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Protective Effects of Lupine Extract Against Cadmium Induced Reproductive Toxicity, Sperm Abnormalities, and Hormonal Imbalance in Male Albino Rats

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Abstract

Background: Cadmium is a widely distributed environmental pollutant with well-documented testicular toxicity. Lupinus albus seed extract is rich in flavonoids, phenolic acids and tocopherols, and has been suggested to support male reproductive function, but its protective role against cadmium-induced testicular injury remains incompletely characterised. The aim was to evaluate whether oral co-administration of Lupinus albus seed extract attenuates cadmium chloride-induced reproductive toxicity in male albino rats.

Methods: Thirty-two adult male albino rats were randomly allocated into four groups (n = 8): control (normal saline), cadmium chloride (5 mg/kg, p.o.), lupine extract (15 mg/kg, p.o.), and cadmium + lupine, treated daily for 45 days. Body and reproductive organ weights, sperm parameters (count, motility, viability, morphology), serum testosterone, LH and FSH (ELISA), testicular oxidative stress markers (MDA, SOD, CAT, GSH), and Johnsen testicular score were assessed.

Results: Cadmium significantly reduced testis weight, sperm count, motility, viability, testosterone, LH, FSH, and antioxidant enzymes, and significantly increased sperm abnormalities and MDA (all $p < 0.001$). Johnsen score fell from 9.05 ± 0.37 to 3.74 ± 0.70. Co-treatment with lupine extract significantly attenuated all of these changes, with partial recovery in most parameters (percentage protection 34.5–76.3%). Strong negative correlations between MDA and sperm count ($r = -0.92$) and Johnsen score ($r = -0.89$) supported an oxidative stress-mediated mechanism.

Conclusion: Lupine extract partially mitigates cadmium-induced testicular damage in rats, plausibly via antioxidant mechanisms. Further dose–response and translational studies are warranted.

التأثيرات الوقائية لمستخلص الترمس ضد السمية التناسلية المحدثة بالكادميوم وتشوهات الحيوانات المنوية والاختلال الهرموني في ذكور الجرذان البيضاء

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الخلاصة

الخلفية: الكادميوم ملوث بيئي واسع الانتشار ذو سمية خصوية موثقة جيداً. ويُعدّ مستخلص بذور الترمس الأبيض (Lupinus albus) غنياً بالفلافونويدات والأحماض الفينولية والتوكوفيرولات، وقد اقتُرح أنه يدعم وظيفة الجهاز التناسلي الذكري، إلا أن دوره الوقائي ضد الأذى الخصوي المحدث بالكادميوم لا يزال غير مُوصَّف بشكل كامل. هدفت الدراسة إلى تقييم ما إذا كان التناول الفموي المتزامن لمستخلص بذور الترمس يخفف من السمية التناسلية المحدثة بكلوريد الكادميوم في ذكور الجرذان البيضاء.

طرائق العمل: وُزّع اثنان وثلاثون جرذاً أبيض بالغاً عشوائياً إلى أربع مجموعات (8 لكل مجموعة): مجموعة ضابطة (محلول ملحي)، وكلوريد الكادميوم (5 ملغم/كغم فموياً)، ومستخلص الترمس (15 ملغم/كغم فموياً)، والكادميوم + الترمس، عُولجت يومياً لمدة 45 يوماً. قُيّمت أوزان الجسم وأعضاء التكاثر، ومعايير الحيوانات المنوية (العدد والحركة والحيوية والشكل)، وهرمونات التستوستيرون و LH و FSH في المصل (بالإليزا)، ومؤشرات الإجهاد التأكسدي الخصوية (MDA و SOD و CAT و GSH)، ودرجة جونسن الخصوية.

النتائج: خفّض الكادميوم بشكل معنوي وزن الخصية وعدد الحيوانات المنوية وحركتها وحيويتها والتستوستيرون و LH و FSH والإنزيمات المضادة للأكسدة، وزاد بشكل معنوي من تشوهات الحيوانات المنوية و MDA (جميعها $p < 0.001$). وانخفضت درجة جونسن من 9.05 ± 0.37 إلى 3.74 ± 0.70. وأدى العلاج المتزامن بمستخلص الترمس إلى تخفيف معنوي لجميع هذه التغيرات، مع تعافٍ جزئي في معظم المعايير (نسبة الحماية 34.5–76.3%). وأيّدت الارتباطات السلبية القوية بين MDA وعدد الحيوانات المنوية ($r = -0.92$) ودرجة جونسن ($r = -0.89$) وجود آلية تتوسطها الإجهاد التأكسدي.

الاستنتاج: يخفف مستخلص الترمس جزئياً من الأذى الخصوي المحدث بالكادميوم في الجرذان، وذلك على الأرجح عبر آليات مضادة للأكسدة. ويُوصى بإجراء مزيد من دراسات الاستجابة للجرعة والدراسات التطبيقية.

1. Introduction

Cadmium (Cd) is a non-essential heavy metal classified by the International Agency for Research on Cancer as a Group 1 human carcinogen, and is a recognised environmental and occupational pollutant. Human exposure occurs through tobacco smoke, contaminated water and food, occupational settings (battery manufacturing, electroplating, pigment industries), and through Cd-contaminated agricultural land — a concern of growing relevance in several Middle Eastern countries, including Iraq, where industrial and agricultural runoff and the legacy of armed conflict have increased the background burden of heavy metals in soil and water (World Health Organization, 2019; Genchi et al., 2020). Once absorbed, Cd has a biological half-life of more than 20 years in humans, accumulates preferentially in the liver, kidneys, and gonads, and exerts toxicity on a wide range of organs (Rafati Rahimzadeh et al., 2017). The male reproductive system is one of the most sensitive targets of Cd toxicity. Experimental and epidemiological studies have consistently shown that Cd exposure is associated with reduced sperm count and motility, increased sperm abnormalities, suppressed serum testosterone, disruption of the hypothalamic–pituitary–gonadal (HPG) axis, and structural injury to the seminiferous epithelium and the blood–testis barrier (Marettová, Maretta and Legáth, 2015; Nna et al., 2017; Wang et al., 2017; Yang et al., 2018). The mechanisms underlying Cd-induced testicular injury are multifactorial and include the generation of reactive oxygen species (ROS), depletion of endogenous antioxidant defences (superoxide dismutase, catalase, reduced glutathione), peroxidation of membrane lipids — reflected in elevated malondialdehyde (MDA) — Leydig cell dysfunction, mitochondrial impairment, DNA damage, and germ-cell apoptosis (Nna et al., 2017; Almeer et al., 2021; Genchi et al., 2024). Because oxidative stress occupies a central position in Cd-induced testicular injury, dietary phytochemicals with strong antioxidant capacity have attracted considerable interest as candidate protective agents. Lupinus albus L. (white lupine) is a legume widely consumed in the Mediterranean basin and in parts of the Middle East, including Iraq, where lupine seeds (locally known as turmus) are part of the traditional diet (Hashim and Mohammed Ali, 2021). Phytochemical analyses of L. albus seeds have identified flavonoids (apigenin and luteolin glycosides), phenolic acids (ferulic, p-coumaric, caffeic), tocopherols, quinolizidine alkaloids (lupanine, sparteine), high-quality protein, and a favourable fatty-acid profile (Cabello-Hurtado et al., 2016; Magalhães et al., 2017). In rodents, hydro-ethanolic L. albus extracts have been reported to enhance serum testosterone, improve sperm count, raise glutathione, reduce MDA, and improve testicular histoarchitecture (Erbas et al., 2014; Hashim and Mohammed Ali, 2021). L. albus extracts have also been shown to attenuate metabolic disturbances, hyperglycaemia and dyslipidaemia in experimental models, all of which are linked to oxidative stress (Mané et al., 2022). Despite this body of work, several knowledge gaps remain. First, although individual Iraqi and regional studies have separately characterised either Cd-induced testicular toxicity (Al-Hilli and Hassan, 2020; Atea and Razooqi, 2025) or the reproductive effects of L. albus (Hashim and Mohammed Ali, 2021), data on the combined exposure model — that is, whether lupine extract can prevent, attenuate or reverse Cd-induced testicular injury — are scarce, and to our knowledge no Iraqi study has yet evaluated this protective interaction. Second, most published Cd protection studies have focused on single endpoints and have not concurrently examined organ weights, sperm parameters, full HPG-axis hormones, the testicular antioxidant network, and quantitative histopathology in the same animals. Third, the relative weight of antioxidant-mediated mechanisms in L. albus protection has not been directly tied to quantitative correlations between oxidative stress markers and reproductive endpoints. Accordingly, the present study was designed to evaluate, in a controlled four-group experimental design, whether oral co-administration of an ethanolic L. albus seed extract (15 mg/kg/day for 45

days) attenuates the reproductive toxicity induced by daily oral cadmium chloride (5 mg/kg) in adult male albino rats. The specific objectives were: (i) to characterise the effects of Cd on body and reproductive organ weights, sperm parameters, serum reproductive hormones, testicular oxidative stress markers, and seminiferous tubule histopathology (Johnsen scoring); (ii) to determine the magnitude of protection afforded by concurrent L. albus extract; and (iii) to test, via correlation analysis, whether the protection observed is quantitatively consistent with an antioxidant-mediated mechanism.

