



Original Research Paper

Redox hormesis in the testis: *Bunium incrassatum* root extract biphasically modulates testosterone levels and spermatogenesis in male rabbits (*Oryctolagus cuniculus*)

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Abstract

Bunium incrassatum (Apiaceae), whose tuber is traditionally consumed in North Africa as a tonic food reputed to support fertility, had never been assessed for male reproductive function. Twenty-four adult male rabbits assigned to four groups received the CH₂Cl₂/MeOH root extract by daily gavage for 15 days at 0, 50, 100 and 200 mg/kg/day. At 100 mg/kg/day, every indicator of gonadal function improved: serum testosterone rose by 50.4%, superoxide dismutase and glutathione peroxidase activities increased in testicular homogenate while malondialdehyde decreased significantly (–20.0%), seminiferous tubule diameter increased from 249.19 ± 3.01 to 290.68 ± 3.99 µm and germinal epithelium height from 73.30 ± 4.55 to 91.00 ± 3.21 µm, and sperm concentration, motility and viability increased concurrently (p < 0.05). The convergence of four methodologically independent parameter families indicates reinforced gonadal antioxidant defences preserving Leydig cell steroidogenic competence. At 200 mg/kg/day, the testicular redox balance reversed: antioxidant enzymes collapsed, malondialdehyde rose by 70.3%, and the endocrine, tissue and sperm gains were not merely abolished but crossed in the opposite direction. The optimum therefore lies at 100 mg/kg/day, a dose coinciding with the safety ceiling previously established for this extract. Blood glucose, which declined monotonically across the whole dose range, confines this hormetic behaviour to the steroidogenic and germinal compartments. *B. incrassatum* thus holds genuine reproductive value, inseparable from control of both dose and administered formulation.

Keywords: *Bunium incrassatum*; hormesis; testicular oxidative stress; testosterone; seminiferous tubule morphometry; sperm quality; male rabbit.

1. Introduction

Male reproductive function depends on a tightly regulated redox balance. When reactive oxygen species production exceeds cellular antioxidant capacity, the result is peroxidation of sperm membranes, DNA fragmentation and germ cell apoptosis; oxidative stress is thus one of the principal molecular bases of male infertility (Agarwal et al., 2014). The testis is particularly vulnerable, since spermatogenesis requires both this balance and adequate testosterone, synthesised by Leydig cells from cholesterol — cells that rank among the most susceptible to oxidative injury (Monageng et al., 2023). A plant extract rich in antioxidant compounds might therefore protect steroidogenesis and spermatogenesis simultaneously.

Bunium incrassatum (Boiss.) Batt. & Trab. (Apiaceae), locally known as Talghouda, is a tuberous species endemic to North Africa, long consumed in Algeria

as a tonic food and traditionally reputed to support vigour and fertility (Aiouaz & Bitam, 2022). Its antioxidant, anti-haemolytic, anti-inflammatory and antimicrobial activities have been documented (Bousetla et al., 2015; El Kolli, 2017; Berroukeche et al., 2022; Toul & Djendar, 2023; Amraoui et al., 2024). The dichloromethane/methanol root extract contains two coumarins, scopoletin and scoparone, together with β-sitosterol, sucrose and oleic acid (Bousetla et al., 2015), whereas the seeds are rich in phenolic acids and flavonoids (Toul et al., 2022). This profile is of direct relevance to reproduction: scopoletin reduces lipid peroxidation (Gao et al., 2024), β-sitosterol protects testicular tissue (Singh et al., 2025), and oleic acid participates in cholesterol handling within Leydig cells (Meikle et al., 1996).

Available reproductive data, however, concern females exclusively. Chentouh et al. (2017) reported an oestrogenic effect in mature does at 50 and 100 mg/kg/day, whereas 200 mg/kg/day increased the

proportion of atretic follicles; Hammoudi et al. (2020) described adrenal zona fasciculata hypertrophy in pregnant does, with an apparent safety threshold around 100 mg/kg/day; Attoui et al. (2021) documented the effects of the root extract on biological and histological parameters in mature female rats. Conducted with the same extract and the same dose range as used here, these studies already reveal, on the female side, a dose-dependent response that is beneficial at moderate doses and unfavourable at the highest one.

A paradox follows: although the traditional reputation of this species rests largely on its reproductive properties, no study has assessed its effect on male reproductive function, nor combined a dose-response analysis with the underlying redox mechanisms. The rabbit (*Oryctolagus cuniculus*) is well suited to this question, since semen collection is repeatable and non-invasive, its reproductive physiology is well characterised, and it is widely used to evaluate the effect of plant supplements on male fertility (Shaath et al., 2025). The present study therefore provides the first assessment of the organic root extract of *B. incrassatum* on male rabbit reproductive function — body and organ weights, hormonal, sperm, histological, biochemical and oxidative stress parameters — at three increasing doses, hypothesising a biphasic response that is beneficial at moderate doses and reversed at the highest.

