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Lupinus Albus Seed Extract Attenuates Cadmium-Induced Reproductive Toxicity in Male Wistar Rats: Dose-Dependent Modulation of Oxidative Stress, Steroidogenesis, Inflammation and Testicular Histoarchitecture

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Abstract

Introduction: Cadmium (Cd) is a widespread environmental and occupational toxicant that impairs male reproductive function through oxidative stress, endocrine disruption and inflammation. *Lupinus albus* is a legume rich in phenolic and flavonoid antioxidants, but its effect on the cadmium-exposed testis has not been examined. Aim of this study was to evaluate the dose-dependent protective effect of *L. albus* seed extract against cadmium-induced reproductive toxicity in male rats.

Methods: Forty adult male Wistar rats were randomly allocated to five groups (n = 8): control; lupine (200 mg/kg); Cd ($CdCl_2$, 5 mg/kg); Cd + lupine 100 mg/kg; and Cd + lupine 200 mg/kg, administered orally for 56 days. Body and reproductive organ weights, epididymal sperm parameters, serum testosterone, luteinising hormone (LH) and follicle-stimulating hormone (FSH), testicular oxidative markers (MDA, GSH, SOD, CAT, GPx, TAC), serum ALT, AST, ALP, urea and creatinine, serum TNF-α, IL-1β and IL-6, and testicular histomorphometry were assessed. Data were analysed by one-way ANOVA with Tukey's test.

Results: Cadmium reduced final body and reproductive organ weights, sperm count, motility, viability and normal morphology, lowered testosterone, LH and FSH, depleted antioxidant enzymes and raised MDA, increased liver and kidney markers and pro-inflammatory cytokines, and reduced seminiferous tubule diameter, germinal epithelium height and Johnsen score (all $P < 0.001$). Co-treatment with lupine attenuated these changes in a dose-dependent manner; at 200 mg/kg most parameters were not significantly different from control.

Conclusion: Within this model, *L. albus* seed extract was associated with dose-dependent attenuation of cadmium-induced reproductive, oxidative, endocrine, inflammatory and histological alterations. These findings support further mechanistic and translational investigation but do not, by themselves, establish clinical benefit.

Keywords: Cadmium, Lupinus albus, Male reproductive toxicity, Oxidative stress, Testosterone, Testis

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Introduction

Cadmium (Cd) is a non-essential heavy metal and a persistent environmental and occupational pollutant released from mining, smelting, electroplating, battery manufacture, phosphate fertilisers and tobacco smoke (1). Because it has a long biological half-life and is poorly excreted, cadmium accumulates in soft tissues, including the testis, where it interferes with normal cellular function (1). The male reproductive system is regarded as one of the most sensitive targets of cadmium toxicity, and exposure has been linked to declining semen quality in experimental and human studies (2,3).

The reproductive toxicity of cadmium is multifactorial. A central event is the excessive generation of reactive oxygen species together with depletion of enzymatic and non-enzymatic antioxidant defences, leading to lipid peroxidation and damage to germ-cell membranes (2,4). Cadmium also disrupts the hypothalamic–pituitary–gonadal axis, lowering circulating testosterone, LH and FSH and impairing Leydig-cell steroidogenesis (5). In parallel, it compromises the blood–testis barrier, promotes germ-cell apoptosis and triggers a pro-inflammatory response, collectively producing seminiferous tubule atrophy and disruption of spermatogenesis (2,3). These reproductive effects are frequently accompanied by hepatic and renal injury, reflecting the systemic distribution of the metal (1).

Because oxidative stress is a unifying mechanism of cadmium injury, dietary antioxidants and plant-derived

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polyphenols have attracted attention as candidate protective agents (6,7,8). A range of natural extracts — including Salvia officinalis and Fragaria ananassa — has been reported to preserve sperm quality, hormone concentrations and testicular structure in cadmium-exposed rodents, largely by restoring antioxidant capacity and limiting lipid peroxidation (6,7). Comparable strategies using plant extracts and antioxidants against heavy-metal reproductive toxicity have also been pursued by regional research groups (9,10).

White lupin (*Lupinus albus* L., Fabaceae) is a widely cultivated grain legume whose seeds are rich in protein, dietary fibre and a spectrum of bioactive phenolic compounds, flavones and flavonoid C-glycosides with documented free-radical-scavenging activity (11,12). The antioxidant capacity of the seed correlates with its total phenolic content (11), and lupin preparations have shown antioxidant and metabolic benefits in animal models (12). Despite this favourable antioxidant profile, the capacity of *L. albus* to protect the male reproductive system against a defined oxidative insult such as cadmium has not, to our knowledge, been directly investigated.

The present study was therefore designed to address this gap. We examined whether oral *L. albus* seed extract, at two dose levels, attenuates $CdCl_2$-induced reproductive toxicity in male Wistar rats, and whether any protection is dose-dependent. To provide a comprehensive assessment, we evaluated reproductive organ weights, epididymal sperm parameters, serum reproductive hormones, testicular oxidative-stress and antioxidant markers, serum hepatic and renal indices, pro-inflammatory cytokines, and quantitative testicular histomorphometry.

Materials and Methods

Study design and setting

This experimental study was carried out at the animal house and research laboratories of the College of Science, University of Kufa, Iraq. The work — comprising a two-week acclimatisation period, the 56-day treatment phase and subsequent laboratory and histological analyses — was conducted between October 2023 and February 2024. All procedures were performed in accordance with internationally accepted standards for the care and use of laboratory animals (13) and were approved by the Scientific and Ethics Committee of Al-Furat Al-Awsat Technical University (Approval No.: 336822; Date: October 2023).

Plant material and extract preparation

Mature *L. albus* seeds were obtained locally, authenticated by a plant taxonomist, cleaned, oven-dried and ground to a fine powder. The powder was extracted with 70% ethanol by maceration for 72 h with intermittent shaking, filtered, and concentrated under reduced pressure in a rotary evaporator; the residue was dried and stored at 4 °C until use. The extract was freshly reconstituted in distilled water immediately before administration and given by oral gavage at 100 or 200 mg/kg body weight, doses selected on the basis of previous lupin studies (12) and a preliminary tolerability check.

Chemicals and cadmium dosing

Analytical-grade cadmium chloride ($CdCl_2$) was dissolved in distilled water and administered orally at 5 mg/kg body weight per day, a dose previously shown to induce reproducible testicular injury in rats (4). All other reagents were of analytical grade.

Animals and experimental groups

Forty adult male Wistar albino rats (10–12 weeks old; 264–268 g) were housed under controlled conditions (22 ± 2 °C; 12-h light/dark cycle) with free access to a standard pellet diet and tap water. After acclimatisation, animals were randomly allocated to five groups of eight: Group I (control) received distilled water (1 mL/kg); Group II received *L. albus* extract (200 mg/kg); Group III received $CdCl_2$ (5 mg/kg); Group IV received $CdCl_2$ (5 mg/kg) plus *L. albus* (100 mg/kg); and Group V received $CdCl_2$ (5 mg/kg) plus *L. albus* (200 mg/kg). All treatments were given orally once daily for 56 consecutive days, a period corresponding to approximately one full cycle of rat spermatogenesis; in the co-treatment groups the extract was administered shortly after $CdCl_2$.

Body and organ weights

Body weight was recorded at the start and end of the experiment. At necropsy the testes, epididymides and seminal vesicles were dissected and weighed; relative organ weights were expressed per 100 g body weight.

Sample collection

At the end of treatment, rats were fasted overnight, anaesthetised with an intraperitoneal ketamine–xylazine combination, and blood was collected by cardiac puncture. Serum was separated by centrifugation (3000 × g, 15 min) and stored at −20 °C for hormonal, biochemical and cytokine assays. One testis from each animal was homogenised in ice-cold phosphate buffer (pH 7.4) and centrifuged; the supernatant was used for oxidative-stress assays, with results normalised to tissue protein. The contralateral testis was fixed for histology.

Epididymal sperm analysis

The cauda epididymis was minced in pre-warmed normal saline to release spermatozoa. Sperm concentration was determined with a Neubauer haemocytometer; progressive motility was assessed under a light microscope (Olympus CX31; Olympus, Japan); viability was evaluated using eosin–nigrosin staining; and morphological abnormalities (head, tail and neck/mid-piece defects) were scored on stained smears, following standard semen-evaluation procedures adapted for the rat (14).

Hormone assays

Serum testosterone (Cusabio CSB-E05100r), LH (CSB-E12654r) and FSH (CSB-E06869r) were measured with rat-specific competitive enzyme-linked immunosorbent

assay (ELISA) kits (Cusabio, China) on a microplate reader (BioTek ELx800; BioTek Instruments, USA), according to the manufacturer's instructions.