2. Materials and Methods

2.1.Study Site and Timeframe

The experimental work was carried out at the Animal House and the laboratories of the College of Veterinary Medicine, University of Al-Qadisiyah, Diwaniyah, Iraq, between March and May 2024. The total study duration was 52 days (7 days acclimatisation + 45 days treatment + sampling on day 46 and subsequent biochemical/histological workup).

2.2.Ethical Approval

All experimental procedures were reviewed and approved by the Scientific Committee and the Institutional Animal Care and Use Committee (IACUC) of the College of Veterinary Medicine, University of Al-Qadisiyah, in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals (8th edition) and the ARRIVE 2.0 guidelines for animal research reporting (Percie du Sert et al., 2020).

2.3.Animals and Housing

Thirty-two adult male albino rats (Wistar strain, Rattus norvegicus), 8–12 weeks old and weighing 180–250 g, were obtained from the local breeding colony of the College of Veterinary Medicine, University of Al-Qadisiyah. Rats were housed in standard polypropylene cages (2–3 animals per cage) under controlled environmental conditions (temperature 22 ± 2 °C, relative humidity 50–60%, and a 12-h light/dark cycle). They were fed standard pelleted rat chow and had free access to drinking water ad libitum. The animals were allowed seven days of acclimatisation before the start of the experiment.

2.4.Chemicals, Plant Material and Extract Preparation

Cadmium chloride ($CdCl_2 \cdot 2.5H_2O$, ≥98% purity, CAS 10108-64-2) was obtained from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany) through an authorised Iraqi scientific supplier. Fresh working solutions of $CdCl_2$ were prepared in distilled water every three days and stored at 4 °C.

Mature, debittered white lupine seeds (Lupinus albus L.) were purchased from local Iraqi markets and authenticated by a botanist at the College of Agriculture, University of Al-Qadisiyah; a voucher specimen was deposited in the departmental herbarium. The seeds (100 g) were finely ground, defatted briefly with n-hexane, and then extracted by maceration in 70% ethanol (1:10, w/v) for 72 h at room temperature with intermittent shaking. The extract was filtered (Whatman No. 1), and the solvent was removed under reduced pressure using a rotary evaporator (Heidolph Hei-VAP, Germany) at 45 °C. The resulting dry, semi-solid extract was stored at 4 °C in light-protected, sealed containers and re-suspended in distilled water immediately before administration. The extraction procedure followed the protocol previously described by Hashim and Mohammed Ali (2021).

2.5.Experimental Design

After acclimatisation, rats were randomly allocated into four groups (n = 8 per group) using a computer-generated random number list: Group 1 (Control) received normal saline (0.9% NaCl), 1 mL/kg body weight, p.o.; Group 2 (Cd) received cadmium chloride, 5 mg/kg body weight, p.o.; Group 3 (Lupine) received L. albus ethanolic extract, 15 mg/kg body weight, p.o.; and Group 4 (Cd + Lupine) received both treatments, with the lupine extract

administered 30 min before cadmium chloride. All treatments were delivered by oral gavage daily, between 09:00 and 11:00, for 45 consecutive days. Doses were selected on the basis of previously published Cd toxicity studies (Nna et al., 2017; Atea and Razooqi, 2025) and lupine pharmacology studies in rodents (Hashim and Mohammed Ali, 2021). Body weight was recorded weekly, and the volume of administration was adjusted accordingly.

2.6.Sample Collection

On day 46, animals were fasted overnight and anaesthetised with intraperitoneal ketamine/xylazine (60/6 mg/kg). Blood samples were collected by cardiac puncture into plain Vacuette® tubes (Greiner Bio-One, Austria), allowed to clot at room temperature for 30 min, and centrifuged at 3,000 rpm for 15 min using a refrigerated centrifuge (Hettich Universal 320R, Germany). Sera were aliquoted and stored at −20 °C until hormonal assay. Animals were then euthanised by cervical dislocation under deep anaesthesia. Both testes and epididymides were rapidly excised, freed of adhering connective tissue, blotted dry, and weighed individually using an analytical balance (Sartorius BL210S, Germany; readability 0.001 g). One testis from each animal was fixed in 10% neutral buffered formalin for histopathology, while the contralateral testis was snap-frozen at −80 °C for biochemical analysis of oxidative stress markers.

2.7.Sperm Parameters

The right cauda epididymis was minced in 2 mL of pre-warmed (37 °C) normal saline in a sterile petri dish and incubated for 5 min at 37 °C to allow sperm dispersion. The resulting suspension was used immediately for the assessment of sperm parameters following the methodology described by the WHO laboratory manual (5th edition) adapted to rats (World Health Organization, 2010).

2.7.1. Sperm count. A 1:20 dilution of the sperm suspension was loaded onto an improved Neubauer haemocytometer (Marienfeld, Germany), and at least 200 sperm were counted under a light microscope (Olympus CX31, Japan) at 400× magnification. Counts were expressed as $\times 10^6$ spermatozoa/mL.

2.7.2. Sperm motility. A drop of the fresh suspension was placed on a pre-warmed slide and examined at 400× under phase-contrast microscopy. The percentage of motile spermatozoa was determined by counting at least 200 sperm in four randomly selected fields.

2.7.3. Sperm viability. Viability was determined by the eosin–nigrosin staining technique. Equal volumes (10 µL each) of sperm suspension, 1% eosin Y, and 5% nigrosin were mixed; a smear was prepared, air-dried, and examined under oil immersion (×1,000). Two hundred spermatozoa per slide were scored; unstained sperm were classified as viable and stained ones as non-viable.

2.7.4. Sperm morphology. Smears stained with eosin–nigrosin were examined at 1,000× to assess head, midpiece and tail abnormalities. At least 200 sperm per animal were evaluated, and the percentage of morphologically abnormal sperm was recorded.

2.8.Serum Reproductive Hormones (ELISA)

Serum concentrations of testosterone, luteinising hormone (LH), and follicle-stimulating hormone (FSH) were measured by enzyme-linked immunosorbent assay (ELISA) using commercially available rat-specific kits, following the manufacturer's protocol. Optical density was read at 450 nm using a BioTek ELx800 microplate reader (BioTek Instruments, Winooski, VT, USA), with curve fitting performed using a four-parameter logistic (4-PL) model. Testosterone was assayed using a Rat/Mouse Testosterone ELISA Kit (IBL-America, Minneapolis, MN, USA; cat. no. RE52151; detection range 0.066–25 ng/mL) (IBL-America, 2020); LH using a Rat LH ELISA Kit (Elabscience, Wuhan, China; cat. no. E-EL-R0026; range 1.56–100 mIU/mL); and FSH using a Rat FSH

ELISA Kit (Cusabio Biotech, Wuhan, China; cat. no. CSB-E06869r; range 0.17–10 mIU/mL). All samples were assayed in duplicate, and intra-assay coefficients of variation were maintained below 10%.

2.9.Testicular Oxidative Stress Markers

Snap-frozen testicular tissue (~100 mg) was homogenised in ice-cold phosphate-buffered saline (0.05 M PBS, pH 7.4) at a 1:10 (w/v) ratio using a Teflon–glass homogeniser. Homogenates were centrifuged at 10,000 × g for 15 min at 4 °C (Hettich Mikro 220R, Germany), and the supernatants were used for the assays. Total protein concentration was determined by the Bradford method (Bradford, 1976) using bovine serum albumin (BSA) as the standard.

2.9.1. Malondialdehyde (MDA) was determined by the thiobarbituric acid reactive substances (TBARS) method of Ohkawa, Ohishi and Yagi (1979); the pink chromogen was read at 532 nm (Shimadzu UV-1800, Japan) and expressed as nmol/mg protein.

2.9.2. Superoxide dismutase (SOD) was assayed by the nitroblue tetrazolium (NBT) inhibition method (Asada, 1984); one unit was defined as the amount of enzyme causing 50% inhibition of NBT reduction, expressed as U/mg protein.

2.9.3. Catalase (CAT) was assayed by the kinetic method of Aebi (1984), following H_2O_2 decomposition at 240 nm, expressed as U/mg protein. **Reduced glutathione (GSH)** was quantified by Ellman's method with DTNB (Ellman, 1959); absorbance was read at 412 nm and expressed as µmol/g tissue.

