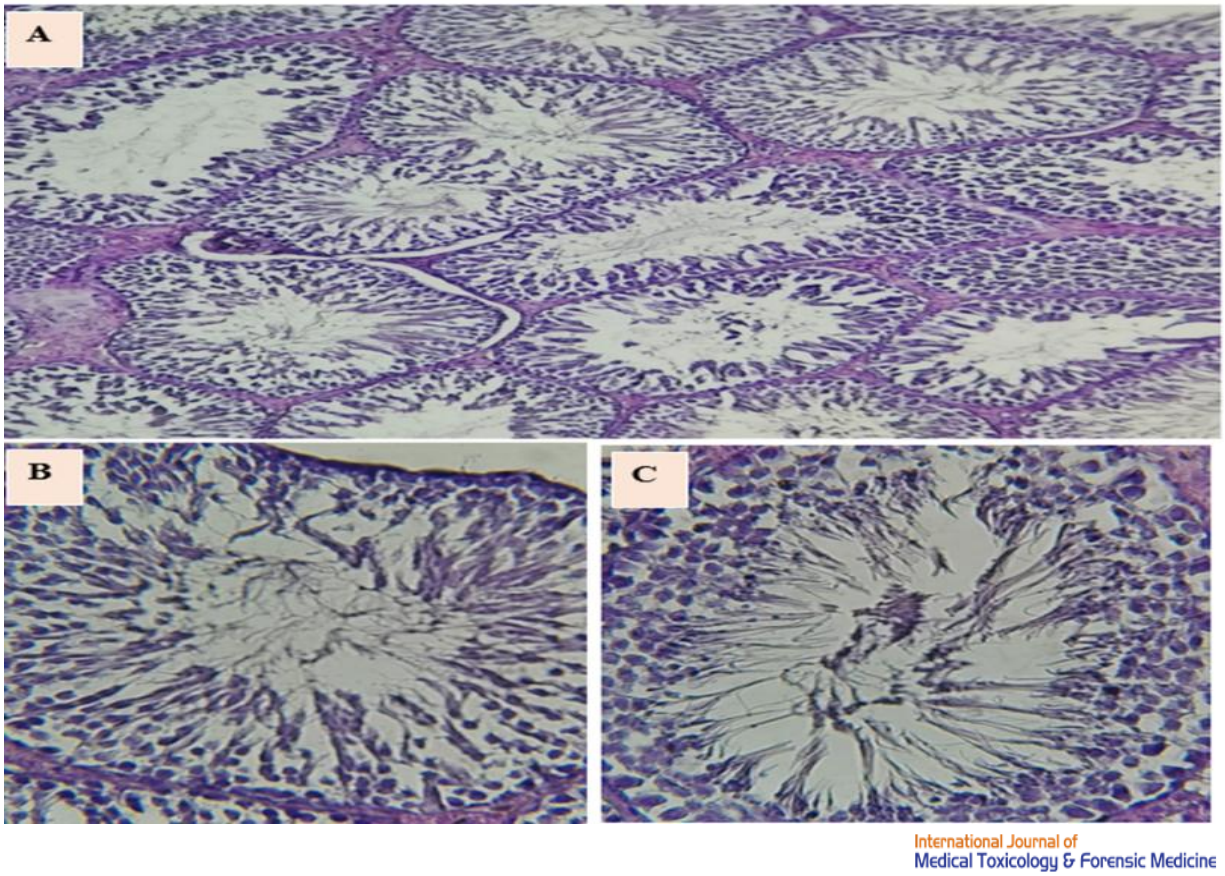


Figure 4. Representative H&E-stained testicular sections from OS-480 (HFD plus orlistat, 480 mg/kg/day). (A) Broad-field view showing organized seminiferous tubules. (B, C) Higher-detail views showing germinal epithelium and luminal spermatozoa. Images illustrate qualitative architecture.