2. Materials and Methods

2.1. Plant material and extract preparation

Tubers of *Bunium incrassatum* (Boiss.) Batt. & Trab. (Apiaceae), locally known as Talghouda, were collected in September 2015 at Meskiana (Oum El Bouaghi Province, eastern Algeria) and authenticated by Prof. Amar Zellagui (Department of Biology, University of Oum El Bouaghi); a voucher specimen was deposited at the University of Constantine under the number ZA 103. After separation from the aerial parts, the roots were washed, air-dried at room temperature protected from light, and ground to a fine powder.

Extraction followed the protocol of Boussetla et al. (2015). Root powder (800 g) was macerated in dichloromethane-methanol (1:1, v/v) at room temperature in the dark for 24 h. After filtration through Whatman 3MM paper, the filtrate was concentrated under reduced pressure (BÜCHI Rotavapor® R-300, 60 °C) to a dry residue, yielding 5 g of crude extract (0.63% w/w). The extract was stored at 4 °C in an airtight container, protected from light and moisture. Since this organ-solvent combination reproduces the conditions under which Boussetla et al. (2015) characterised the root of this species, the composition of the present extract is referred to that reference profile.

2.2. Animals and housing conditions

Twenty-four adult male rabbits (*Oryctolagus cuniculus*) of local population, sexually mature and weighing 2,850 to 3,200 g, were obtained from the Technical Institute of Livestock (ITELV, Hamma Bouziane, Constantine, Algeria). The study was conducted in

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autumn 2016. Animals were housed in metal cages under natural conditions of temperature, photoperiod and humidity, with free access to drinking water and to pelleted feed supplied by the National Office for Animal Feed (O.N.A.B., Algeria), following a seven-day acclimatisation period. The protocol was approved by the Animal Care Committee of the Biotechnology Research Centre (CRBt, Constantine, Algeria) and conducted in accordance with the recommendations of the International Bioethics Committee, the European Institute of Bioethics and the World Organisation for Animal Health.

2.3. Experimental design and treatment

Animals were randomly allocated to four groups of six (n = 6). The control group (C) received the vehicle alone (distilled water), while groups G1, G2 and G3 received 50, 100 and 200 mg/kg/day of *B. incrassatum* organic extract, respectively. Dose selection was based on previous work in rabbits (Chentouh et al., 2017; Rahmouni et al., 2019; Daoudi et al., 2025; Ositadimma et al., 2025) and on preliminary acute toxicity trials carried out in our laboratory.

The extract was freshly resuspended each morning in distilled water at 50, 100 and 200 mg/mL, so as to deliver the target dose in a constant gavage volume of 1 mL/kg body weight in all groups, including the control. Administration was oral, by gavage tube, once daily for 15 consecutive days. Body weight was recorded every two days.

2.4. Euthanasia and sample collection

Animals were euthanised by exsanguination on the day following the last gavage. Blood was collected into polyethylene tubes without anticoagulant, allowed to clot, then centrifuged at 3,000 rpm for 15 min; serum was aliquoted and stored at -20 °C until assay. Testes were excised, cleared of adipose and connective tissue, rinsed in cold phosphate buffer, blotted and weighed individually to the nearest 0.001 g; relative testis weight was calculated as: relative weight (%) = (testis weight / final body weight) × 100. The right testis was fixed in 10% neutral buffered formalin for histology, the left testis frozen at -80 °C for homogenate preparation, and the left cauda epididymidis collected separately for semen analysis.

2.5. Serum biochemical analyses

Serum glucose, total cholesterol and triglycerides were determined by enzymatic colorimetric assay using commercial SPINREACT kits (Sant Esteve de Bas, Spain): GOD-POD (ref. 41010), CHOD-POD (ref. 1001091) and GPO-POD (ref. 1001311), respectively, with results expressed in g/L. Serum testosterone was measured by enzyme-linked immunosorbent assay (ELISA) using a MyBioSource kit (ref. MBS766199, San Diego, CA, USA) according to the manufacturer's instructions, with concentrations expressed in ng/mL.

2.6. Assessment of testicular oxidative status

Testicular fragments were homogenised on ice

in 50 mM phosphate buffer (pH 7.4) at a tissue-to-buffer ratio of 1:9 (w/v), then centrifuged at 10,000 × g for 10 min at 4 °C; supernatants were stored at -80 °C until assay. Total protein was quantified by the Bradford (1976) method at 595 nm, using bovine serum albumin as standard.

Superoxide dismutase activity was determined according to Marklund and Marklund (1974), based on inhibition of pyrogallol autoxidation (0.2 mM) in 50 mM Tris-HCl buffer pH 8.2 containing 1 mM EDTA, monitored at 420 nm for 3 min at 25 °C; one unit corresponds to 50% inhibition. Glutathione peroxidase activity was measured by the glutathione reductase-coupled method of Flohé and Günzler (1984), with NADPH consumption followed at 340 nm for 3 min at 37 °C; one unit corresponds to the oxidation of 1 µmol NADPH per minute. Both activities are expressed in U/mL. Lipid peroxidation was estimated by malondialdehyde assay according to Ohkawa et al. (1979): reaction with 0.8% thiobarbituric acid under acidic conditions (8.1% SDS, 20% acetic acid, pH 3.5) at 95 °C for 60 min, extraction of the complex with n-butanol/pyridine (15:1, v/v) and reading at 532 nm, with 1,1,3,3-tetraethoxypropane as calibration standard; results are expressed in nM.