Oxidative-stress and antioxidant markers

Testicular malondialdehyde (MDA; Elabscience E-BC-K025-M, thiobarbituric acid method) (15), reduced glutathione (GSH; E-BC-K030-M, DTNB method) (16), superoxide dismutase (SOD; E-BC-K020-M) (17), catalase (CAT; ZellBio ZB-CAT-96A) (18), glutathione peroxidase (GPx; E-BC-K096-S) (19) and total antioxidant capacity (TAC; ZellBio ZB-TAC-96A) were determined colorimetrically using commercial kits (Elabscience, China; ZellBio, Germany) and a UV-Vis spectrophotometer (APEL PD-303; APEL, Japan), with absorbances read at the kit-specified wavelengths; assay principles followed established colorimetric methods (15–19).

Hepatic and renal function

Serum alanine aminotransferase (ALT; Spinreact 1001170), aspartate aminotransferase (AST; 1001160) and alkaline phosphatase (ALP; 1001130) (Spinreact, Spain), and serum urea (Biosystems COD 11516) and creatinine (Biosystems COD 11734, compensated Jaffe method) (BioSystems, Spain) were measured on a semi-automated chemistry analyser (BioSystems BTS-350; BioSystems, Spain).

Pro-inflammatory cytokines

Serum TNF-α (Cusabio CSB-E11987r), IL-1β (CSB-E04610r) and IL-6 (CSB-E04640r) were quantified with rat-specific sandwich ELISA kits (Cusabio, China) on the same microplate reader.

Histopathology and histomorphometry

Testes fixed in Bouin's solution were processed, embedded in paraffin, sectioned at 5 µm with a rotary microtome (Leica RM2125; Leica, Germany) and stained with haematoxylin and eosin (20). Sections were examined under a light microscope by an examiner blinded to group allocation. Seminiferous tubule diameter and germinal epithelium height were measured on 30 round tubular profiles per animal using a calibrated ocular micrometer/image-analysis system, and spermatogenic activity was graded on 100 tubules per animal using the Johnsen testicular biopsy score (1–10) (21).

Statistical analysis

Continuous variables were assessed for normality (Shapiro–Wilk) and homogeneity of variance (Levene) and compared among the five groups by one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post-hoc test; the individual animal was the experimental unit (n = 8 per group). Effect sizes were expressed as omega-squared (ω^2). The dose-dependence of lupine protection across the cadmium co-treated groups (0, 100, 200 mg/kg) was tested by orthogonal polynomial (linear and quadratic) trend contrasts. Percentage protection was calculated as [(mean Cd+lupine − mean Cd)/(mean control − mean Cd)] × 100. Significance was set at $P < 0.05$. Analyses were performed using SPSS version 29 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 10 (GraphPad Software, USA).

Results

Body weight and reproductive organ weights

Initial body weight did not differ among groups ($F(4,35) = 0.11$, $P = 0.977$), confirming comparable baselines (Table 1). By the end of the study, cadmium exposure was associated with significantly lower final body weight and lower absolute and relative testis, epididymis and seminal vesicle weights than control ($P < 0.001$), with large effect sizes ($\omega^2 = 0.60–0.74$). Co-treatment with lupine raised these weights in a dose-graded fashion; at 200 mg/kg, final body weight and most organ weights were not significantly different from control (shared superscript "a"). The lupine-only group did not differ from control for any weight parameter, indicating no adverse effect of the extract alone on growth or reproductive organ mass.

Epididymal sperm parameters

Cadmium markedly reduced sperm count, motility, viability and the proportion of morphologically normal spermatozoa relative to control (all $P < 0.001$), and increased head, tail and neck/mid-piece abnormalities (Table 2), with very large effect sizes ($\omega^2 = 0.82–0.96$). As shown in Figure 1, both lupine doses partially restored

Table 1. Effect of lupine extract on body weight and reproductive organ weights in cadmium-exposed male rats

Parameter	Unit	Control	Lupine	Cd	Cd + Lup-L	Cd + Lup-H	F	*P*	ω²
Initial body weight	g	265.0 ± 13.2[a]	268.0 ± 13.4[a]	264.0 ± 13.2[a]	266.0 ± 13.3[a]	267.0 ± 13.4[a]	0.11	0.977	0.00
Final body weight	g	298.0 ± 14.9[a]	305.0 ± 15.2[a]	252.0 ± 12.6[c]	275.0 ± 13.8[b]	290.0 ± 14.5[a]	17.56	<0.001	0.62
Absolute testis weight	g	1.14 ± 0.11[a]	1.18 ± 0.12[a]	0.72 ± 0.07[c]	0.92 ± 0.09[b]	1.08 ± 0.11[a]	27.69	<0.001	0.73
Relative testis weight	g/100 g	0.38 ± 0.03[a]	0.39 ± 0.03[a]	0.29 ± 0.02[c]	0.34 ± 0.03[b]	0.37 ± 0.03[a]	16.30	<0.001	0.60
Absolute epididymis weight	g	0.42 ± 0.04[a]	0.44 ± 0.04[a]	0.28 ± 0.03[c]	0.34 ± 0.03[b]	0.40 ± 0.04[a]	25.94	<0.001	0.71
Relative epididymis weight	g/100 g	0.14 ± 0.01[a]	0.14 ± 0.01[a]	0.11 ± 0.01[b]	0.12 ± 0.01[b]	0.14 ± 0.01[a]	16.00	<0.001	0.60
Absolute seminal vesicle weight	g	0.85 ± 0.10[a]	0.92 ± 0.11[a]	0.48 ± 0.06[c]	0.65 ± 0.08[b]	0.80 ± 0.10[a]	29.41	<0.001	0.74
Relative seminal vesicle weight	g/100 g	0.28 ± 0.03[a]	0.30 ± 0.03[a]	0.19 ± 0.02[c]	0.24 ± 0.02[b]	0.28 ± 0.03[a]	21.94	<0.001	0.68

Data are expressed as mean ± SD (n = 8 animals per group). Means in the same row not sharing a common superscript letter (a–d) differ significantly at $P < 0.05$ (one-way ANOVA followed by Tukey's HSD post-hoc test). F, ANOVA F-statistic (df = 4, 35); ω², omega-squared effect size. Cd, cadmium chloride (5 mg/kg); Lup-L, lupine (100 mg/kg); Lup-H, lupine (200 mg/kg)

Table 2. Effect of lupine extract on epididymal sperm quality parameters in cadmium-exposed male rats

Parameter	Unit	Control	Lupine	Cd	Cd + Lup-L	Cd + Lup-H	F	*P*	ω²
Sperm count	×10⁶/mL	352.0 ± 42.2ᵃ	378.0 ± 45.4ᵃ	165.0 ± 19.8ᵈ	248.0 ± 29.8ᶜ	320.0 ± 38.4ᵇ	45.24	<0.001	0.82
Sperm motility	%	76.50 ± 6.12ᵃ	81.20 ± 6.50ᵃ	35.80 ± 2.86ᵈ	54.20 ± 4.34ᶜ	70.30 ± 5.62ᵇ	99.94	<0.001	0.91
Sperm viability	%	84.20 ± 6.74ᵃ	88.50 ± 7.08ᵃ	38.50 ± 3.08ᵈ	58.60 ± 4.69ᶜ	78.40 ± 6.27ᵇ	104.4	<0.001	0.91
Normal morphology	%	95.80 ± 2.87ᵃ	96.50 ± 2.90ᵃ	62.40 ± 1.87ᵈ	78.50 ± 2.35ᶜ	91.20 ± 2.74ᵇ	253.3	<0.001	0.96
Head abnormalities	%	1.85 ± 0.37ᶜ	1.42 ± 0.28ᶜ	14.80 ± 2.96ᵃ	8.65 ± 1.73ᵇ	3.45 ± 0.69ᶜ	103.8	<0.001	0.91
Tail abnormalities	%	1.20 ± 0.24ᶜ	0.95 ± 0.19ᵈ	12.50 ± 2.50ᵃ	6.80 ± 1.36ᵇ	2.85 ± 0.57ᶜ	111.4	<0.001	0.92
Neck/mid-piece abnormalities	%	1.15 ± 0.25ᶜ	0.85 ± 0.19ᶜ	10.30 ± 2.27ᵃ	6.05 ± 1.33ᵇ	2.50 ± 0.55ᶜ	87.46	<0.001	0.90