2.10. Testicular Histopathology and Johnsen Scoring

Formalin-fixed testicular samples were processed in an automatic tissue processor (Leica TP1020, Germany), embedded in paraffin wax, sectioned at 5 µm using a rotary microtome (Leica RM2125 RTS, Germany), and stained with haematoxylin and eosin (H&E) following standard protocols. Slides were examined under a light microscope (Olympus BX43, Japan) equipped with a digital camera, and photomicrographs were captured at 100× and 400× magnification. For semi-quantitative evaluation, the Johnsen scoring system (Johnsen, 1970) was used; at least 30 round seminiferous tubule cross-sections per animal were scored on a 1–10 scale (1 = no cells; 10 = complete spermatogenesis with many spermatozoa). All slides were scored by a single experienced pathologist blinded to the group allocation.

2.11. Statistical Analysis

Statistical analyses were performed using Python (v3.11) with the SciPy (v1.11) and scikit-posthocs (v0.9) libraries; the same workflow can be reproduced in SPSS (v26 or later) or GraphPad Prism (v9). Results are expressed as mean ± standard deviation (SD) for n = 8 animals/group. Normality was assessed by the Shapiro–Wilk test and homogeneity of variance by Levene's test. When data met parametric assumptions, one-way ANOVA followed by Tukey's HSD post-hoc test was used; when variances were heterogeneous, Welch's ANOVA followed by the Games–Howell test was applied; non-normal data were analysed by the Kruskal–Wallis H test followed by Dunn's post-hoc test with Bonferroni correction. Effect sizes were estimated as η^2 (parametric) or ε^2 (non-parametric), with values > 0.14 considered large. Pearson's correlation coefficients (r) were calculated between all measured parameters across the four groups (N = 32). The protective effect of lupine was quantified as percentage change of the Cd group relative to control and percentage protection, calculated as [(Cd+Lupine − Cd) / (Control − Cd)] × 100. A two-tailed p-value < 0.05 was considered statistically significant.

3. Results

3.1. Experimental Design

The four-group, randomised parallel design and the dosing scheme used in the present study are summarized in Table1.

Table1: Experimental Design and Dosing Scheme

Group	Designation	Treatment	Dose & route	n
G1	Control	Normal saline	1 mL/kg, oral, 45 days	8
G2	Cd	Cadmium chloride	5 mg/kg, oral, 45 days	8
G3	Lupine	Lupine seed extract	15 mg/kg, oral, 45 days	8
G4	Cd + Lupine	Cadmium + lupine	5 + 15 mg/kg, oral, 45 days	8
All animals were treated by oral gavage once daily for 45 consecutive days following a 7-day acclimatization period				

3.2.Body Weight and Reproductive Organ Weights

As shown in Table2 and Figure1, daily oral administration of cadmium chloride for 45 days produced a significant reduction in final body weight (207.25 ± 13.42 g vs 244.88 ± 13.75 g in controls), testis weight (0.82 ± 0.09 g vs 1.27 ± 0.10 g), and epididymis weight (0.34 ± 0.04 g vs 0.53 ± 0.05 g) (all $p < 0.001$). Concurrent administration of Lupinus albus extract significantly attenuated these reductions, with body weight, testis weight and epididymis weight in the Cd + Lupine group reaching 92.9%, 84.3% and 77.4% of control values respectively. Lupine alone produced values numerically equal to or higher than the saline control, with the highest mean testis weight observed in this group (1.49 ± 0.07 g). The percentage protection conferred by lupine ranged from 34.5% (epididymis weight) to 56.7% (testis weight).

Table2: Effect of Cadmium Chloride And Lupine Extract on Body Weight and Reproductive Organ Weights in Male Albino Rats (Mean ± SD, N = 8).

Parameter	Control	Cd	Lupine	Cd + Lupine	F / H	p-value
Body weight (g)	244.88 ± 13.75	207.25 ± 13.42	265.49 ± 20.04	227.73 ± 12.96	20.94	< 0.001
Testis weight (g)	1.27 ± 0.10	0.82 ± 0.09	1.49 ± 0.07	1.07 ± 0.09	80.68	< 0.001
Epididymis weight (g)	0.53 ± 0.05	0.34 ± 0.04	0.57 ± 0.05	0.41 ± 0.05	24.15	< 0.001
Values within the same row differ significantly at $p < 0.05$ (one-way ANOVA + Tukey's HSD or Welch's ANOVA + Games–Howell, as appropriate).						

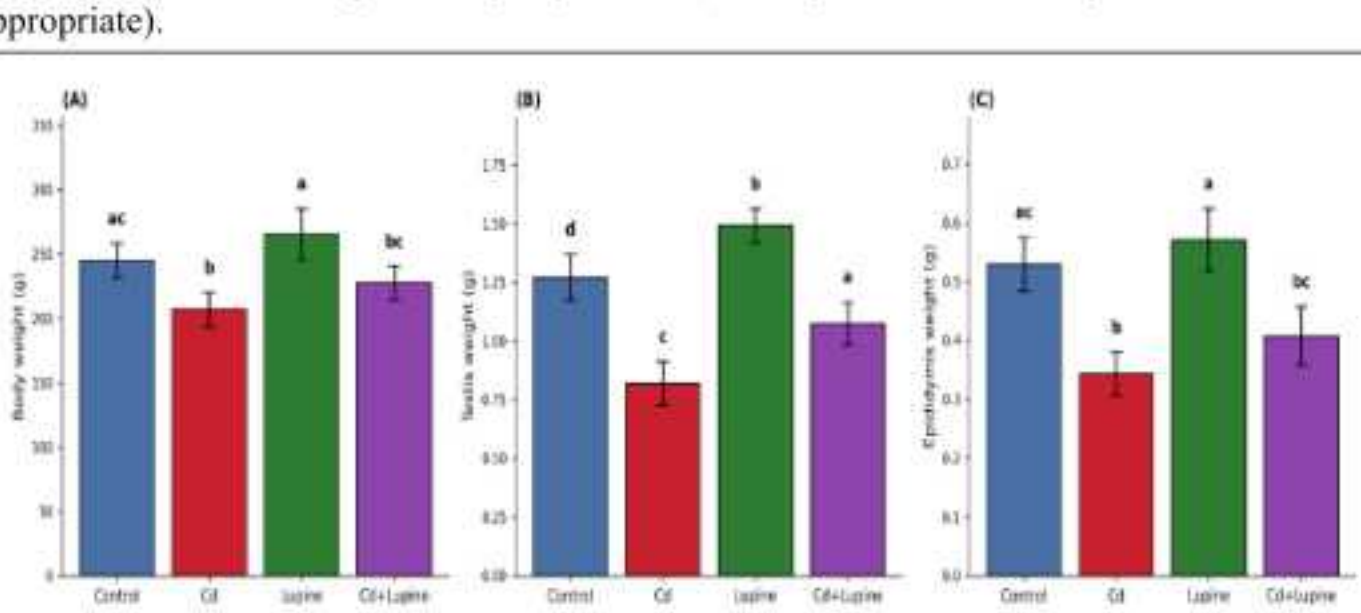

Figure1: Effect of Cadmium Chloride and Lupine Extract on (A) body weight, (B) testis weight and (C) epididymis weight in male albino rats. Values are mean ± SD (n = 8). Bars bearing different letters differ significantly at $p < 0.05$.

3.3.Sperm Parameters

The effects of the four treatments on sperm parameters are summarized in Table3 and illustrated in Fig.2. Cadmium produced the most pronounced reductions among all measured endpoints in sperm count (−79.1%; 11.83 ± 4.31 vs 56.56 ± 3.72 ×10⁶/mL), motility (−65.2%) and viability (−54.9%), together with a striking ~6.5-fold increase in the percentage of morphologically abnormal sperm (8.40 ± 2.66% to 55.04 ± 7.26%). Co-administration of lupine extract significantly improved all four sperm parameters, with the Cd + Lupine group restoring sperm count to 35.43 ± 4.72 ×10⁶/mL (62.6% of control), motility to 40.26 ± 8.76% (62.9% of control), viability to 55.96 ± 6.63% (65.8% of control), and reducing abnormality from 55.04% to 32.67%. Lupine alone produced numerically higher sperm count and motility than control values.

Table3: Effect of Cadmium Chloride and Lupine Extract on Epididymal Sperm Parameters in Male Albino Rats (Mean ± SD, n = 8).