2.7. Histology and testicular histomorphometry

Testes fixed in 10% neutral buffered formalin were dehydrated through graded ethanol, cleared in xylene, embedded in paraffin and sectioned at 4–5 µm. Sections were stained with haematoxylin–eosin according to Luna (1968) and examined under an OPTIKA B-150 light microscope fitted with a digital camera.

Measurements were performed on digital images using ImageJ (version 1.54g; NIH, Bethesda, MD, USA; Schneider et al., 2012), calibrated at each magnification against a stage micrometer. Thirty seminiferous tubules per animal, circular or slightly oval in outline with a clearly visible lumen, were analysed, oblique sections being excluded: mean seminiferous tubule diameter (MSTD) was taken as the average of two perpendicular diameters, and seminiferous epithelium height (HST) as the average of four radial measurements from the basement membrane to the lumen (Wing & Christensen, 1982). Leydig cells were counted in ten fields per animal at ×400 magnification (Iczkowski et al., 1991). Johnsen scores were assigned to each tubule on the 1–10 scale (Johnsen, 1970), the value reported per animal being the mean of individual scores; the spermatogenesis index (SI) was defined, in the present study, as the percentage of tubules showing complete

2.8. Semen analysis

The left cauda epididymidis was incised to collect a drop of semen, immediately diluted in 1 mL of physiological saline (0.9% NaCl). Sperm parameters were assessed according to World Health Organization guidelines (WHO, 1992). For concentration, semen was diluted 1:50 and 10 µL of the dilution loaded onto a Malassez counting chamber; after 10 to 15 min sedimentation, at least 200 spermatozoa displaying a head, midpiece and flagellum were counted. Motility was assessed immediately after collection by classifying one hundred consecutive spermatozoa across several fields at ×400 magnification. Viability was determined by 1% eosin vital staining: after 30 s contact, a smear was prepared and at least one hundred spermatozoa examined at ×400 magnification, dead spermatozoa appearing pink and live ones unstained. Results are expressed in ×10⁶/mL for concentration and as percentages for motility and viability.

2.9. Statistical analysis

Data are expressed as mean ± standard deviation. Normality of distributions was verified by the Shapiro–Wilk test and homogeneity of variances by Levene's test; both conditions being satisfied, between-group comparisons were performed by one-way analysis of variance, with dose as a fixed factor at four levels (0, 50, 100 and 200 mg/kg/day). Where ANOVA indicated a significant effect, pairwise comparisons were made using Tukey's HSD post hoc test; in the tables, means sharing no superscript letter differ significantly. Analyses were carried out with SPSS Statistics version 17.0 (SPSS Inc., Chicago, IL, USA), with significance set at $p < 0.05$.

3. Results

3.1. Body weight and testis weight

Body weight gain increased dose-dependently in all treated groups ($p < 0.001$), reaching its highest value in G3 (340 ± 21.00 g), and the rate of weight increase followed the same trend, from 5.76% in controls to 11.73% in G3 (Table 1). Absolute testis weight, by contrast, showed a biphasic profile ($p < 0.001$): it rose to a peak in G2 (8.36 ± 0.06 g) and then fell in G3 (6.34 ± 0.05 g), 6.6% below the control value. Relative testis weight remained stable from control to G2 and decreased significantly only in G3 ($0.19 \pm 0.008\%$; -15.6% ; $p < 0.001$).

Table 1. Body weight changes and relative testis weight in control and treated rabbits.

Parameter	Control	G1	G2	G3
Initial body weight (g)	2850 ± 50 a	3120 ± 158.75 b	3200 ± 100 b	2900 ± 100 ab
Final body weight (g)	3014 ± 43.31 a	3371 ± 153.84 c	3490 ± 103.59 c	3240 ± 105.08 b
Weight gain (g)	164 ± 8.72 a	251 ± 5.29 b	290 ± 5.57 c	340 ± 21.00 d
Rate of increase (%)	5.76 ± 0.39 a	8.06 ± 0.56 b	9.07 ± 0.22 c	11.73 ± 0.77 d
Absolute testis weight (g)	6.79 ± 0.17 b	7.77 ± 0.13 c	8.36 ± 0.06 d	6.34 ± 0.05 a
Relative testis weight (%)	0.225 ± 0.003 b	0.23 ± 0.013 b	0.24 ± 0.006 b	0.19 ± 0.008 a

Mean $\pm$ SD (n = 6). Values bearing different superscript letters within the same row differ significantly (one-way ANOVA followed by Tukey's HSD post hoc test, $p < 0.05$). C: control; G1: 50 mg/kg; G2: 100 mg/kg; G3: 200 mg/kg.

3.2. Serum testosterone

Serum testosterone concentration differed significantly among groups ($p < 0.001$; Table 2). It increased slightly in G1 (2.80 ng/mL; $p = 0.059$) and peaked in G2 (3.91 ng/mL), a 50.4% rise over controls ($p < 0.001$). In G3 it fell to 2.37 ng/mL, below the control value (-8.8% ; $p = 0.028$).