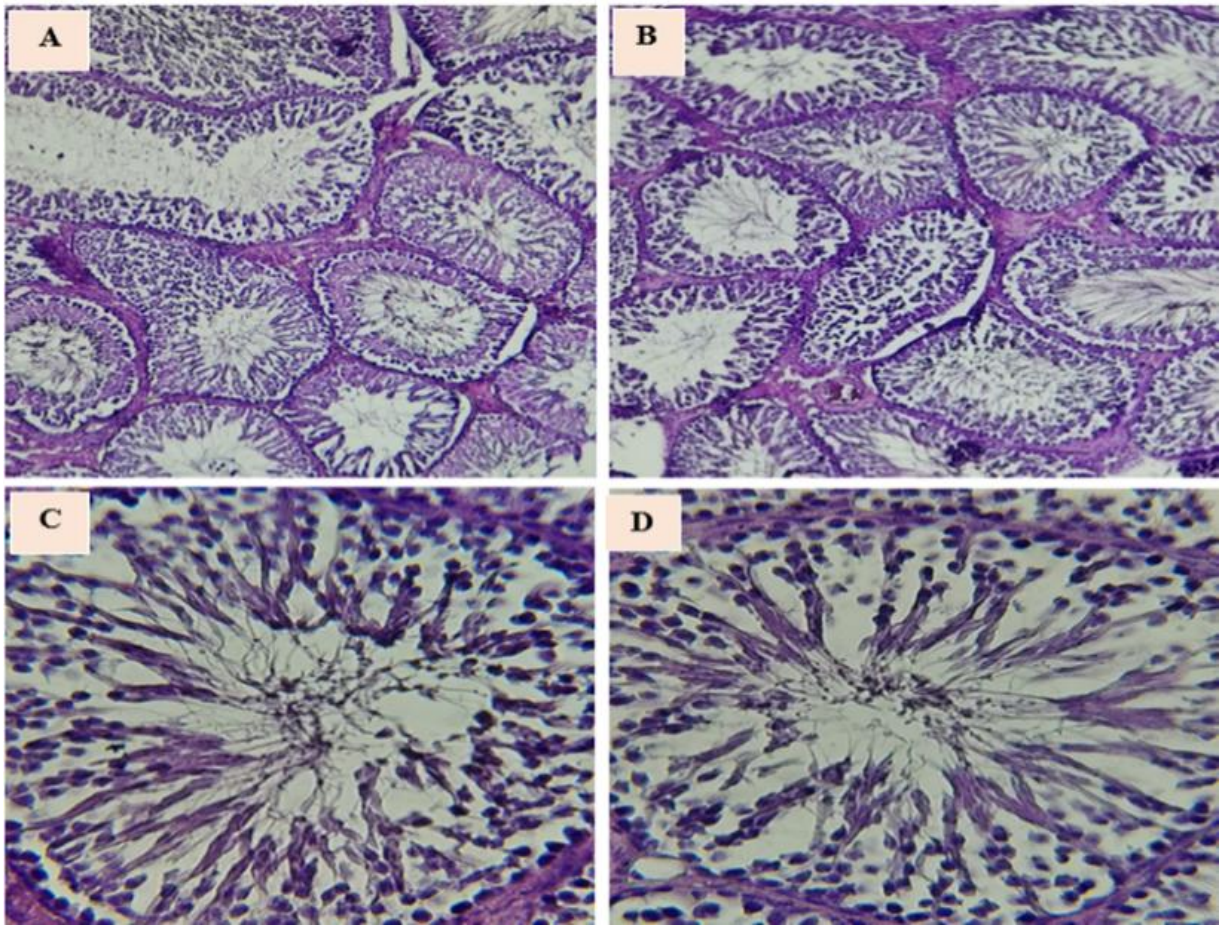


Figure 5. Representative H&E-stained testicular sections from OS-600 (HFD plus orlistat, 600 mg/kg/day). (A, B) Broad-field views of seminiferous tubules. (C, D) Higher-detail views showing focal germ-cell disorganization and variable luminal spermatozoal density. Images illustrate qualitative architecture.

only at 600 mg/kg/day. GST and GSH were also lower at the highest dose, but GPx and SOD did not differ significantly between the HFD control and the treatment groups. Testosterone at 600 mg/kg/day was lower than in the HFD control and close to the standard-diet control value. These observations emphasize that a favorable change in a lipid-peroxidation measure does not necessarily coincide with better sperm function. Each endpoint should therefore be interpreted separately rather than combined into a single claim of reproductive protection.

The HFD control showed higher TBARS, sperm-abnormality percentages, and testosterone than the standard-diet control, but motility and viability were numerically higher, with no significant control-group differences in the reported Tukey analysis. This discordant pattern differs from the generalized expectation that HFD-associated metabolic stress uniformly impairs reproductive function. Obesity-related reproductive effects are heterogeneous in both human and experimental studies [1–3]. In the present experiment, HFD exposure is the appropriate model description; the control-group pattern alone does not establish an obese phenotype or a compensatory endocrine mechanism. Differences in dietary formulation, exposure duration, strain, biological variation, and sampling may contribute to variation between studies, but the current observations do not distinguish these explanations.