Data are expressed as mean ± SD (n = 8 animals per group). Means in the same row not sharing a common superscript letter (a–d) differ significantly at $P < 0.05$ (one-way ANOVA followed by Tukey's HSD post-hoc test). F, ANOVA F-statistic (df = 4, 35); ω², omega-squared effect size. Cd, cadmium chloride (5 mg/kg); Lup-L, lupine (100 mg/kg); Lup-H, lupine (200 mg/kg)

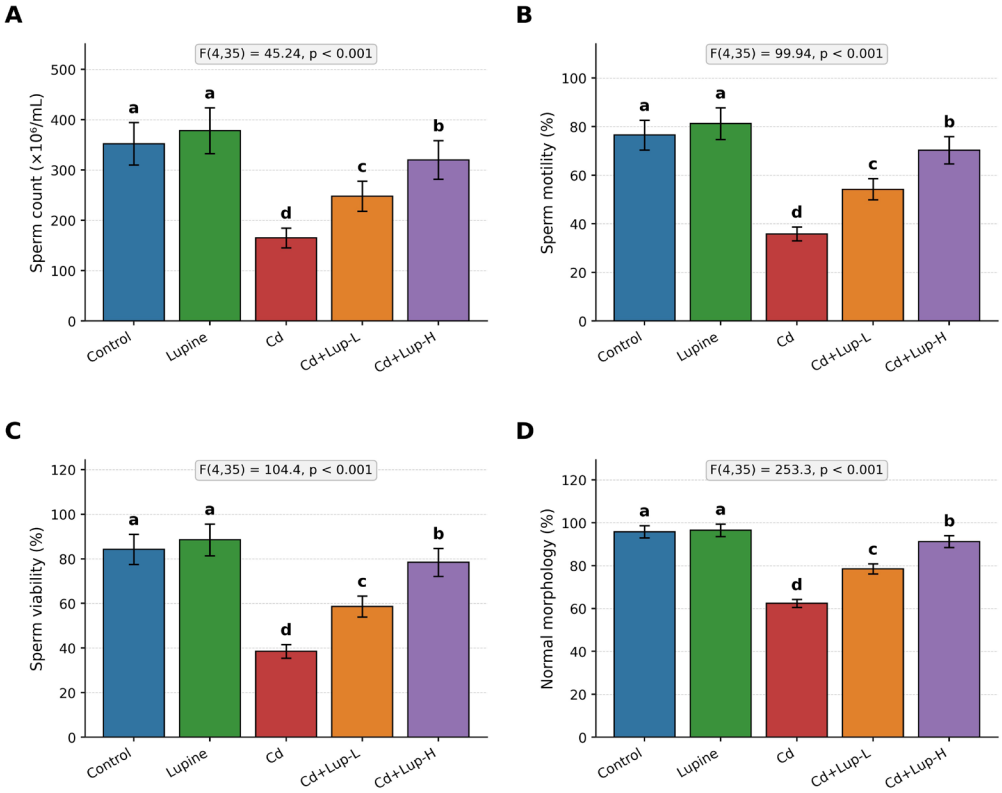

Figure 1. Effect of lupine extract on epididymal sperm quality in cadmium-exposed rats. (A) Sperm count, (B) sperm motility, (C) sperm viability and (D) normal sperm morphology. Data are mean ± SD (n = 8 per group); bars not sharing a common letter (a–d) differ at $P < 0.05$ (one-way ANOVA with Tukey's HSD). The ANOVA F-statistic and p-value are shown in each panel

sperm count, motility, viability and normal morphology, and the high dose produced values that, while still below control, were significantly higher than the low dose. Figure 2 shows a parallel, dose-dependent reduction in each category of morphological abnormality with co-treatment; at 200 mg/kg the abnormality frequencies were statistically indistinguishable from control. The lupine-only group was comparable to control throughout.

Serum reproductive hormones

Serum testosterone, LH and FSH were significantly lower in the cadmium group than in control ($P < 0.001$; $\omega^2 = 0.67$–0.80) (Table 3). Co-treatment with lupine increased all three hormones dose-dependently (Figure 3). For testosterone and LH the high-dose group remained significantly below control, whereas FSH in the high-dose group returned to a value not significantly different from control (shared superscript "a"), indicating partial endocrine recovery.

Testicular oxidative stress and antioxidant defence

Cadmium produced a pronounced oxidative imbalance in testicular tissue, with an approximately 2.8-fold higher MDA concentration and substantially lower GSH, SOD, CAT, GPx and TAC than control (all $P < 0.001$; $\omega^2 = 0.74$–0.93) (Table 4). Figure 4 shows that lupine co-treatment lowered MDA and restored antioxidant markers in a dose-dependent manner; at 200 mg/kg, MDA and several antioxidant indices were not significantly different from control. The lupine-only group showed slightly lower MDA and higher GSH and TAC than control, consistent with an intrinsic antioxidant action of the extract.

Serum hepatic and renal function markers

Cadmium significantly elevated serum ALT, AST, ALP,

Table 3. Effect of lupine extract on serum reproductive hormone concentrations in cadmium-exposed male rats

Parameter	Unit	Control	Lupine	Cd	Cd + Lup-L	Cd + Lup-H	F	*P*	ω²
Testosterone	ng/mL	3.15 ± 0.47^{a}	3.52 ± 0.53^{a}	1.25 ± 0.19^{d}	2.05 ± 0.31^{c}	2.85 ± 0.43^{b}	40.66	<0.001	0.80
LH	mIU/mL	2.12 ± 0.32^{a}	2.38 ± 0.36^{a}	0.98 ± 0.15^{d}	1.45 ± 0.22^{c}	1.92 ± 0.29^{b}	32.13	<0.001	0.76
FSH	ng/mL	2.48 ± 0.37^{a}	2.65 ± 0.40^{a}	1.35 ± 0.20^{c}	1.82 ± 0.27^{b}	2.28 ± 0.34^{a}	21.30	<0.001	0.67

Data are expressed as mean ± SD (n = 8 animals per group). Means in the same row not sharing a common superscript letter (a–d) differ significantly at $P < 0.05$ (one-way ANOVA followed by Tukey's HSD post-hoc test). F, ANOVA F-statistic (df = 4, 35); ω², omega-squared effect size. Cd, cadmium chloride (5 mg/kg); Lup-L, lupine (100 mg/kg); Lup-H, lupine (200 mg/kg). LH, luteinising hormone; FSH, follicle-stimulating hormone

Table 4. Effect of lupine extract on testicular oxidative stress and antioxidant defence markers in cadmium-exposed male rats

Parameter	Unit	Control	Lupine	Cd	Cd + Lup-L	Cd + Lup-H	F	*P*	ω²
MDA	nmol/mg prot.	2.45 ± 0.29^{c}	2.05 ± 0.25^{d}	6.85 ± 0.82^{a}	4.65 ± 0.56^{b}	2.95 ± 0.35^{c}	124.5	<0.001	0.93
GSH	nmol/mg prot.	5.25 ± 0.79^{b}	6.45 ± 0.97^{a}	2.15 ± 0.32^{d}	3.55 ± 0.53^{c}	5.05 ± 0.76^{b}	43.91	<0.001	0.81
SOD	U/mg prot.	4.15 ± 0.62^{a}	4.85 ± 0.73^{a}	1.65 ± 0.25^{d}	2.85 ± 0.43^{c}	3.85 ± 0.58^{b}	41.36	<0.001	0.80
CAT	U/mg prot.	52.50 ± 7.88^{a}	58.50 ± 8.78^{a}	24.50 ± 3.67^{d}	38.50 ± 5.77^{c}	48.50 ± 7.27^{b}	29.82	<0.001	0.74
GPx	U/mg prot.	36.50 ± 5.47^{a}	41.50 ± 6.22^{a}	15.50 ± 2.32^{d}	25.50 ± 3.82^{c}	33.50 ± 5.02^{b}	36.56	<0.001	0.78
TAC	nmol/mg prot.	105.0 ± 15.8^{b}	125.0 ± 18.8^{a}	42.00 ± 6.30^{d}	72.00 ± 10.80^{c}	95.00 ± 14.30^{b}	42.32	<0.001	0.81

Data are expressed as mean ± SD (n = 8 animals per group). Means in the same row not sharing a common superscript letter (a–d) differ significantly at $P < 0.05$ (one-way ANOVA followed by Tukey's HSD post-hoc test). F, ANOVA F-statistic (df = 4, 35); ω², omega-squared effect size. Cd, cadmium chloride (5 mg/kg); Lup-L, lupine (100 mg/kg); Lup-H, lupine (200 mg/kg). MDA, malondialdehyde; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; TAC, total antioxidant capacity; prot., protein