Table 2. Serum testosterone concentration (ng/mL) in control and treated rabbits.

Parameter	Control	G1	G2	G3	p
Testosterone (ng/mL)	2.60 $\pm$ 0.10 b	2.80 $\pm$ 0.10 b	3.91 $\pm$ 0.20 c	2.37 $\pm$ 0.07 a	< 0.001

Mean $\pm$ SD (n = 6). Values bearing different superscript letters within the same row differ significantly (one-way ANOVA followed by Tukey's HSD post hoc test, $p < 0.05$). C: control; G1: 50 mg/kg; G2: 100 mg/kg; G3: 200 mg/kg.

3.3. Serum biochemical parameters

Serum triglycerides varied moderately among groups ($p = 0.002$); the only significant change was a

decrease in G2 (0.45 $\pm$ 0.04 g/L; -16.7% vs control; $p < 0.01$), while G1 showed only a slight reduction and G3 did not differ from controls. Serum cholesterol, by contrast, increased markedly and dose-dependently ($p < 0.001$), from 0.22 $\pm$ 0.026 g/L in controls to 0.35 $\pm$ 0.030 g/L in G3, a 59.1% rise. Blood glucose decreased progressively with dose ($p < 0.001$), from 1.10 $\pm$ 0.082 g/L in controls to 0.90 $\pm$ 0.111 g/L in G3 (-18.2%), the reductions in G2 and G3 being statistically significant (Table 3).

Table 3. Serum triglycerides, cholesterol and glucose in control and treated rabbits.

Parameter	Control	G1	G2	G3	p
Triglycerides (g/L)	0.54 $\pm$ 0.04 b	0.53 $\pm$ 0.04 b	0.45 $\pm$ 0.04 a	0.54 $\pm$ 0.05 b	0.002
Cholesterol (g/L)	0.22 $\pm$ 0.026 a	0.267 $\pm$ 0.021 b	0.29 $\pm$ 0.010 b	0.35 $\pm$ 0.030 c	< 0.001
Glucose (g/L)	1.10 $\pm$ 0.082 b	1.043 $\pm$ 0.006 b	0.927 $\pm$ 0.064 a	0.90 $\pm$ 0.111 a	< 0.001

Mean $\pm$ SD (n = 6). Values bearing different superscript letters within the same row differ significantly (one-way ANOVA followed by Tukey's HSD post hoc test, $p < 0.05$). C: control; G1: 50 mg/kg; G2: 100 mg/kg; G3: 200 mg/kg.

3.4. Antioxidant enzymes and lipid peroxidation

Superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities followed a similar pattern. Relative to controls (2.181 $\pm$ 0.150 U/mL for SOD and 187.44 $\pm$ 4.07 U/mL for GPx), both enzymes increased at moderate doses: SOD reached 2.469 $\pm$ 0.067 U/mL in G1 and 2.607 $\pm$ 0.051 U/mL in G2 ($+13.2\%$ and $+19.5\%$), the difference from controls being significant only in G2; GPx rose to 220.65 $\pm$ 8.70 and 222.72 $\pm$ 7.13 U/mL ($+17.7\%$

Malondialdehyde (MDA) showed the opposite profile. It decreased from control values (1.615 $\pm$ 0.263 nM) through G1 (1.450 $\pm$ 0.062 nM) to G2 (1.291 $\pm$ 0.133 nM), where the 20.0% reduction became significant ($p < 0.05$), with no difference between G1 and G2. Conversely, G3 showed a marked increase (2.750 $\pm$ 0.145 nM), 70.3% above controls ($p < 0.001$), indicating intensified oxidative stress (Table 4). The mirror-image profiles of the antioxidant enzymes and of lipid peroxidation across the dose range are shown in Figure 1.

Table 4. SOD and GPx activities and MDA levels in testicular homogenate of control and treated rabbits.

Parameter	Control	G1	G2	G3	p
SOD (U/mL)	2.181 $\pm$ 0.150 b	2.469 $\pm$ 0.067 bc	2.607 $\pm$ 0.051 c	1.208 $\pm$ 0.429 a	< 0.001
GPx (U/mL)	187.44 $\pm$ 4.07 b	220.65 $\pm$ 8.70 c	222.72 $\pm$ 7.13 c	100.76 $\pm$ 9.04 a	< 0.001
MDA (nM)	1.615 $\pm$ 0.263 b	1.450 $\pm$ 0.062 ab	1.291 $\pm$ 0.133 a	2.750 $\pm$ 0.145 c	< 0.001

Mean $\pm$ SD (n = 6). Values bearing different superscript letters within the same row differ significantly (one-way

ANOVA followed by Tukey's HSD post hoc test, $p < 0.05$). C: control; G1: 50 mg/kg; G2: 100 mg/kg; G3: 200 mg/kg.