Primary HFD-orlistat studies provide useful context. Suleiman et al. studied 24 male Sprague–Dawley rats in groups of six, using 10 mg/kg/day orlistat either concurrently with HFD for 12 weeks or during the final 6 weeks after obesity induction. They reported HFD-associated reductions in testicular antioxidant-enzyme activity and increases in inflammatory and apoptotic markers, with improvements under preventive and therapeutic orlistat schedules [3]. Galaly et al. used a 35%-fat diet for 16 weeks, followed by 6 weeks of treatment with 2 mg/kg/day orlistat, and reported lower testicular MDA, along with higher GSH and SOD, than in HFD controls [6]. The lower lipid-peroxidation measures in the present treatment groups are directionally compatible with those reports, whereas the antioxidant pattern differs. This distinction matters because the present

doses were substantially higher, the exposure schedule was shorter, and the diet, strain, group size, and measurement scales differed.

A related experiment by Suleiman et al. used 10 mg/kg/day orlistat in preventive and post-induction schedules and assessed obesity using body-size indices. HFD-associated reductions in sperm motility, viability, normal morphology, and steroidogenic endpoints were improved with treatment [17]. Another study from the same group reported restoration of intratesticular lactate-transport measures and fertility-related endpoints in obese rats receiving orlistat [18]. Those molecular and functional measurements provide a mechanistic framework for their respective experiments, but cannot be assumed to mediate the present findings. The contrasting sperm patterns across these reports and the current study favor a design-specific interpretation rather than a universal beneficial effect of orlistat.

The biochemical findings require an endpoint-specific interpretation. TBARS is an assay for thiobarbituric acid reactive products, commonly expressed as MDA equivalents [10]. The TBARS and separately reported MDA columns should therefore not be treated as two independent demonstrations of a mechanism. Erel's TOS assay measures composite oxidant status rather than individual reactive oxygen species [11], so it is not interchangeable with direct ROS quantification. Lower GST, GSH, GPx, or SOD values also do not establish antioxidant exhaustion: they describe assay outcomes and do not identify whether substrate availability, enzyme regulation, cellular composition, or another process accounts for the pattern. Inhibition of fatty acid synthase has been demonstrated in cellular systems [5], but the current experiment does not establish that this mechanism caused changes in testicular redox balance or sperm function.

The testosterone pattern should likewise be distinguished from physiological normalization. OS-600 and Control A had similar mean testosterone concentrations, but proximity to a comparator does not establish restoration of endocrine function or explain the sperm findings. Furthermore, the dose-group ordering was not identical for all endpoints: GST was higher in OS-480 than in OS-360, and the MDA means in OS-480 and OS-600 were similar. Although the reported Tukey comparisons identify specific treatment–control differences, they do not constitute a formal trend test. The descending means for motility and viability therefore do not by themselves establish a monotonic pharmacological dose-response or identify a therapeutic optimum.

The histological findings complement, but do not replace, the sperm and biochemical observations. Figure 1 provides the standard-diet control image. The representative sections show focal disorganization in the HFD control, generally preserved organization in OS-360 and OS-480, and focal disorganization in OS-600. These appearances should not be interpreted as proof that OS-480 was structurally superior or that histological preservation ensured better sperm function. A qualitative image-based assessment captures tissue architecture, whereas motility and viability assess distinct functional properties.

Several design considerations limit precision and generalizability. With five rats per group, individual animals can substantially influence the group summaries. Shared housing also creates a potential cage-level contribution to between-group differences. If each treatment group occupied a single cage, cage and treatment effects would be inseparable; more generally, within-cage dependence can reduce effective independent replication. A cage effect could exaggerate or attenuate a treatment-associated difference, and the reported summaries cannot quantify its direction or magnitude. Blinding of semen and histological assessment reduces observer-knowledge bias but does not remove potential housing or sampling effects. Larger studies with independent cage replication, defined primary outcomes, calibrated quantitative histology, and transparent effect estimates would strengthen the precision and interpretation of these observations.

Conclusion

Orlistat-associated changes in testicular biochemical markers did not translate into uniformly improved reproductive function in HFD-fed rats. These findings underscore the importance of evaluating sperm function alongside biochemical and endocrine endpoints when assessing reproductive effects. Further dose-ranging studies are warranted before mechanistic conclusions or clinical dosing recommendations can be established.

Ethical Approval

The experimental protocol and animal handling procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (8th edition, 2011). The study was reviewed and approved by the Institutional Animal Care and Use Committee at the College of Science, University of Al-Qadisiyah, Diwaniyah, Iraq (approval code UOQ-SCI-IACUC-2023-012; approval date March 15, 2023).