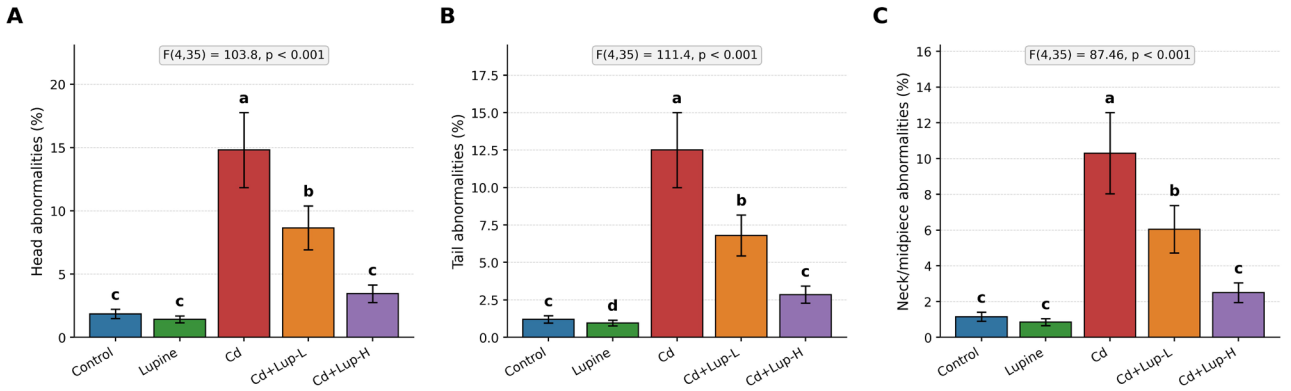

Figure 2. Effect of lupine extract on sperm morphological abnormalities. (A) Head, (B) tail and (C) neck/mid-piece abnormalities. Data are mean ± SD (n = 8 per group); bars not sharing a common letter (a–d) differ at $P < 0.05$ (one-way ANOVA with Tukey's HSD)

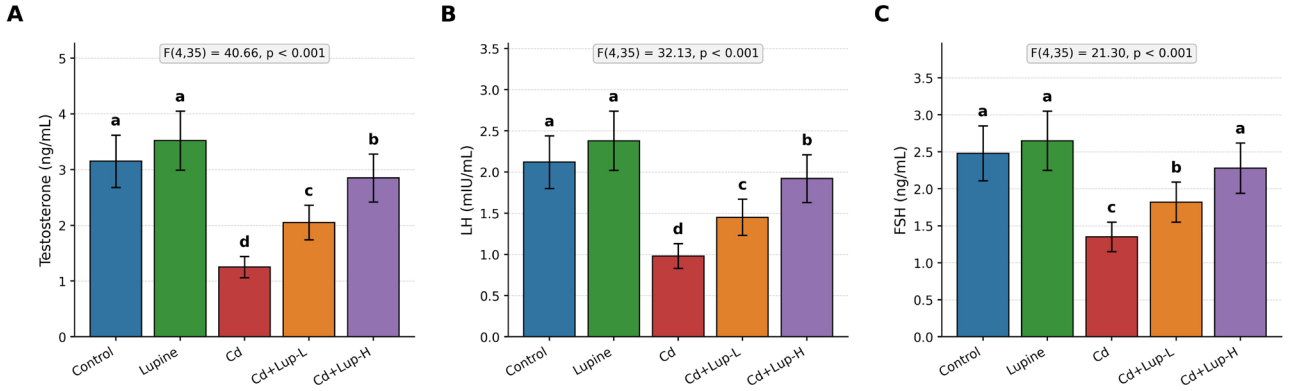

Figure 3. Effect of lupine extract on serum reproductive hormones. (A) Testosterone, (B) LH and (C) FSH. Data are mean ± SD (n = 8 per group); bars not sharing a common letter (a–d) differ at $P < 0.05$ (one-way ANOVA with Tukey's HSD)

urea and creatinine compared with control ($P < 0.001$; $\omega^2 = 0.68–0.84$), indicating concurrent hepatic and renal involvement (Table 5; Figure 5). Lupine co-treatment attenuated these increases dose-dependently; at 200 mg/kg, ALT, AST, urea and creatinine were not significantly different from control, while ALP remained modestly elevated.

Serum pro-inflammatory cytokines

Serum TNF-α, IL-1β and IL-6 were several-fold higher in the cadmium group than in control (all $P < 0.001$; $\omega^2 = 0.91–0.92$) (Table 6). As shown in Figure 6, lupine co-treatment reduced all three cytokines in a dose-dependent manner, and at the high dose their concentrations were statistically comparable to control.

Table 5. Effect of lupine extract on serum hepatic and renal function markers in cadmium-exposed male rats

Parameter	Unit	Control	Lupine	Cd	Cd + Lup-L	Cd + Lup-H	F	*P*	ω²
ALT	U/L	34.50 ± 4.14^{c}	32.50 ± 3.90^{c}	68.50 ± 8.22^{a}	52.50 ± 6.30^{b}	40.50 ± 4.86^{c}	54.70	<0.001	0.84
AST	U/L	54.50 ± 6.54^{c}	51.50 ± 6.18^{c}	95.50 ± 11.46^{a}	75.50 ± 9.06^{b}	62.50 ± 7.50^{c}	37.00	<0.001	0.78
ALP	U/L	118.0 ± 11.8^{c}	112.0 ± 11.2^{c}	168.0 ± 16.8^{a}	145.0 ± 14.5^{b}	128.0 ± 12.8^{b}	22.29	<0.001	0.68
Urea	mg/dL	28.50 ± 2.85^{c}	27.20 ± 2.72^{d}	48.50 ± 4.85^{a}	39.50 ± 3.95^{b}	32.50 ± 3.25^{c}	47.78	<0.001	0.82
Creatinine	mg/dL	0.55 ± 0.06^{c}	0.53 ± 0.05^{d}	0.95 ± 0.10^{a}	0.78 ± 0.08^{b}	0.64 ± 0.06^{c}	47.28	<0.001	0.82

Data are expressed as mean ± SD (n = 8 animals per group). Means in the same row not sharing a common superscript letter (a–d) differ significantly at $P < 0.05$ (one-way ANOVA followed by Tukey's HSD post-hoc test). F, ANOVA F-statistic (df = 4, 35); ω², omega-squared effect size. Cd, cadmium chloride (5 mg/kg); Lup-L, lupine (100 mg/kg); Lup-H, lupine (200 mg/kg). ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase