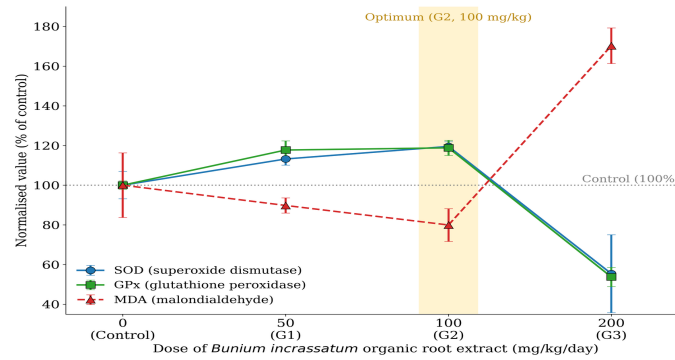


Figure 1. Biphasic (hormetic) dose-response of the three oxidative stress markers, expressed as a percentage of the control value (100%). Data are mean $\pm$ SD ($n = 6$).

3.5. Testicular histomorphometry

Mean seminiferous tubule diameter (MSTD) differed significantly among groups ($p < 0.001$), reaching a maximum in G2 ($290.68 \pm 3.99 \mu\text{m}$), significantly above controls ($249.19 \pm 3.01 \mu\text{m}$), then decreasing in G3 ($241.62 \pm 6.64 \mu\text{m}$) without differing significantly from controls ($p = 0.078$). Seminiferous epithelium height (HST) followed a comparable ascending pattern ($p < 0.001$), with equivalent values in G1 ($88.00 \pm 2.60 \mu\text{m}$) and G2 ($91.00 \pm 3.21 \mu\text{m}$), but diverged at the highest dose: a significant reduction was recorded in G3 ($57.66 \pm 2.50 \mu\text{m}$), 21.3% below controls ($p < 0.001$).

Leydig cell counts per field increased significantly ($p < 0.001$), peaking in G2 (16.00 ± 2.45) above controls ($p = 0.001$), while G3 showed the lowest density (9.00 ± 1.50). Johnsen scores increased significantly from control to G2, with no difference between G1 and G2 ($p = 0.428$), before falling in G3 (8.303 ± 0.44 ; $p < 0.001$). Consistently, the percentage of seminiferous tubules with complete spermatogenesis (SI) showed the same biphasic profile ($p < 0.001$), with a maximum in G2 ($96 \pm 3\%$) and a significant reduction in G3 ($73 \pm 4\%$; $p = 0.001$ vs control) (Figure 2). Expressed as a percentage of control values, the five parameters peak consistently at G2 and drop at G3 (Figure 3).

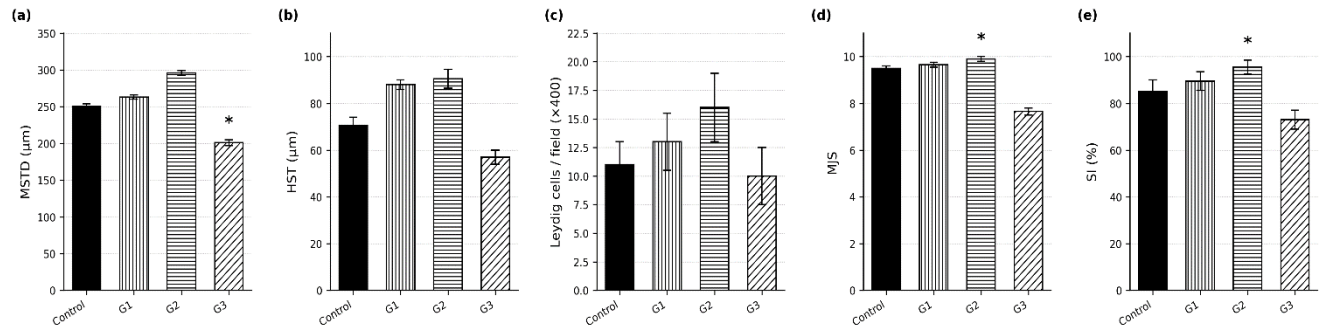


Figure 2. Testicular histomorphometric and semi-quantitative parameters in control and treated rabbits (G1: 50 mg/kg/day; G2: 100 mg/kg/day; G3: 200 mg/kg/day).

Data are expressed as mean $\pm$ SD ($n = 6$). MSTD: mean seminiferous tubule diameter; HST: seminiferous epithelium height; Johnsen score: mean Johnsen score; SI: percentage of tubules with complete spermatogenesis. Bars

sharing no letter differ significantly (one-way ANOVA followed by Tukey's HSD post hoc test, $p < 0.05$).

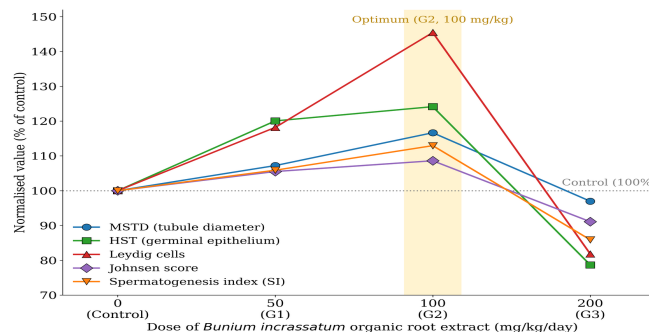


Figure 3. Biphasic (hormetic) dose–response of the five histomorphometric and semi-quantitative parameters, expressed as a percentage of the control value (100%).