Parameter	Control	Cd	Lupine	Cd + Lupine	F / H	p-value
Sperm count (×10⁶/mL)	56.56 ± 3.72	11.83 ± 4.31	62.28 ± 4.53	35.43 ± 4.72	223.33	< 0.001
Sperm motility (%)	64.05 ± 5.59	22.31 ± 4.59	71.09 ± 4.85	40.26 ± 8.76	27.68	< 0.001
Sperm viability (%)	85.10 ± 3.38	38.36 ± 5.93	88.21 ± 4.18	55.96 ± 6.63	169.93	< 0.001
Sperm abnormality (%)	8.40 ± 2.66	55.04 ± 7.26	8.84 ± 2.79	32.67 ± 6.95	26.29	< 0.001
Values within the same row differ significantly at $p < 0.05$.						

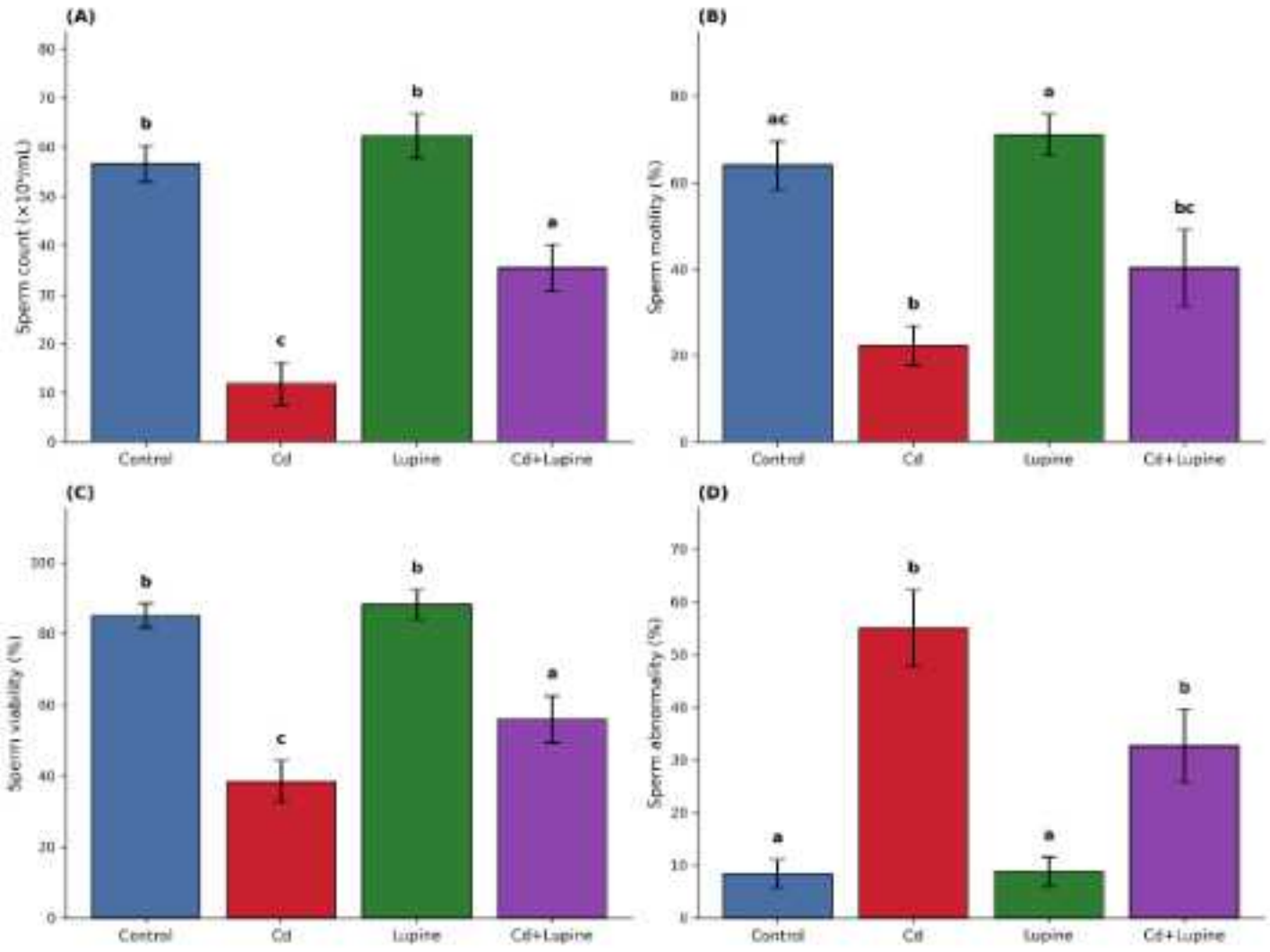

Figure2: Effect of cadmium chloride and lupine extract on epididymal sperm parameters: (A) sperm count, (B) progressive motility, (C) viability and (D) morphological abnormality. Bars bearing different letters differ significantly at $p < 0.05$.

3.4. Serum Reproductive Hormones

Cadmium produced a profound disruption of the hypothalamic–pituitary–gonadal axis (Table4, Fig.3). Serum testosterone showed the largest single percentage change of any parameter studied (−87.1%; 6.25 ± 1.57 ng/mL → 0.81 ± 0.21 ng/mL). LH and FSH were reduced by 72.7% and 69.1% respectively. Co-treatment with lupine partially restored all three hormones; the strongest hormonal recovery was for LH (74.5% protection), followed by FSH (54.2%) and testosterone (44.0%). The lupine-alone group again showed numerically higher mean values than control for all three hormones (testosterone 8.69 ± 1.24 ng/mL; LH 7.22 ± 1.90 mIU/mL; FSH 7.80 ± 3.37 mIU/mL).

Table4: Effect of Cadmium Chloride and Lupine Extract on Serum Reproductive Hormones in Male Albino Rats (Mean ± SD, n = 8).

Parameter	Control	Cd	Lupine	Cd + Lupine	F / H	p-value
Testosterone (ng/mL)	6.25 ± 1.57	0.81 ± 0.21	8.69 ± 1.24	3.21 ± 0.76	67.77	< 0.001
LH (mIU/mL)	5.14 ± 2.13	1.40 ± 0.68	7.22 ± 1.90	4.19 ± 1.48	35.41	< 0.001
FSH (mIU/mL)	6.71 ± 1.86	2.07 ± 0.70	7.80 ± 3.37	4.59 ± 1.75	18.20	< 0.001
Values within the same row differ significantly at $p < 0.05$.						

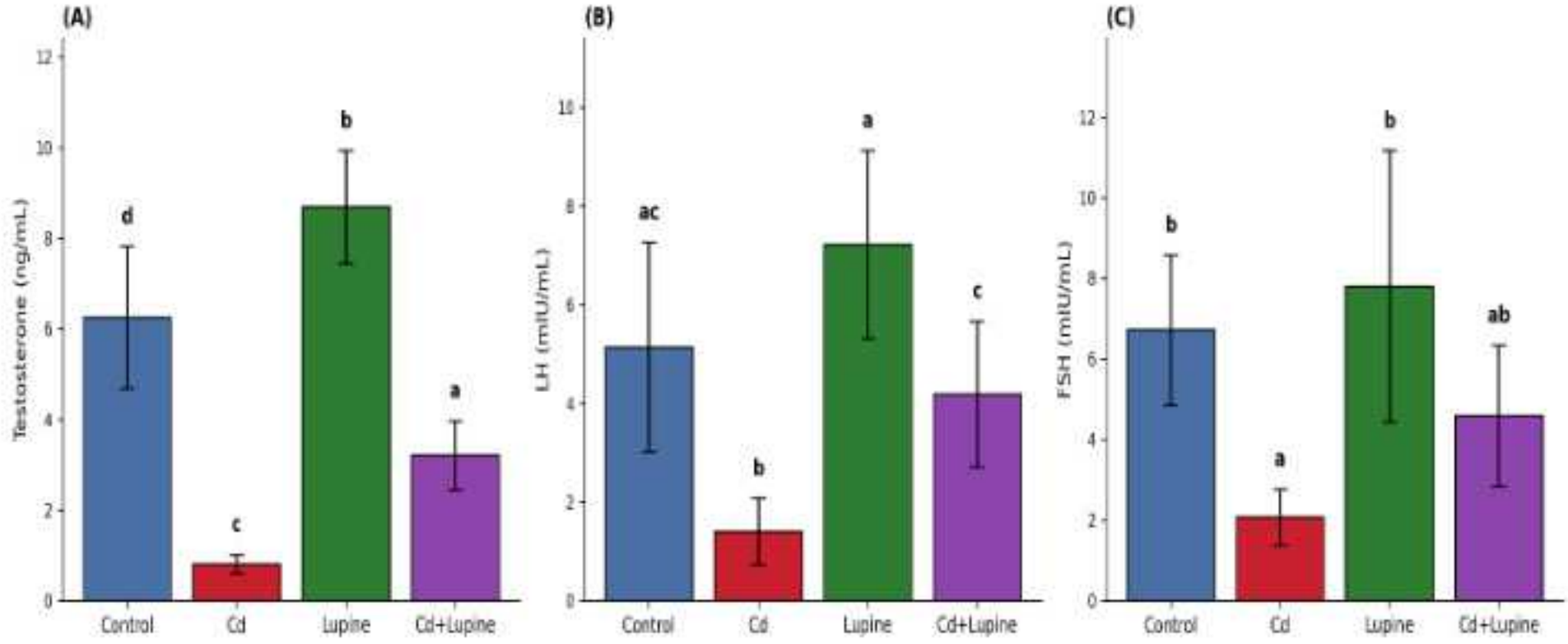

Figure3: Effect of Cadmium Chloride and Lupine Extract on Serum (A) testosterone, (B) luteinizing hormone (LH) and (C) follicle-stimulating hormone (FSH). Bars bearing different letters differ significantly at $p < 0.05$.