Acknowledgment

The authors thank all individuals who assisted with the conduct of the study.

Funding

No external funding was received for this research.

Conflicts of Interest

The authors report that there are no competing interests to declare.

References

[1] Du Plessis SS, Cabler S, McAlister DA, Sabanegh E, Agarwal A. The effect of obesity on sperm disorders and male infertility. Nat Rev Urol. 2010;7(3):153-61. [DOI: 10.1038/nrurol.2010.6]

[2] Aggerholm AS, Thulstrup AM, Toft G, Ramlau-Hansen CH, Bonde JP. Is overweight a risk factor for reduced semen quality and altered serum sex hormone profile? Fertil Steril. 2008;90(3):619-26. [DOI: 10.1016/j.fertnstert.2007.07.1292]

[3] Suleiman JB, Nna VU, Zakaria Z, Othman ZA, Bakar ABA, Mohamed M. Obesity-induced testicular oxidative stress, inflammation and apoptosis: protective and therapeutic effects of orlistat. Reprod Toxicol. 2020;95:113-22. [DOI: 10.1016/j.reprotox.2020.05.009]

[4] Rössner S, Sjöström L, Noack R, Meinders AE, Noseda G; European Orlistat Obesity Study Group. Weight loss, weight maintenance, and improved cardiovascular risk factors after 2 years' treatment with orlistat for obesity. Obes Res. 2000;8(1):49-61. [DOI: 10.1038/oby.2000.8]

[5] Kridel SJ, Axelrod F, Rozenkrantz N, Smith JW. Orlistat is a novel inhibitor of fatty acid synthase with antitumor activity. Cancer Res. 2004;64(6):2070-5. [DOI: 10.1158/0008-5472.CAN-03-3645]

[6] Galaly SR, Hozayen WG, Amin KA, Ramadan SM. Effects of orlistat and herbal mixture extract on brain and testicular function and oxidative stress biomarkers in a rat model of a high-fat diet. Beni-Suef Univ J Basic Appl Sci. 2014;3(2):93-105. [DOI: 10.1016/j.bjbas.2014.05.002]

[7] Altunkaynak Z. Effects of high-fat diet-induced obesity on female rat livers (a histochemical study). Eur J Gen Med. 2005;2(3):100-9. [DOI: 10.29333/ejgm/82319]

[8] Seed J, Chapin RE, Clegg ED, Dostal LA, Foote RH, Hurtt ME, et al. Methods for assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: a consensus report. Reprod Toxicol. 1996;10(3):237-44. [DOI: 10.1016/0890-6238(96)00028-7]

[9] Björndahl L, Söderlund I, Kvist U. Evaluation of the one-step eosin-nigrosin staining technique for human sperm vitality assessment. Hum Reprod. 2003;18(4):813-6. [DOI: 10.1093/humrep/deg199]

[10] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-8. [DOI: 10.1016/0003-2697(79)90738-3]

[11] Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005;38(12):1103-11. [DOI: 10.1016/j.clinbiochem.2005.08.008]

[12] Mohsin NY, Demir H, Hadwan MH, Hadwan AM, Mohammed RM. A new fluorescent method for measuring peroxiredoxin enzyme activity using monobromobimane. J Fluoresc. 2025;35(8):6357-65. [DOI: 10.1007/s10895-024-03991-4]

[13] Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J Biol Chem. 1974;249(22):7130-9. [DOI: 10.1016/S0021-9258(19)42083-8]

[14] Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968;25(1):192-205. [DOI: 10.1016/0003-2697(68)90092-4]

[15] Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science. 1973;179(4073):588-90. [DOI: 10.1126/science.179.4073.588]

[16] Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974;47(3):469-74. [DOI: 10.1111/j.1432-1033.1974.tb03714.x]

[17] Suleiman JB, Nna VU, Othman ZA, Zakaria Z, Bakar ABA, Mohamed M. Orlistat attenuates

obesity-induced decline in steroidogenesis and spermatogenesis by up-regulating steroidogenic genes. Andrology. 2020;8(5):1471-85. [DOI: 10.1111/andr.12824]

[18] Suleiman JB, Nna VU, Zakaria Z, Othman ZA, Bakar ABA, Usman UZ, et al. Orlistat reverses intratesticular lactate transport decline and infertility in male obese rats. Reproduction. 2020;160(6):863-72. [DOI: 10.1530/REP-20-0381]