3.6. Testicular histopathology

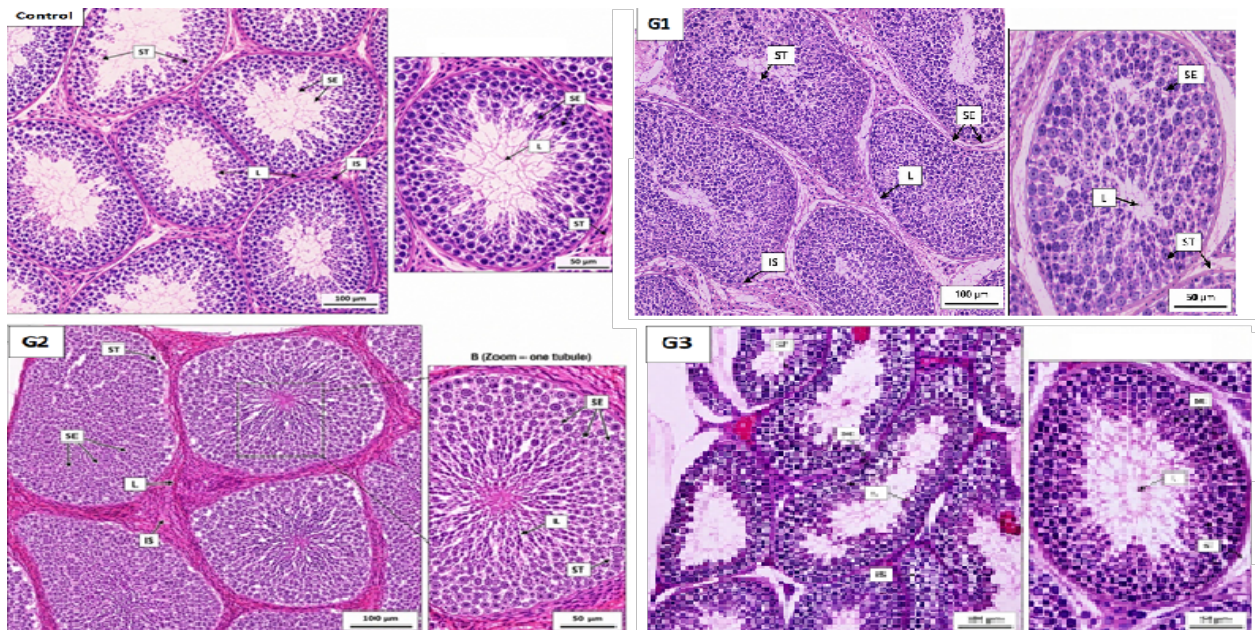
Histological examination revealed a dose-related effect. Control animals displayed normal testicular architecture, with circular to slightly oval seminiferous tubules regularly distributed throughout the parenchyma (Figure 4A). Basement membranes appeared intact and delimited a thick, well-organised seminiferous epithelium in which the germ cell stages were readily identifiable: spermatogonia against the basement membrane, followed by primary and secondary spermatocytes, and finally round and elongated spermatids bordering the tubular lumen. Interstitial spaces were narrow and contained morphologically normal Leydig cells; no abnormality was detected.

At 50 mg/kg/day, tubular architecture remained largely preserved, with a slightly more compact germinal epithelium than in controls and clearly visible spermatocytes (Figure 4B). At 100 mg/kg/day, the organisation appeared optimal: seminiferous tubules were enlarged, the epithelium thickened, the tubular lumen abundantly supplied with spermatids and spermatozoa, and Leydig cell density increased within a well-vascularised interstitium (Figure 4C). At 200 mg/kg/day, in contrast, the seminiferous epithelium showed moderate focal disorganisation, germ cell depletion and marked thinning, together with widening of the interstitial space; these alterations remained localised, without extensive vacuolisation, necrosis or destruction of the tubular architecture (Figure 4D).

hematoxylin–eosin (HE) in male rabbits treated for two weeks by daily gavage with the organic root extract of *Bunium incrassatum*. Magnification: $\times 200$ (main panels) and $\times 400$ (insets).

(A) Control: regular tubular contours, stratified seminiferous epithelium, spermatids in tubular lumen, Leydig cell clusters in moderate interstitial space. **(B) 50 mg/kg/day:** preserved tubular architecture, visible spermatocytes, slight increase in interstitial cellularity. **(C) 100 mg/kg/day:** enlarged seminiferous tubules, thickened epithelium with active spermatogenesis, increased Leydig cell density - optimal histological appearance. **(D) 200 mg/kg/day:** reduced tubular diameter, thinned germinal epithelium, focal disorganization, enlarged interstitial space - consistent with functional withdrawal without massive apoptotic destruction. **ST:** seminiferous tubule; **SE:** seminiferous epithelium; **SC:** spermatocytes; **L:** Leydig cells; **IS:** interstitial space.

Figure 4. Testicular histological sections stained with



3.7. Sperm parameters

The three sperm parameters followed a biphasic profile, with a maximum in G2 and a decline in G3 (Table

Table 5. Sperm concentration, motility and viability in control and treated rabbits.