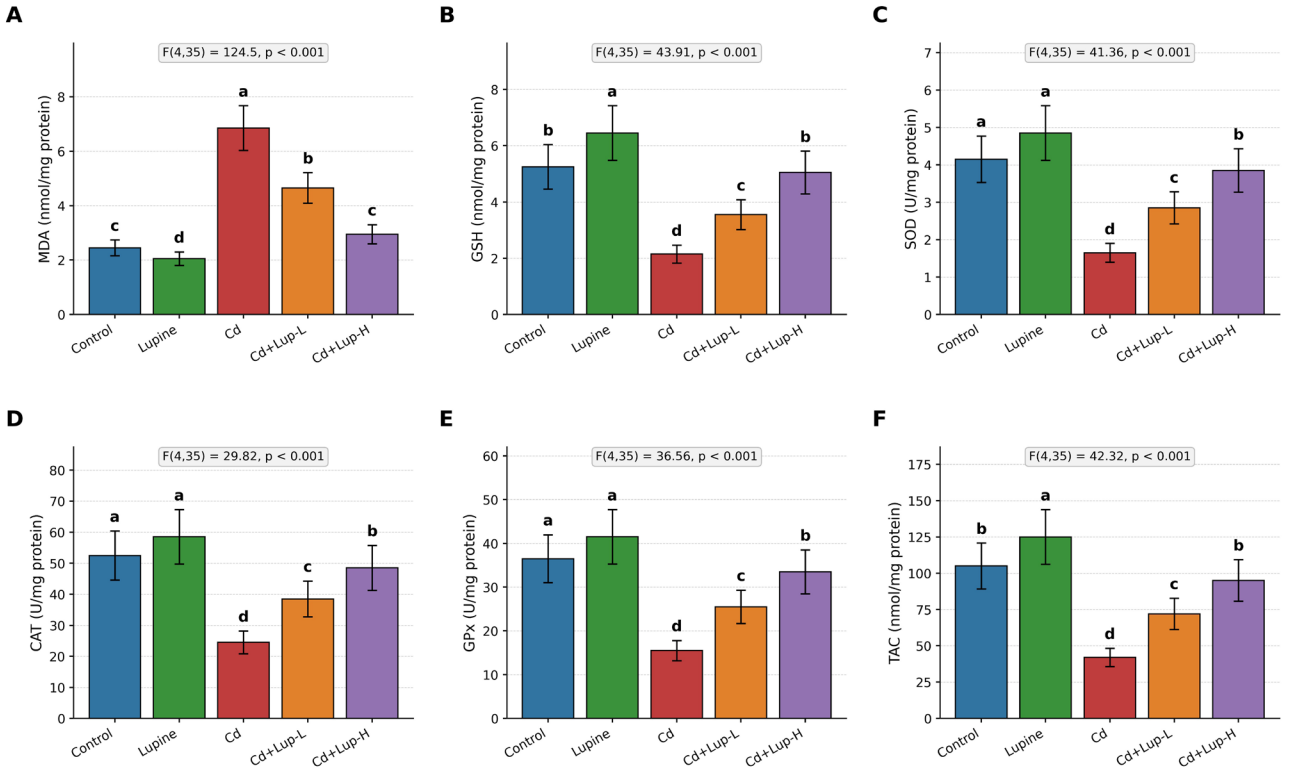

Figure 4. Effect of lupine extract on testicular oxidative stress and antioxidant markers. (A) MDA, (B) GSH, (C) SOD, (D) CAT, (E) GPx and (F) TAC. Data are mean ± SD (n = 8 per group); bars not sharing a common letter (a–d) differ at $P < 0.05$ (one-way ANOVA with Tukey's HSD)

Testicular histomorphometry and histopathology

Quantitative analysis showed that cadmium reduced seminiferous tubule diameter, germinal epithelium height and the Johnsen score relative to control (all $P < 0.001$; $\omega^2 = 0.89–0.95$) (Table 7; Figure 7). Representative photomicrographs (Figure 8) showed normal tubular architecture with organised germinal epithelium and luminal spermatozoa in the control and lupine groups, whereas cadmium-exposed testes exhibited tubular atrophy, epithelial disorganisation and depletion, germ-cell sloughing and interstitial changes; co-treatment with lupine was associated with progressive preservation of tubular architecture, most evident at the high dose.

Dose-dependent protective efficacy

The magnitude of protection is summarised in Table 8 and Figure 9. Across representative biomarkers, percentage restoration toward control values rose from 42–50% at 100 mg/kg to 83–94% at 200 mg/kg. Orthogonal polynomial contrasts indicated a significant linear trend for every biomarker examined ($P < 0.001$), with no significant quadratic component, consistent with an approximately linear dose–response over the range tested. The correlation matrix (Figure 10) showed strong positive associations among the beneficial parameters (sperm indices, hormones, antioxidants and histomorphometry) and strong negative associations between these and the markers of injury (MDA, abnormal sperm, liver and kidney enzymes and cytokines). Principal component analysis (Figure 11) separated the cadmium group from the control and lupine groups along the first component, with the cadmium + lupine groups positioned between them in a dose-ordered manner.

Discussion

In this study, oral administration of $CdCl_2$ for 56 days produced a consistent pattern of male reproductive injury in Wistar rats — reduced reproductive organ weights, impaired sperm parameters, suppressed reproductive hormones, testicular oxidative stress, systemic inflammation, hepatic and renal disturbance,

Table 6. Effect of lupine extract on serum pro-inflammatory cytokine levels in cadmium-exposed male rats

Parameter	Unit	Control	Lupine	Cd	Cd + Lup-L	Cd + Lup-H	F	*P*	ω²
TNF-α	pg/mL	12.50 ± 1.88^{c}	10.80 ± 1.62^{d}	42.50 ± 6.38^{a}	28.50 ± 4.27^{b}	16.80 ± 2.52^{c}	98.67	<0.001	0.91
IL-1β	pg/mL	8.50 ± 1.27^{c}	7.20 ± 1.08^{d}	32.50 ± 4.88^{a}	20.50 ± 3.07^{b}	11.50 ± 1.72^{c}	114.5	<0.001	0.92
IL-6	pg/mL	15.50 ± 2.32^{c}	13.20 ± 1.98^{d}	55.50 ± 8.32^{a}	35.50 ± 5.33^{b}	21.50 ± 3.23^{c}	104.8	<0.001	0.91

Data are expressed as mean ± SD (n = 8 animals per group). Means in the same row not sharing a common superscript letter (a–d) differ significantly at $P < 0.05$ (one-way ANOVA followed by Tukey's HSD post-hoc test). F, ANOVA F-statistic (df = 4, 35); ω², omega-squared effect size. Cd, cadmium chloride (5 mg/kg); Lup-L, lupine (100 mg/kg); Lup-H, lupine (200 mg/kg). TNF-α, tumour necrosis factor-alpha; IL-1β, interleukin-1 beta; IL-6, interleukin-6

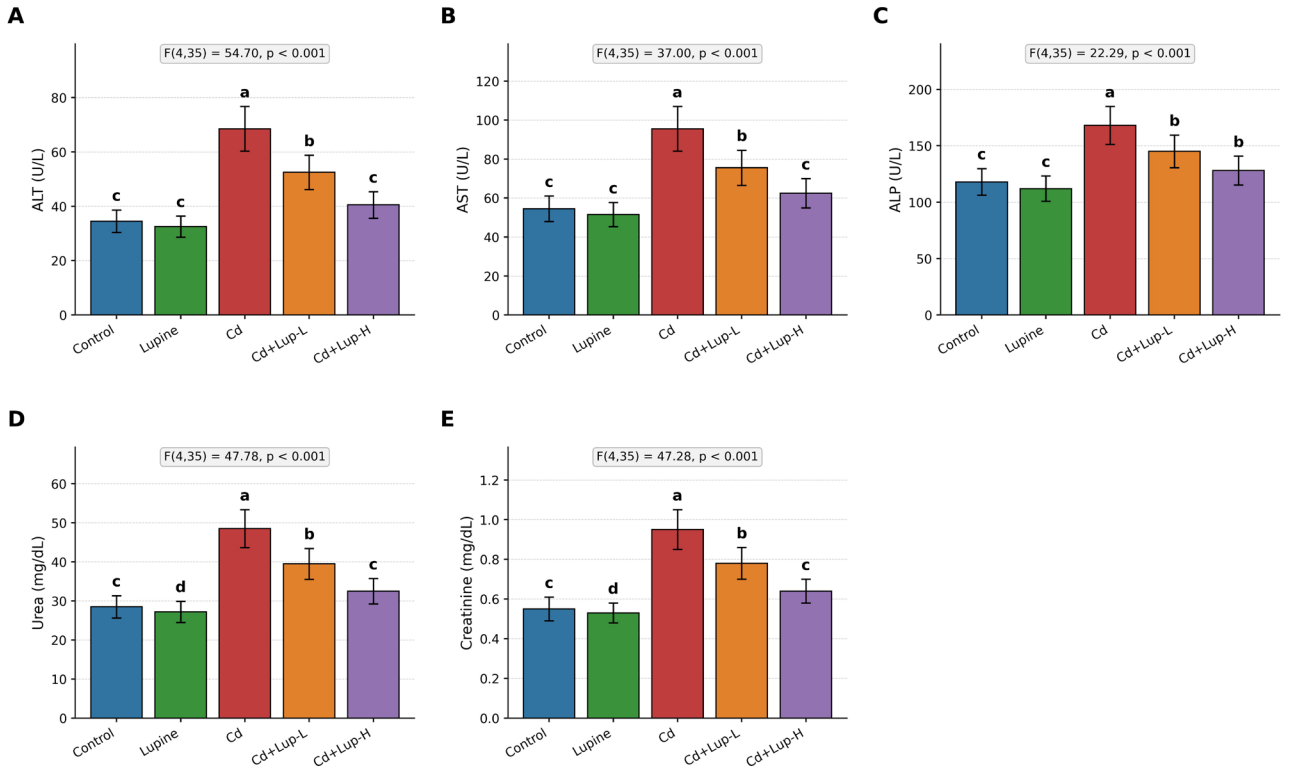

Figure 5. Effect of lupine extract on serum hepatic and renal function markers. (A) ALT, (B) AST, (C) ALP, (D) urea and (E) creatinine. Data are mean ± SD (n = 8 per group); bars not sharing a common letter (a–d) differ at $P < 0.05$ (one-way ANOVA with Tukey's HSD)