3.5.Testicular Oxidative Stress Markers

The oxidative stress profile (Table5, Fig.4) was consistent with the central role of redox imbalance in cadmium-induced testicular injury. Cd increased testicular MDA by 174.6% (1.53 ± 0.36 → 4.21 ± 0.64 nmol/mg protein) and reduced SOD, CAT and GSH by 65.7%, 56.1% and 67.0% respectively. Co-administration of lupine extract was associated with the strongest percentage protection observed in this study for CAT (76.3%) and MDA (66.4%), and substantial protection for GSH (64.2%) and SOD (49.9%). In rats receiving lupine alone, all four antioxidant indices were numerically more favourable than in the saline control.

Table5: Effect of Cadmium Chloride and Lupine Extract on Testicular Oxidative Stress Markers in Male Albino Rats (Mean ± SD, n = 8).

Parameter	Control	Cd	Lupine	Cd + Lupine	F / H	p-value
MDA (nmol/mg protein)	1.53 ± 0.36	4.21 ± 0.64	1.17 ± 0.13	2.43 ± 0.64	26.30	< 0.001
SOD (U/mg protein)	39.40 ± 8.41	13.50 ± 2.43	47.97 ± 6.41	26.43 ± 6.40	53.25	< 0.001
CAT (U/mg protein)	9.88 ± 2.07	4.34 ± 1.17	13.82 ± 2.17	8.57 ± 1.62	37.72	< 0.001
GSH (µmol/g tissue)	8.12 ± 1.43	2.68 ± 0.63	10.35 ± 1.67	6.17 ± 0.95	54.97	< 0.001

MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; GSH, reduced glutathione. Values within the same row differ significantly at $p < 0.05$.

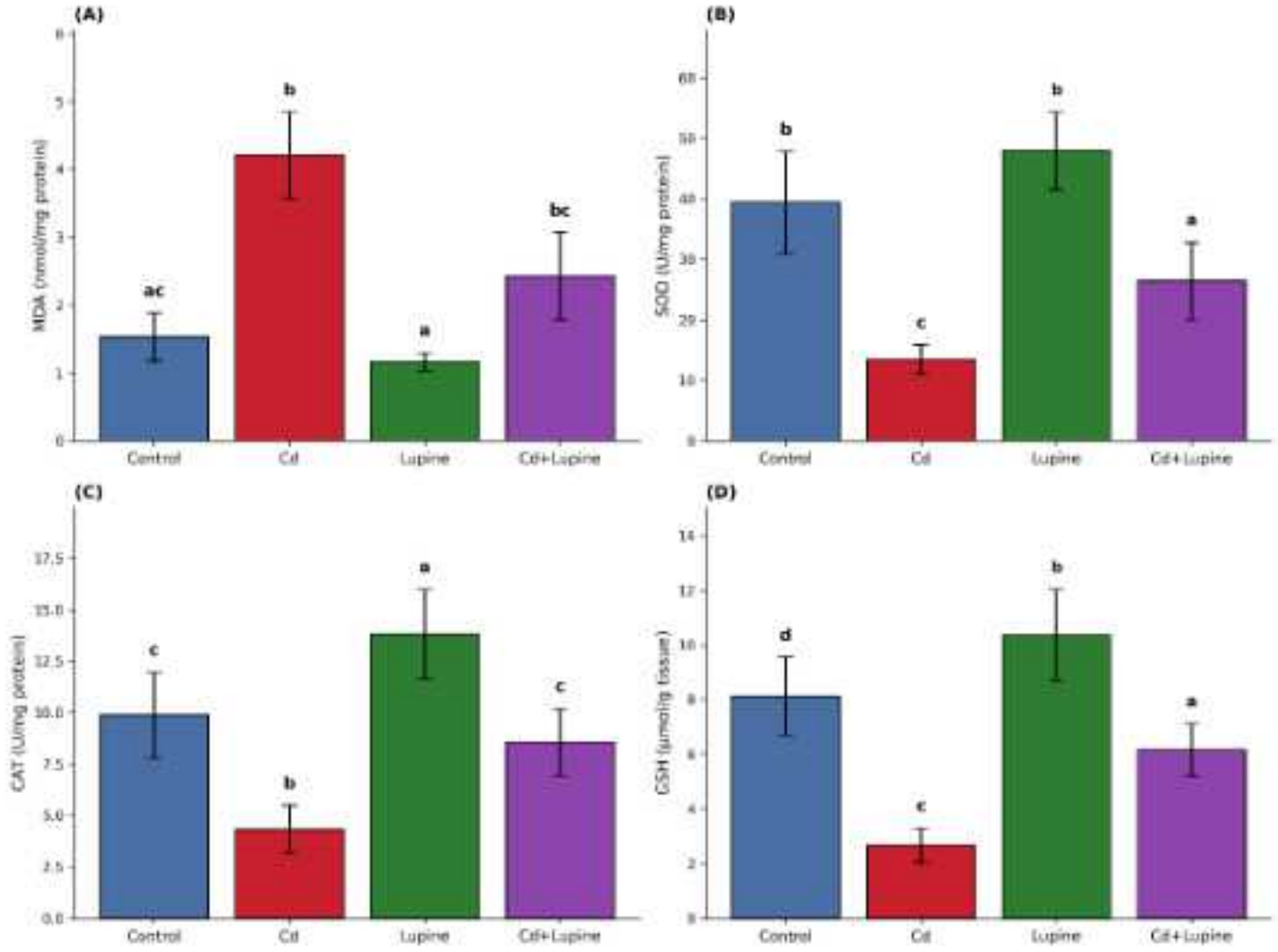

Figure 4: Effect of Cadmium Chloride and Lupine Extract on Testicular Oxidative Stress Markers: (A) MDA, (B) SOD, (C) CAT and (D) GSH. Bars bearing different letters differ significantly at $p < 0.05$.

3.6. Testicular Histopathology

Histopathological evaluation of the seminiferous tubules using the Johnsen score is presented in Table6 and Fig5 and Fig.6. The control testes showed normal seminiferous architecture, complete spermatogenesis and abundant spermatozoa in the tubular lumen, with a mean Johnsen score of 9.05 ± 0.37. In the Cd group, the score fell to 3.74 ± 0.70 (−58.7%), corresponding to widespread tubular atrophy, disorganisation of germinal epithelium, loss of mature germ cells and depletion of spermatozoa in the lumen. The Lupine-alone group exhibited well-preserved tubular architecture with active spermatogenesis (Johnsen 9.40 ± 0.24). The Cd + Lupine group showed substantial recovery of testicular histoarchitecture, with a mean Johnsen score of 6.54 ± 0.83 — a value significantly higher than the Cd group and representing 52.7% protection — although tubular organisation did not return to control levels.

Table6: Effect of Cadmium Chloride and Lupine Extract on The Johnsen Testicular Biopsy Score (n = 8)

Statistic	Control	Cd	Lupine	Cd + Lupine
Mean ± SD	9.05 ± 0.37	3.74 ± 0.70	9.40 ± 0.24	6.54 ± 0.83
Median (IQR)	9.00 (8.78–9.25)	3.55 (3.38–3.73)	9.35 (9.20–9.60)	6.45 (5.70–7.25)
IQR, interquartile range. Means differ significantly at $p < 0.05$.				