Parameter	Control	G1	G2	G3
Concentration ($\times 10^6/\text{mL}$)	144.10 ± 1.15 a	151.40 ± 13.51 a	180.87 ± 5.83 b	137.73 ± 4.35 a
Motility (%)	66.00 ± 3.61 b	76.33 ± 9.02 c	87.33 ± 3.51 d	52.00 ± 2.65 a
Viability (%)	70.00 ± 2.00 b	81.33 ± 4.16 c	85.33 ± 3.51 c	60.00 ± 6.24 a

Mean $\pm$ SD (n = 6). Values bearing different superscript letters within the same row differ significantly (one-way ANOVA followed by Tukey's HSD post hoc test, $p < 0.05$). C: control; G1: 50 mg/kg; G2: 100 mg/kg; G3: 200 mg/kg.

4. Discussion

To our knowledge, this is the first assessment of *Bunium incrassatum* on male reproductive function. Earlier in vivo studies dealt exclusively with females (Chentouh et al., 2017; Hammoudi et al., 2020; Attoui et al., 2021; Aiouaz & Bitam, 2022), and the only work that examined the testis was limited to organ weighing and qualitative histological description (Berroukeche et al., 2022). Four parameter families, testicular redox markers, serum testosterone, tubular histomorphometry and sperm characteristics, were assessed separately using independent methods, and all traced the same inverted U-shaped curve, peaking at 100 mg/kg and reversing at 200 mg/kg. Such concordance is difficult to reconcile with a chance accumulation of independent pharmacological effects; it points instead to a single upstream determinant, consistent with the hormetic pattern in which low doses of plant secondary metabolites mobilise cellular defences whereas higher doses shift towards a pro-oxidant action (Calabrese & Mattson, 2017; Perrone & D'Angelo, 2025).

This determinant is first apparent in testicular homogenate, and its dynamics precede the other changes. Up to 100 mg/kg, superoxide dismutase and glutathione peroxidase rose together while malondialdehyde declined, the reduction becoming significant at this dose: protection therefore does not merely maintain a basal level of peroxidation but actively lowers it within the gonad itself. At 200 mg/kg the pattern reversed point for point, both enzyme activities falling below control values while testicular lipid peroxidation rose by 70.3%. Malondialdehyde thus traces an inverted curve of its own, which argues against a simple tolerance threshold being exceeded and in favour of a genuine shift in redox balance. This reciprocity reproduces the sequence established as causal by Shokoohi et al. (2018) in the rat testis, where the fall in antioxidant enzymes and the rise in malondialdehyde precede the shift in the Bax/Bcl-2 ratio, germ cell apoptosis, the decline in testosterone and tubular shrinkage; Taha et al. (2023) described the same chain traversed in the opposite direction under protective treatment. In rabbits, Shaat et al. (2025) showed that elevated catalase and superoxide dismutase activities in testicular homogenate accompany an intact seminiferous epithelium, whereas their collapse coincides with vacuolisation and necrosis. Normalised to control values, this reciprocity appears as two mirror-image curves

crossing between 100 and 200 mg/kg/day (Figure 1).

Which constituents carry this effect? Bousetla et al. (2015) identified, in roots extracted with $\text{CH}_2\text{Cl}_2/\text{MeOH}$, the same organ and solvent as in the present work, two coumarins, scopoletin and scoparone, alongside β -sitosterol, sucrose and oleic acid. Scopoletin and scoparone have documented radical-scavenging and cytoprotective activities (Gao et al., 2024), consistent with the protection observed in vivo for this extract against oxidative haemolysis (Amraoui et al., 2024) and sodium fluoride toxicity (Bouzid et al., 2024). The seeds of this species also contain several phenolic acids and flavonoids, including quercetin (Toul et al., 2022), recognised as a modulator of testicular function through its antioxidant, anti-inflammatory and anti-apoptotic properties (Yang et al., 2020); Tufarelli et al. (2018) showed in male rabbits that quercetin supplementation preserves semen quality under heat stress. β -Sitosterol, however, cannot account for the androgenic response: its established effect, inhibition of 5α -reductase, runs counter to the observed rise in testosterone (Buř et al., 2024), and its contribution more plausibly concerns sterol metabolism.

The rise in testosterone at 100 mg/kg is most plausibly explained by preservation of Leydig cell mitochondrial competence rather than by direct stimulation of steroidogenesis: Leydig cell density, serum testosterone and testicular antioxidant activity varied in parallel, suggesting that protection of these cells sustains the pathway involving StAR and CYP11A1 (Xiong et al., 2022; Monageng et al., 2023). This benefit parallels the findings of Shaat et al. (2025), who reported in rabbits a concurrent rise in testosterone, androgen receptor immunoreactivity and interstitial cell proliferation under an oleic acid-rich oil, as well as the steroidogenic effects previously described for this extract in does (Hammoudi et al., 2020) and its gametogenic effect in females (Chentouh et al., 2017).