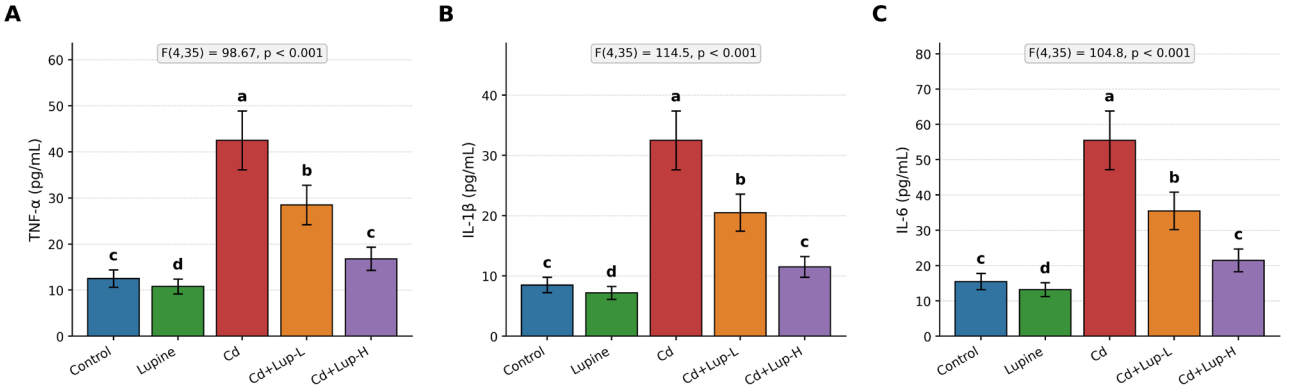

Figure 6. Effect of lupine extract on serum pro-inflammatory cytokines. (A) TNF-α, (B) IL-1β and (C) IL-6. Data are mean ± SD (n = 8 per group); bars not sharing a common letter (a–d) differ at $P < 0.05$ (one-way ANOVA with Tukey's HSD)

and disrupted testicular histoarchitecture. Concurrent oral *L. albus* seed extract attenuated each of these changes in a dose-dependent manner, with the 200 mg/kg dose returning many endpoints to values not statistically different from control. These findings are consistent with, and extend, a substantial body of literature on cadmium reproductive toxicity and on the protective potential of plant antioxidants.

The oxidative imbalance we observed — elevated testicular MDA with depletion of SOD, CAT, GPx, GSH and TAC — mirrors the widely reported mechanism by which cadmium injures the testis. Reviews by Ali et al. and Bhardwaj et al. describe reactive oxygen species generation and antioxidant depletion as central to cadmium-induced Sertoli-cell and germ-cell damage (2,3), and Yi et al. reported dose-dependent reductions in testicular SOD

Table 7. Effect of lupine extract on quantitative testicular histomorphometric indices in cadmium-exposed male rats

Parameter	Unit	Control	Lupine	Cd	Cd + Lup-L	Cd + Lup-H	F	*P*	ω²
Seminiferous tubule diameter	µm	252.0 ± 15.1^{a}	258.0 ± 15.5^{a}	158.0 ± 9.5^{d}	198.0 ± 11.9^{c}	238.0 ± 14.3^{b}	78.67	< 0.001	0.89
Germinal epithelium height	µm	62.50 ± 5.00^{a}	65.50 ± 5.24^{a}	28.50 ± 2.28^{d}	42.50 ± 3.40^{c}	56.50 ± 4.52^{b}	106.1	< 0.001	0.91
Johnsen score	1–10	9.45 ± 0.38^{a}	9.55 ± 0.38^{a}	5.85 ± 0.23^{d}	7.45 ± 0.30^{c}	9.05 ± 0.36^{b}	181.2	< 0.001	0.95

Data are expressed as mean ± SD (n = 8 animals per group). Means in the same row not sharing a common superscript letter (a–d) differ significantly at $P < 0.05$ (one-way ANOVA followed by Tukey's HSD post-hoc test). F, ANOVA F-statistic (df = 4, 35); ω², omega-squared effect size. Cd, cadmium chloride (5 mg/kg); Lup-L, lupine (100 mg/kg); Lup-H, lupine (200 mg/kg). Tubule diameter and epithelium height were measured on 30 round tubular profiles per animal; the Johnsen score was assigned to 100 tubules per animal

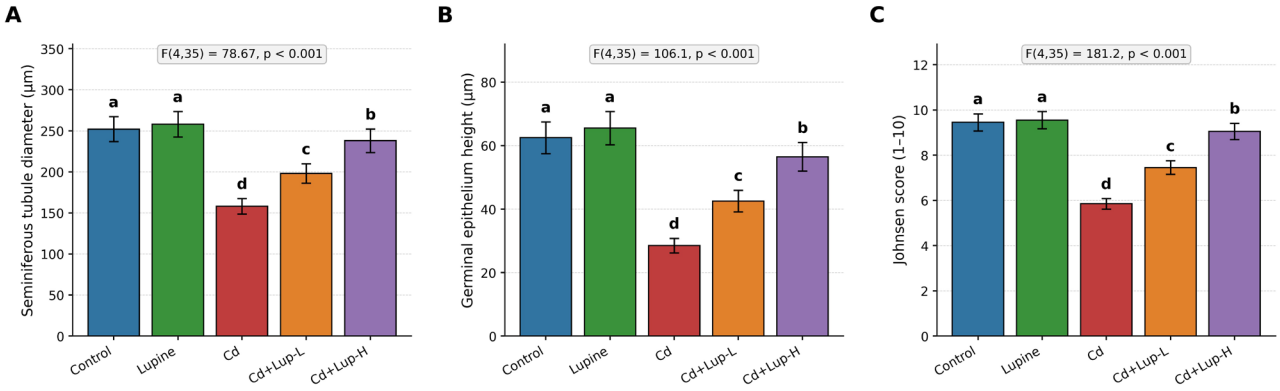

Figure 7. Effect of lupine extract on quantitative testicular histomorphometry. (A) Seminiferous tubule diameter, (B) germinal epithelium height and (C) Johnsen testicular biopsy score. Data are mean ± SD (n = 8 per group); bars not sharing a common letter (a–d) differ at $P < 0.05$ (one-way ANOVA with Tukey's HSD)

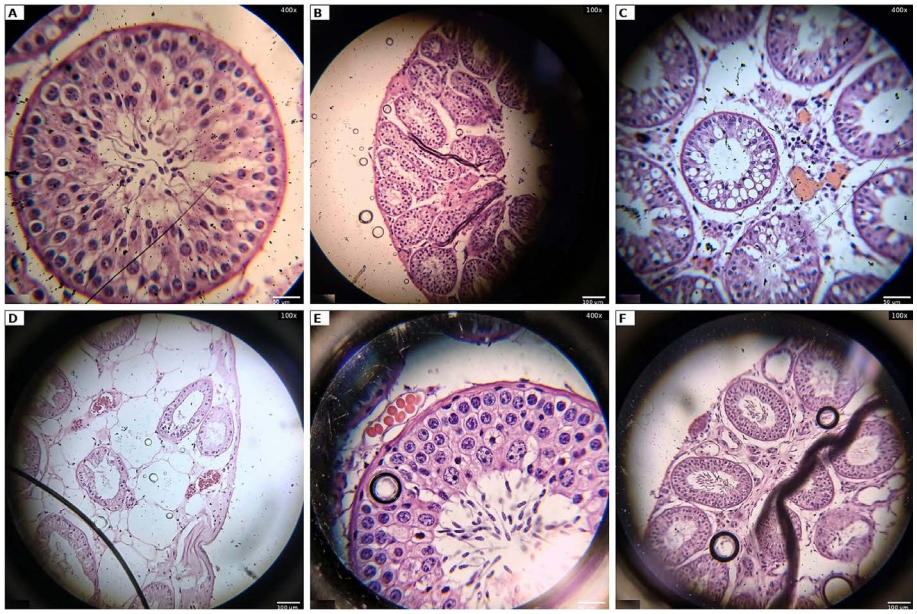

Figure 8. Representative photomicrographs of testicular histology (haematoxylin and eosin). (A) Control and (B) lupine sections show normal seminiferous tubule architecture with an organised germinal epithelium and abundant luminal spermatozoa. (C, D) Cadmium-exposed sections show tubular atrophy, germinal epithelium disorganisation and depletion, sloughing of germ cells into the lumen and interstitial oedema/congestion. (E, F) Co-treatment with lupine progressively restores tubular architecture. Original magnification × 100 and × 400 as indicated; scale bars = 100 µm (× 100) and 50 µm (× 400). Sections were read blinded by a qualified examiner

and GPx with increased MDA in pubertal rats (4). The dose-dependent normalisation of these markers by lupine, together with the modest antioxidant improvement seen in the lupine-only group, is in keeping with the recognised phenolic and flavonoid antioxidant capacity of the seed (11,12) and supports a redox-based mechanism of protection. The pattern also accords with reports that other plant extracts, such as Fragaria ananassa and Salvia officinalis, limit cadmium-induced lipid peroxidation and restore antioxidant enzymes in rat testes (6,7).