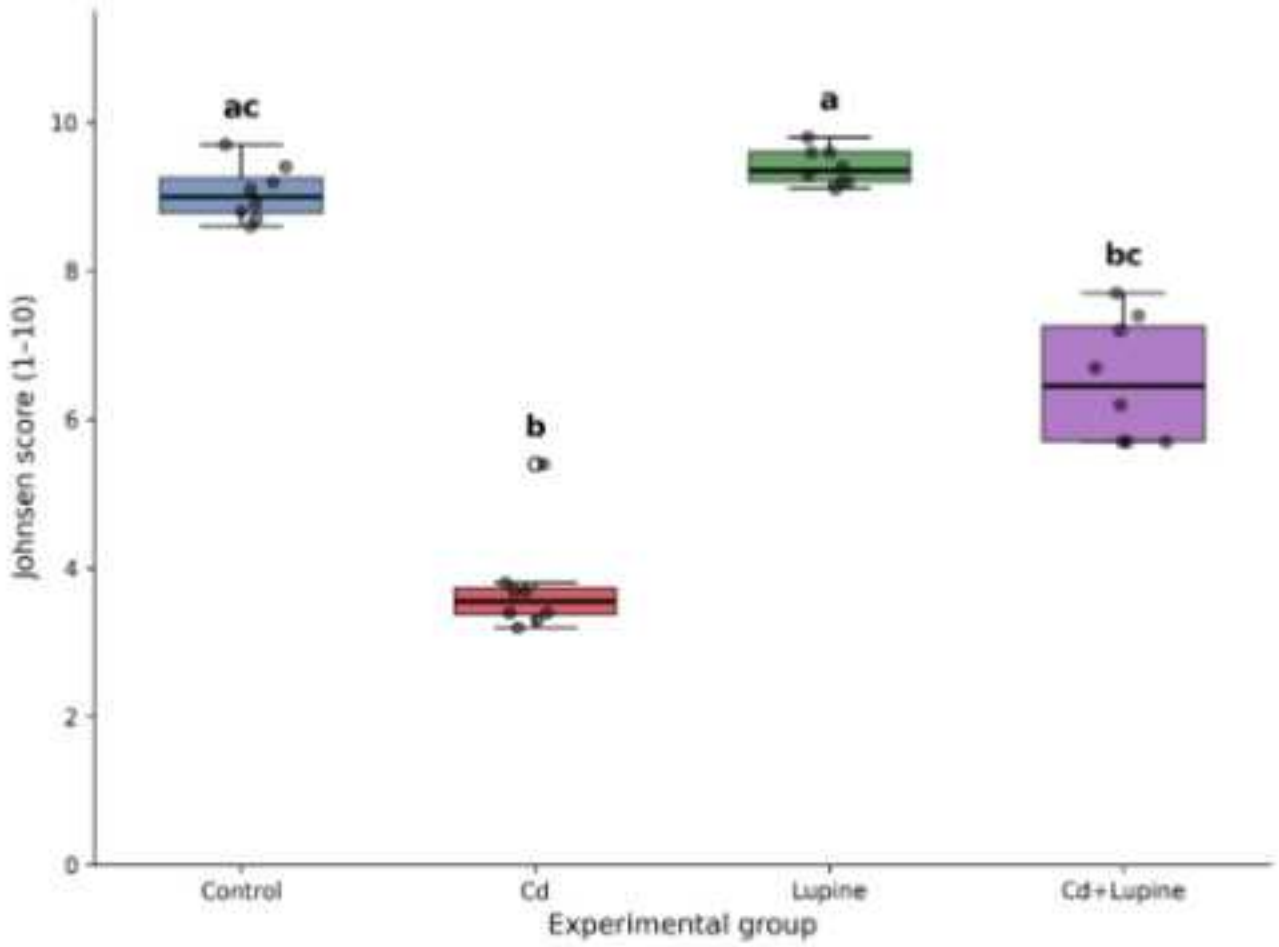

Figure5: Johnsen Testicular Biopsy Score Across the Four Experimental Groups. Box Plots Show Median, Interquartile Range and Individual Values for N = 8 Animals Per Group. Boxes Bearing Different Letters Differ Significantly at P < 0.05.

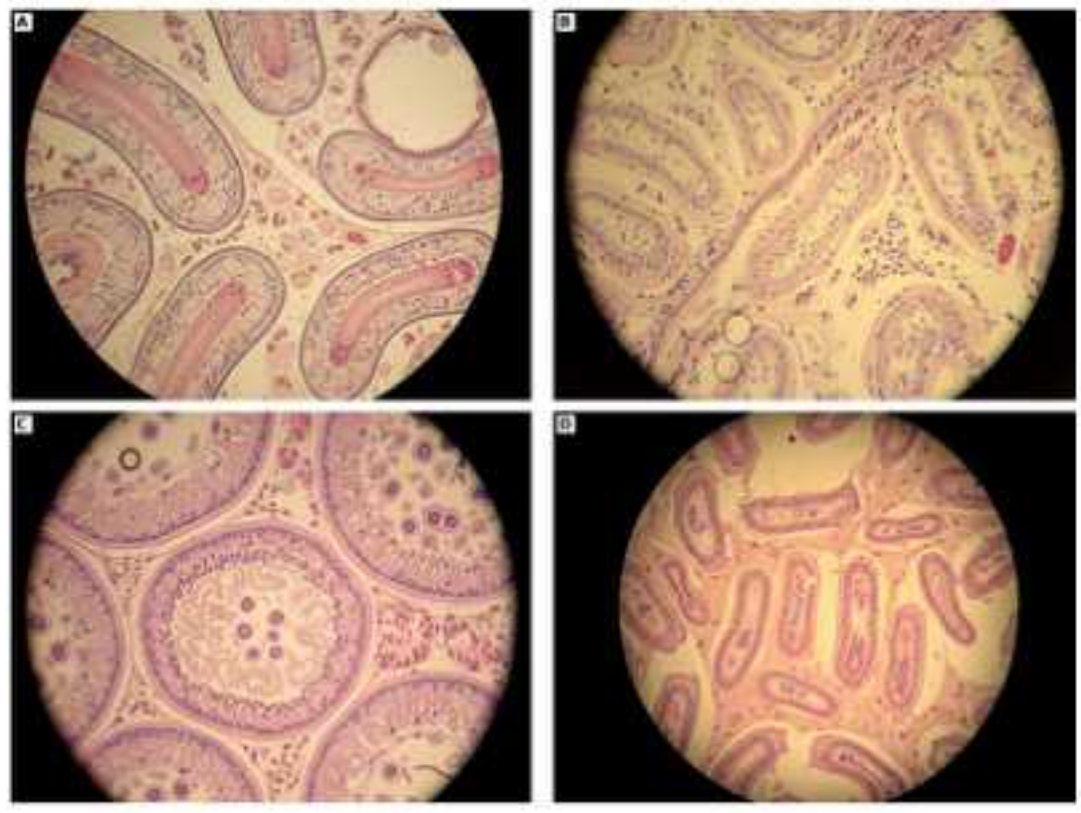

Figure6: Photomicrographs of Hematoxylin and Eosin-Stained Rat Testes (100×, Scale Bar 100Mm). (A) Control: normal seminiferous tubule architecture with complete spermatogenesis. (B) Cd-treated: widespread tubular atrophy, germ-cell depletion and intra-tubular vacuolation. (C) Lupine-treated: preserved seminiferous architecture with active spermatogenesis. (D) Cd + Lupine: partial restoration of tubular organization and germ-cell layering. Johnsen scores (mean ± SD) are shown below each panel.

3.7.Correlation between Oxidative Stress and Reproductive Parameters

Pearson correlation analysis across the four groups (N = 32) is depicted in Fig.7. MDA showed strong negative correlations with sperm count (r = −0.92), sperm viability (r = −0.92), sperm motility (r = −0.87), testosterone (r = −0.84) and Johnsen score (r = −0.89), and a strong positive correlation with sperm abnormality (r = 0.92). Conversely, antioxidant enzymes (SOD, CAT, GSH) were strongly and positively correlated with sperm count (r = 0.83–0.88), testosterone (r = 0.82–0.86), and Johnsen score (r = 0.79–0.89). These patterns are quantitatively consistent with an oxidative stress-mediated mechanism of cadmium-induced reproductive injury, which is mitigated by L. albus extract.