Cadmium lowered serum testosterone, LH and FSH, consistent with the established role of the hypothalamic–pituitary–gonadal axis as a target of cadmium toxicity (5) and with the Leydig-cell steroidogenic suppression documented in recent mechanistic work (2). Lupine co-treatment raised all three hormones dose-dependently. Restoration of the endocrine profile by antioxidant-rich extracts has been reported in several cadmium models

Table 8. Dose-dependent protective efficacy of lupine extract against cadmium-induced changes, expressed as percentage restoration toward control values

Parameter	% protection G4 (100 mg/kg)	% protection G5 (200 mg/kg)	Linear trend *P*	Quadratic trend *P*	Interpretation
Sperm count	44%	83%	<0.001	0.729	Significant linear dose-dependent recovery
Sperm motility	45%	85%	<0.001	0.617	Significant linear dose-dependent recovery
Sperm viability	44%	87%	<0.001	0.952	Significant linear dose-dependent recovery
Normal morphology	48%	86%	<0.001	0.136	Significant linear dose-dependent recovery
Testosterone	42%	84%	<0.001	1.000	Significant linear dose-dependent recovery
MDA	50%	89%	<0.001	0.257	Significant linear dose-dependent recovery
GSH	45%	94%	<0.001	0.872	Significant linear dose-dependent recovery
TAC	48%	84%	<0.001	0.564	Significant linear dose-dependent recovery
TNF-α	47%	86%	<0.001	0.487	Significant linear dose-dependent recovery
Seminiferous tubule diameter	43%	85%	<0.001	1.000	Significant linear dose-dependent recovery
Johnsen score	44%	89%	<0.001	1.000	Significant linear dose-dependent recovery

Percentage protection = [(mean Cd+lupine − mean Cd)/(mean control − mean Cd)] × 100; 100% denotes complete restoration to the control level. Linear and quadratic trend p-values were obtained from orthogonal polynomial contrasts across the ordered lupine doses under cadmium (G3 = 0, G4 = 100, G5 = 200 mg/kg). G4, Cd + low-dose lupine; G5, Cd + high-dose lupine

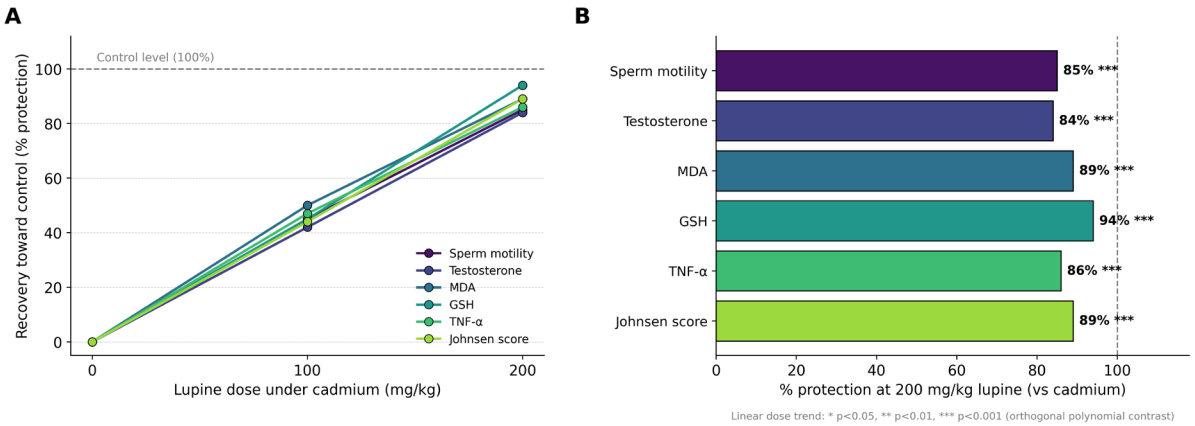

Figure 9. Dose-dependent protective effect of lupine extract against cadmium reproductive toxicity. (A) Percentage recovery toward control values as a function of lupine dose under cadmium (0, 100, 200 mg/kg) for representative biomarkers; the dashed line marks the control level (100%). (B) Percentage protection achieved at the high lupine dose (200 mg/kg); asterisks denote the significance of the linear dose trend (orthogonal polynomial contrast: * $P<0.05$, ** $P<0.01$, *** $P<0.001$).

(6), and probably reflects both protection of Leydig-cell function from oxidative damage and preservation of the pituitary signalling that drives steroidogenesis. Notably, testosterone and LH did not fully return to control at the high dose, indicating partial rather than complete endocrine recovery — a point we are careful not to overstate.

The marked rise in serum TNF-α, IL-1β and IL-6 with cadmium is consistent with the inflammatory component of cadmium reprotoxicity, in which oxidative stress activates redox-sensitive transcription factors and pro-inflammatory cytokine release that contribute to germ-cell apoptosis (2,3). The dose-dependent reduction of these cytokines by lupine suggests an anti-inflammatory contribution to its protective effect, plausibly secondary to its antioxidant action; flavonoid-rich preparations have shown comparable cytokine-lowering effects against heavy-metal testicular toxicity (22).

The deterioration in sperm count, motility, viability and morphology, and the reductions in seminiferous tubule diameter, germinal epithelium height and Johnsen score, are characteristic of cadmium-induced spermatogenic disruption (2,4). Comparable findings have been reported by Iraqi and regional groups. Khafaji demonstrated that hydroethanolic *Cyperus esculentus* and *Euterpe oleracea* extracts improved reproductive efficacy against cadmium-induced testicular toxicity in male rats (10) and, in a related model, that kaempferol and vitamin E exerted antioxidant, anti-inflammatory and anti-reprotoxic effects against lead-induced testicular toxicity (22). Al-Okaily and Murad reported that alpha-lipoic acid protected rat testes against lead toxicity using a 56-day oral design closely comparable to ours, with preservation of testosterone, FSH, sperm parameters and testicular histomorphometry (9). Neamah et al. similarly found that pumpkin (*Cucurbita pepo*) seed extract mitigated heavy-metal-induced reproductive toxicity through an antioxidant mechanism (23), and Ali Hameed et al. described cadmium-induced testicular degeneration in rats (24). Our results align with this regional evidence base and add *L. albus* to the list of plant materials capable of attenuating heavy-metal reproductive injury.

The protective profile of *L. albus* is biologically plausible. Its seeds contain phenolic acids and apigenin-derived flavone C-glycosides whose antioxidant activity

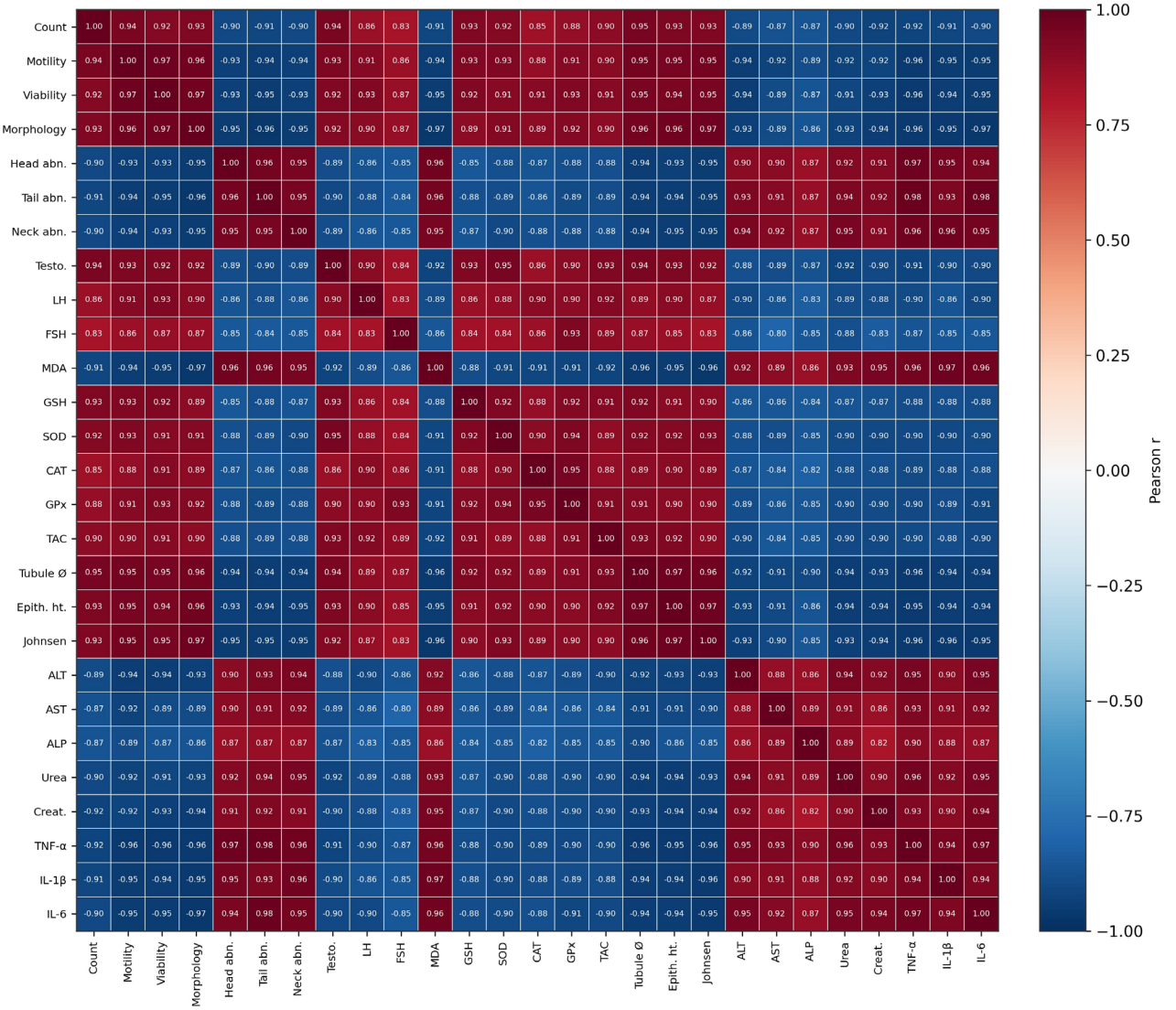

Figure 10. Correlation matrix of reproductive, oxidative, endocrine, histological and systemic toxicity biomarkers. Heatmap of pairwise Pearson correlation coefficients (r) across all animals (n = 40); the colour scale runs from blue (r = −1) to red (r = +1), and coefficients with $|r| \geq 0.45$ are annotated

correlates with total phenolic content (11), and lupin preparations have improved tissue antioxidant status in other rat models (12). To our knowledge, only one previous report has examined *L. albus* seed extract in relation to male reproductive and redox endpoints, observing dose-dependent improvements in reproductive hormones and oxidative markers in male rodents (25); we interpret that single prior report with caution and corroborate its direction with the established antioxidant literature on lupin (11,12). Importantly, the pentacyclic triterpenoid lupeol — although similarly named — is not a constituent specific to *Lupinus albus*, and protective effects attributed to lupeol in cadmium models should not be conflated with those of lupin seed extract.

Beyond the reproductive system, cadmium increased serum ALT, AST, ALP, urea and creatinine, indicating hepatic and renal involvement consistent with the systemic toxicity of the metal (1). Lupine attenuated these changes dose-dependently, suggesting that its benefit is not confined to the testis; this broader protection is in line with reports that antioxidant plant extracts ameliorate cadmium-induced hepatorenal dysfunction alongside reproductive endpoints (6).

From a translational perspective, these results identify *L. albus* seed extract as a dietary antioxidant worth further study as a candidate protective agent against environmental cadmium exposure. However, the data derive from a controlled rodent co-exposure model using specific oral doses, and should not be interpreted as evidence of clinical efficacy in humans; the clinical relevance of the findings remains to be established.

Strengths of the study include the comprehensive, multi-domain assessment (organ weights, sperm, hormones, oxidative status, inflammation, systemic toxicity and quantitative histomorphometry), the inclusion of an extract-only group to detect intrinsic effects, the use of two extract doses to characterise dose-dependence, and the reporting of effect sizes and trend analysis alongside conventional significance testing. Several limitations should be acknowledged. First, the protective mechanism was inferred from biomarker patterns rather than measured directly; molecular endpoints (for example, NF-κB signalling, apoptosis markers and gene or protein expression) and testicular cadmium concentrations were

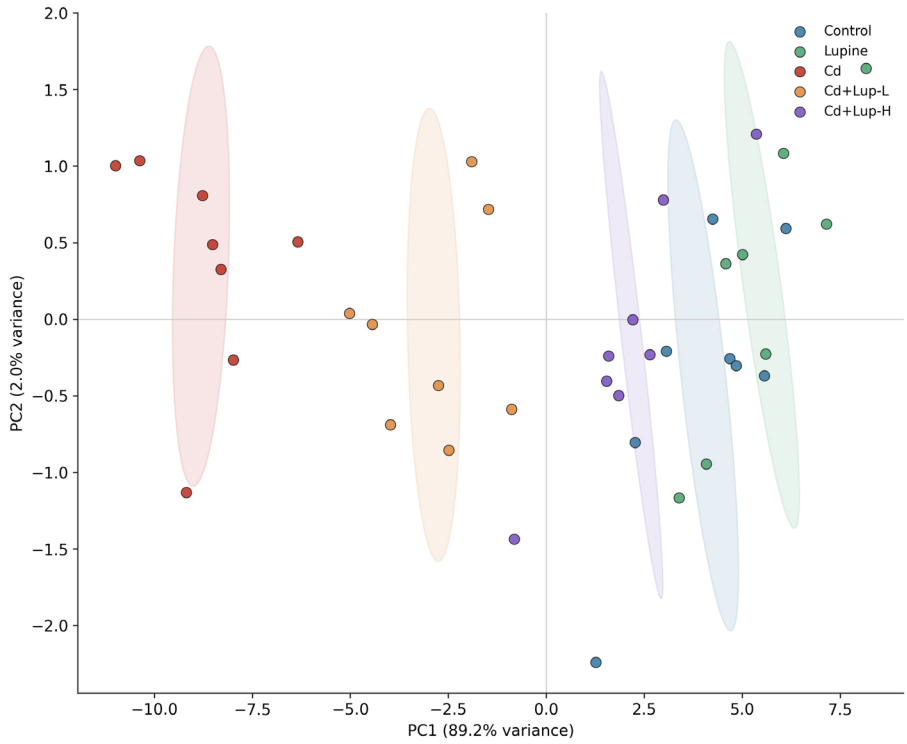

Figure 11. Principal component analysis (PCA) of the multivariate biomarker profile. Score plot of the first two principal components; each point represents one animal, coloured by treatment group, with shaded 1-SD confidence ellipses. PC1 captures the dominant cadmium-toxicity-to-recovery axis, separating the cadmium group from the control and lupine groups, with the cadmium + lupine groups positioned in between in a dose-dependent manner

not determined. Second, the extract was not subjected to detailed phytochemical quantification or standardisation in this study, so the active constituents responsible for the effect cannot be specified. Third, only a co-administration (protective) design was tested; therapeutic administration after established injury, and recovery after withdrawal, were not examined. Fourth, the correlation and principal component analyses are exploratory and were used only to illustrate the overall structure of the data. Finally, findings in rats may not translate directly to humans (26-28).

Future work should include phytochemical standardisation of the extract, measurement of testicular and systemic cadmium burden, mechanistic assessment of oxidative, apoptotic and inflammatory signalling pathways, evaluation of therapeutic (post-exposure) dosing, longer-term and recovery studies, and dose-finding beyond 200 mg/kg to define the upper limit of protection.

Conclusion

Under the conditions of this study, oral *L. albus* seed extract attenuated cadmium-induced reproductive toxicity in male Wistar rats in a dose-dependent manner. Co-treatment limited the cadmium-associated loss of reproductive organ mass, the decline in sperm quality, the suppression of reproductive hormones, testicular oxidative stress, systemic inflammation and hepatorenal disturbance, and helped preserve testicular histoarchitecture, with the 200 mg/kg dose returning many endpoints to values not significantly different from control. These results support further investigation of *L. albus* as a dietary antioxidant against cadmium reproductive toxicity but do not, by themselves, establish clinical benefit; mechanistic and translational studies are required.

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Competing Interests

The authors declare that they have no competing interests.

Consent for Publication

Not applicable.

Data Availability Statement

The datasets generated and analysed during the current study are

available from the corresponding author on reasonable request.

Ethical Approval

All animal procedures were approved by the Scientific and Ethics Committee of Al-Furat Al-Awsat Technical University, Iraq (Approval No.: 336822; Date: October 2023), and were conducted in accordance with recognized guidelines for the care and use of laboratory animals (13). No human participants were involved.

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