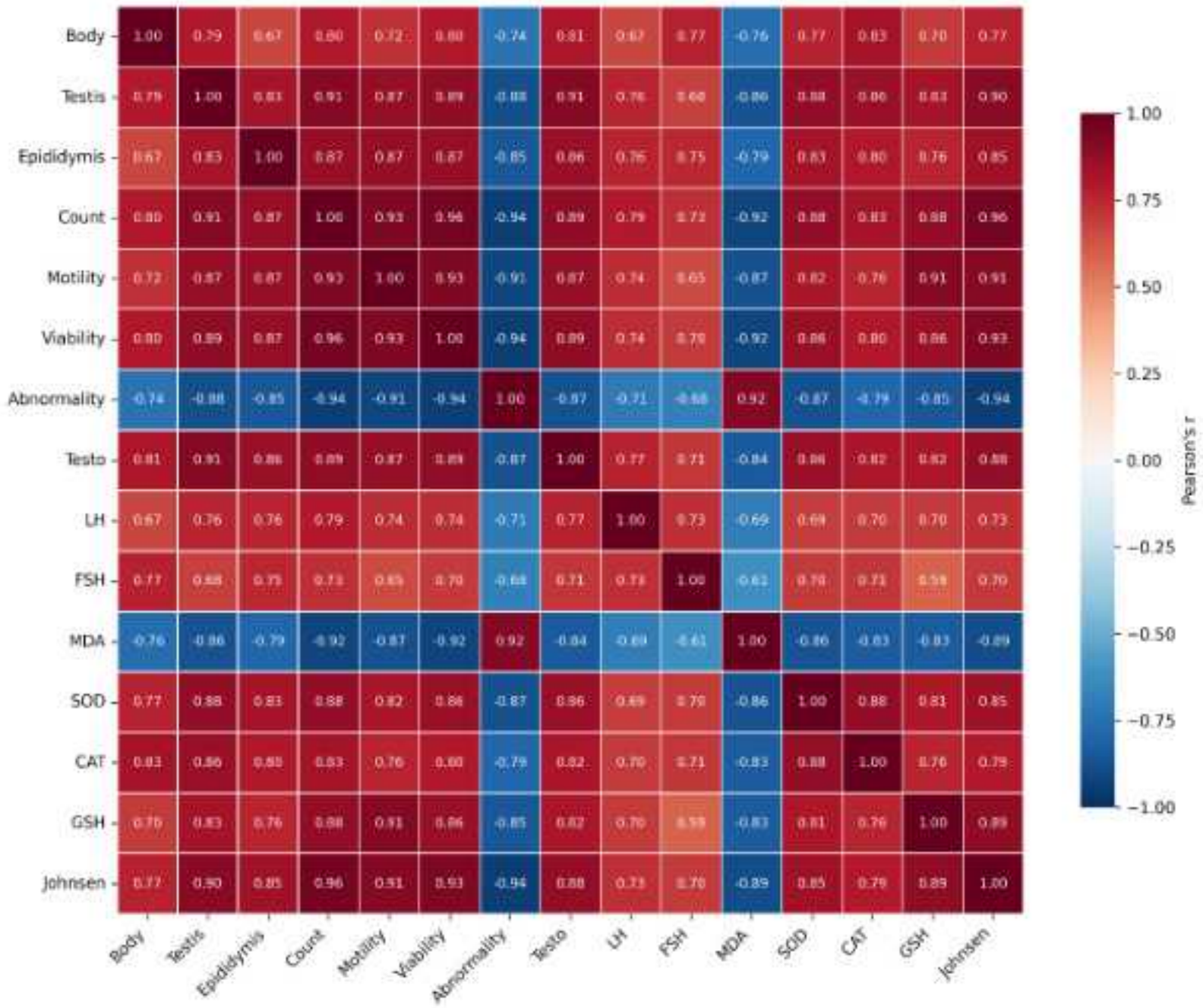

Figure7: Pearson Correlation Heat-Map of the 15 Measured Parameters Across All 32 Animals. Color Intensity Reflects the Strength of The Correlation; Red, Positive; Blue, Negative.

3.8. Magnitude of Cadmium Effects and Lupine Protection

A summary of percentage changes and percentage protection across all 15 parameters is given in Table7. All 15 measured parameters differed significantly across the four groups (all $p < 0.001$), and effect sizes (η^2 or ε^2) were uniformly large (≥ 0.54), indicating biologically meaningful differences. Cadmium's largest deleterious effects were observed on sperm abnormality (+555.2%), testosterone (−87.1%), sperm count (−79.1%), LH (−72.7%) and FSH (−69.1%). The largest percentage protections conferred by lupine were on CAT (76.3%), LH (74.5%), MDA (66.4%), GSH (64.2%) and testis weight (56.7%).

Table7: Magnitude of Cadmium-Induced Changes and Percentage Protection Conferred by Concurrent Lupine Extract for the 15 Measured Parameters.

Parameter	% Change (Cd vs Control)	% Protection (Cd + Lupine)	Direction
Body weight	−15.4%	54.4%	↓
Testis weight	−35.6%	56.7%	↓
Epididymis weight	−35.2%	34.5%	↓
Sperm count	−79.1%	52.8%	↓
Sperm motility	−65.2%	43.0%	↓
Sperm viability	−54.9%	37.7%	↓
Sperm abnormality	+555.2%	47.9%	↑
Testosterone	−87.1%	44.0%	↓
LH	−72.7%	74.5%	↓
FSH	−69.1%	54.2%	↓
MDA	+174.6%	66.4%	↑
SOD	−65.7%	49.9%	↓
CAT	−56.1%	76.3%	↓
GSH	−67.0%	64.2%	↓
Johnsen score	−58.7%	52.7%	↓

% Change = (Cd − Control) / Control × 100; % Protection = (Cd + Lupine − Cd) / (Control − Cd) × 100. All between-group comparisons were significant at $p < 0.001$.

4. Discussion

The present study confirms that subchronic oral exposure of male albino rats to cadmium chloride (5 mg/kg/day for 45 days) produces a coherent pattern of reproductive toxicity — comprising significant reductions in testis and epididymis weight, sperm count, motility and viability, marked increase in sperm abnormalities, suppression of serum testosterone, LH and FSH, depletion of testicular antioxidant enzymes, lipid peroxidation, and severe disruption of the seminiferous epithelium (Johnsen score 3.74 ± 0.70 vs 9.05 ± 0.37 in controls). Concurrent oral administration of Lupinus albus (15 mg/kg/day) significantly attenuated all of these alterations, with percentage protection values ranging from 34.5% to 76.3% across the 15 endpoints assessed.

Cadmium-Induced Reproductive Toxicity

Our findings on Cd are in close agreement with those reported by Iraqi and regional groups. Atea and Razooqi (2025), working at Tikrit University, reported that 30-day oral $CdCl_2$ administration (5 and 10 mg/kg) to male Wistar rats produced a dose-dependent reduction in serum testosterone and sperm count, a rise in MDA, and a fall in SOD, CAT and GSH. Although their exposure period was shorter than ours, the magnitude and direction of the changes are highly comparable, supporting the reproducibility of the Cd model across Iraqi institutions. Hashim and Mohammed Ali (2021), in their work on L. albus in male mice, similarly noted reductions in testosterone and antioxidant capacity following oxidative challenge. Iraqi work on related heavy-metal models, such as that of Al-Saaidi and colleagues on lead

acetate-induced reproductive toxicity (Al-Saaidi, Al-Khafaji and Khudair, 2013), and on selenium- or vitamin E-mediated protection of male reproduction in rats (Aboubakr et al., 2020; Hamza et al., 2021), collectively reinforce the view that environmental metal exposure in the Iraqi setting represents a credible threat to male reproductive health.

Internationally, our results are aligned with the substantial body of work demonstrating Cd reproductive toxicity. Nna et al. (2017) reported that 5 mg/kg $CdCl_2$ produced severe testicular injury, depletion of antioxidants, and significant reductions in testosterone and sperm count in male Wistar rats; Wang et al. (2017) showed that Cd impairs sperm function in mice by disrupting CatSper channels and inducing oxidative stress; and Ekhoye, Olerimi and Ehebha (2013) demonstrated dose-dependent decreases in testicular weight and testosterone in Cd-exposed Wistar rats. More recent (2020–2025) studies have confirmed that Cd disrupts the blood–testis barrier, induces autophagy and apoptosis in germ cells via ROS/NF-κB and Nrf2 pathways, and impairs Leydig cell steroidogenic capacity (Ji et al., 2010; Mouro et al., 2020; Ali et al., 2022). In a 2025 study, Bashandy et al. (2025) showed that chronic Cd exposure caused profound spermiotoxicity and steroidotoxicity, with persistent oxidative damage even after withdrawal. The magnitude of suppression we observed in testosterone (−87.1%) and sperm count (−79.1%) is at the upper end of effects reported in those studies, which likely reflects the relatively long (45-day) exposure period used here.

Mechanisms of Cadmium Reproductive Injury

Several converging mechanisms account for the testicular toxicity of Cd, and our data are internally consistent with each. First, Cd^{2+} ions accumulate in Leydig and Sertoli cells, displace zinc from key zinc-finger proteins (including steroidogenic transcription factors), and generate ROS through Fenton-like reactions and mitochondrial dysfunction (Rafati Rahimzadeh et al., 2017; Genchi et al., 2024). The 175% rise in testicular MDA and the marked depletion of SOD, CAT and GSH that we observed are direct evidence of this oxidative shift. Second, Cd disrupts steroidogenesis by reducing the expression of StAR, P450scc and 3β-HSD in Leydig cells, leading to the steep fall in testosterone we observed (Almeer et al., 2021). Third, Cd damages the blood–testis barrier by disrupting tight-junction proteins (occludin, claudin-11, ZO-1), explaining the disorganised seminiferous epithelium seen in our histology and the precipitous fall in Johnsen score (Yang et al., 2018; Mouro et al., 2020). Fourth, Cd-induced ROS also disrupt sperm membrane integrity and CatSper-mediated calcium signalling, accounting for the dramatic increase in morphologically abnormal sperm (8.4% → 55.0%) observed here and previously (Wang et al., 2017; Ali et al., 2022).

Protective Effect of Lupine Extract

The co-administration of L. albus extract resulted in a reduction of all 15 endpoints evaluated for Cd toxicity. The most significant protective effects were noted for CAT (76.3%) and MDA (66.4%), which serve as direct indicators of redox balance, thereby reinforcing the hypothesis that the observed protection is largely mediated by antioxidant mechanisms. This interpretation is further supported by

the correlation analysis, which reveals strong negative correlations between MDA and sperm count ($r = -0.92$), Johnsen score ($r = -0.89$), and testosterone ($r = -0.84$). Additionally, the robust positive correlations between antioxidant enzymes and reproductive endpoints suggest that the testicular redox state is closely linked to reproductive integrity across the subjects studied.

The findings align with earlier studies regarding L. albus. Hashim and Mohammed Ali (2021) demonstrated that an alcoholic extract of L. albus, administered at dosages of 5–15 mg/kg, resulted in increased testosterone levels, elevated GSH, decreased MDA, and enhanced Leydig cell density in male mice; our observations reflect a similar trend. Numerous recent investigations conducted globally from 2020 to 2025, employing various phytochemical interventions to combat Cd-induced testicular injury, have indicated comparable percentages of protection. For instance, grape seed proanthocyanidins improved sperm quality and antioxidant levels in rats exposed to Cd (Alkhedaide et al., 2016), while quercetin was found to restore testosterone levels and promote spermatogenesis (Nna et al., 2017). Additionally, selenium nanoparticles were reported to alleviate Cd-induced oxidative damage in testicular tissue (Saleh et al., 2022; Wang et al., 2022). Furthermore, thymoquinone and resveratrol have also been documented to reduce Cd-induced testicular injury in reports from 2021 to 2024 (Aly et al., 2021; El-Khadragy et al., 2021). The level of protection provided by L. albus in our study, averaging approximately 54% across all parameters, is consistent with the protective effects observed with these established phytochemicals.

The biochemical foundation for the protective properties of L. albus can be attributed to its bioactive composition. Flavonoids, specifically apigenin and luteolin glycosides, along with phenolic acids such as ferulic, p-coumaric, and caffeic acids, are recognized for their roles as radical scavengers and activators of Nrf2 (Cabello-Hurtado et al., 2016; Magalhães et al., 2017). Tocopherols serve to safeguard membrane lipids against peroxidation, whereas quinolizidine alkaloids, including lupanine and sparteine, have demonstrated anti-inflammatory and metal-chelating properties in vitro (Cortés-Avendaño et al., 2020). Additionally, L. albus protein hydrolysates comprise peptides that have shown in vitro antioxidant effects (Mané et al., 2022), and the seed coat is abundant in dietary fiber, which may diminish the intestinal absorption of divalent cations like Cd^{2+}. Together, these components are likely to operate through various mechanisms—such as luminal chelation, systemic scavenging of reactive oxygen species (ROS), stimulation of endogenous antioxidant enzyme production, and the maintenance of Leydig and Sertoli cell functionality.

It is important to highlight that L. albus alone yielded values that were either numerically comparable to or exceeded those of the control group across the majority of parameters, such as testosterone levels (8.69 vs 6.25 ng/mL), sperm count (62.3 vs 56.6 $\times 10^6$/mL), and Johnsen score (9.40 vs 9.05). This trend, which has also been noted by Hashim and Mohammed Ali (2021), implies that L. albus may have a natural supportive influence on the male reproductive axis in healthy subjects. Nevertheless, it is noteworthy that the differences from the control group were not consistently statistically significant, and these findings should be regarded as generating hypotheses rather than providing conclusive

evidence of a fertility-enhancing effect; additional dose-response studies are necessary before any such claims can be substantiated.

Comparison with Iraqi Studies

In addition to the work of Atea and Razooqi (2025) and Hashim and Mohammed Ali (2021) discussed above, our findings can be placed in the wider context of Iraqi reproductive toxicology research. Studies from Iraqi universities have repeatedly highlighted the vulnerability of the male reproductive axis to environmental and chemical insult — including lead (Al-Saaidi, Al-Khafaji and Khudair, 2013), aluminium (Hamza et al., 2021), cadmium (Al-Hilli and Hassan, 2020; Atea and Razooqi, 2025), and pesticides (Aboubakr et al., 2020) — and the protective potential of indigenous botanical agents such as Nigella sativa, Trigonella foenum-graecum and Lupinus albus (Hashim and Mohammed Ali, 2021; Hassan and Al-Hilli, 2021). The present work contributes to this regional literature by providing the first integrated dataset on L. albus protection against Cd-induced testicular toxicity in Iraqi rats and by quantitatively linking oxidative stress markers to reproductive endpoints using correlation analysis. Such regional data are valuable because lupine seeds are a familiar, affordable and locally available functional food in Iraq, and the doses used here are within the range of human dietary exposure.

Clinical Implications

While direct translation from rodent models to clinical practice should be made with caution, our findings have several implications. First, occupational and environmental exposure to cadmium — including from cigarette smoke, contaminated water and certain agricultural products — remains a relevant concern for male reproductive health in Iraq and elsewhere (World Health Organization, 2019; Genchi et al., 2020). Second, dietary or supplemental strategies built around antioxidant-rich legumes such as L. albus may form part of broader interventions aimed at supporting male reproductive health in exposed populations, but only after proper clinical investigation. Third, the strong correlation between oxidative stress markers and reproductive endpoints in our data supports the use of seminal oxidative stress markers as a useful biomarker panel in evaluating men exposed to heavy metals. These suggestions are exploratory and should not be interpreted as established clinical recommendations.

Strengths and Limitations

The strengths of this study include the parallel measurement of 15 complementary endpoints in the same animals; the inclusion of all four relevant experimental groups (control, toxicant only, protective agent only, toxicant + protective agent); the use of standardised, validated biochemical methods; quantitative correlation analysis linking redox state to reproductive endpoints; and blinded histological scoring. Several limitations should also be acknowledged. First, only a single dose of cadmium and a single dose of lupine extract were tested, precluding dose–response inference. Second, we used an oral subchronic exposure regimen (45 days) and did not assess longer-term recovery after withdrawal, nor did we measure tissue cadmium concentrations. Third, although our correlation analysis is consistent with an antioxidant mechanism, we did not directly measure molecular mediators (e.g., Nrf2, NF-κB, StAR, apoptosis markers), so the mechanistic interpretation remains inferential. Fourth, the lupine extract was prepared from a single batch of locally sourced seeds, and the absence of a detailed chemical fingerprint limits inter-study comparability. Fifth, our findings are limited to male reproductive endpoints in adult rats and cannot be extrapolated directly to human exposure scenarios, to female reproduction, or to developmental exposure windows.

Conclusion

In adult male albino rats, daily oral cadmium chloride (5 mg/kg) for 45 days produced significant deterioration of body and reproductive organ weights, sperm parameters, serum testosterone, LH and FSH, and testicular antioxidant defences, accompanied by lipid peroxidation and severe disruption of seminiferous tubule architecture. Concurrent oral administration of Lupinus albus seed extract (15 mg/kg) significantly attenuated all of these changes, with percentage protection of 34.5–76.3% across the 15 endpoints assessed and the strongest protection observed for testicular catalase, malondialdehyde and luteinising hormone. The strong correlations between oxidative stress markers and reproductive endpoints are consistent with an antioxidant-mediated mechanism of protection. These findings suggest that L. albus extract has a meaningful protective role against cadmium-induced testicular injury in this rat model and provide an evidence base for further mechanistic and translational investigation. Given that only a single dose of each agent was tested and that molecular mediators were not directly measured, our conclusions should be regarded as preliminary.

Ethics Approval and Consent to Participate

All experimental procedures were reviewed and approved by the Scientific Committee and the Institutional Animal Care and Use Committee of the College of Veterinary Medicine, University of Al-Qadisiyah, Iraq, in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals (8th edition) and the ARRIVE 2.0 guidelines. No human participants were involved.

Consent for Publication

Not applicable.

Availability of Data and Materials

The full anonymised dataset analysed in the current study is available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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