At tissue level, the increase in tubular diameter and epithelial height at moderate doses, together with greater cellular density, indicates stimulation of spermatogenic activity rather than tissue damage. It is consistent with observations in rats and rabbits given flavonoid- or polyphenol-rich plant extracts (Kooti et al., 2014; El-Ratel et al., 2021; Khalaf et al., 2024; Shaat et al., 2025), and with the trophic effects reported on a healthy testis by Shokoohi et al. (2018). The configuration

observed at 200 mg/kg, by contrast, warrants close reading: tubular diameter returned to control level without differing significantly from it, whereas germinal epithelium height fell by 21.3% and Leydig cell density, Johnsen score and spermatogenesis index all decreased significantly. The regression therefore affects the cellular content of the tubule rather than its calibre. Set against the apoptotic alterations described by Shaat et al. (2025) under a pro-oxidant regimen, this picture suggests a reversible functional decline rather than cell destruction; joint assessment of tubular diameter, epithelial height and interstitial space, following the approach of Olawuyi et al. (2018), is precisely what allows these two situations to be distinguished. Normalised to control values, the five histomorphometric parameters peak without exception at G2 and drop sharply at G3, the germinal epithelium and Leydig cell density showing the steepest decline (Figure 3).

Organ weighing provides an independent check. Body weight gain increased monotonically across the whole dose range, whereas absolute testis weight was biphasic and fell 6.6% below the control value at the highest dose; relative weight, corrected for somatic growth, remained stable up to 100 mg/kg and decreased significantly only at 200 mg/kg (−15.6%): gonadal regression is therefore not a scaling artefact. The modest magnitude of this mass loss, against a 21.3% fall in epithelial height, reflects the composite nature of organ weight, which sums the tubular, interstitial, vascular and fluid compartments: epithelial thinning may be partly offset by widening of the interstitial space, as confirmed by the maintenance of tubular calibre. Comparable dissociations between somatic growth and gonadal response have been reported in male rodents (Ehigior & Adikwu, 2020; Uwejiho et al., 2025) and under *B. incrassatum* itself (Berroukeche et al., 2022).

The three sperm parameters reproduce the same curve, as expected for variables dependent on both androgen support and membrane integrity: completion of spermatogenesis requires sustained intratesticular testosterone, while the high polyunsaturated fatty acid content of the sperm membrane makes it a preferential target of peroxidation (Pourtezari et al., 2014; Nasiri Boroujeni et al., 2022), a dependence quantified in rabbits by Zeweil and El-Gindy (2017) through a negative correlation between motility and seminal plasma malondialdehyde. The profile of the high-dose group deserves attention: motility and viability remained below control values while concentration merely returned to them. Sperm output reflects germinal yield over preceding weeks, whereas motility and viability report the current state of the gamete membrane. This selective impairment suggests a terminal peroxidative event rather than a block in spermatogenesis, a reading consistent with the histology, which at this dose showed neither maturation arrest nor extensive germ cell loss.

Several studies do not report this reversal, and the divergence deserves examination. Kooti et al. (2014) observed a strictly monotonic increase in sperm counts up to 200 mg/kg, Thang et al. (2020) considered 360 mg/kg fully beneficial in rabbits, and El-Ratel et al. (2021) obtained their maximal effect at the highest dose tested. Three differences reconcile these observations with ours.

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First, the chemical nature of the preparations: a hydroalcoholic leaf extract rich in water-soluble polyphenols is not equivalent to a non-polar organic extract. Second, the route of administration: daily bolus gavage produces tissue peaks that prolonged dietary incorporation, which spreads exposure over the 24-hour cycle, does not generate. Third, the type of protocol: several of these studies rest on a lesion model, and restoration of damaged tissue has an amplitude that stimulation of healthy tissue does not. The non-monotonic character of certain phytochemical responses is in any case established elsewhere (Derbak et al., 2021). Most notably, Hammoudi et al. (2020), working with the same extract and species, set the safety threshold at 100 mg/kg/day — the exact dose at which all our parameters peak.

The lipid profile presents the most informative discrepancy with the literature. Serum cholesterol rose dose-dependently to 59.1% above controls at 200 mg/kg while triglycerides remained unchanged, a dissociation that rules out generalised dyslipidaemia in favour of a targeted disturbance of cholesterol handling. This result agrees with Hammoudi et al. (2020) but conflicts with Chentouh et al. (2017) in does and, more directly, with the data of Shaat et al. (2025), in which an oleic acid-rich oil lowered cholesterol, LDL and triglycerides while raising HDL. The contradiction is all the more striking as the predominant fatty acid is the same on both sides; it may be resolved by separating two scales of action. Systemically, supplied as a dietary lipid, oleic acid improves the circulating lipid profile; at cellular level, it inhibits cholesterol esterase and could thereby hinder mobilisation of esterified cholesterol towards testosterone synthesis in Leydig cells (Meikle et al., 1996). Administered as a concentrated bolus, as here, this second effect may become predominant: cholesterol would accumulate in blood at the very moment testosterone collapses, and two apparently unrelated findings would be two facets of a single phenomenon. In Shaat et al. (2025), improved lipid profile and improved testosterone went together; at our highest dose they dissociate, which is precisely what a block situated downstream of absorption and upstream of steroidogenesis would predict. The documented litholytic activity of *B. incrassatum* extracts on cholesterol stones (Mekraf et al., 2025) confirms at least that this material interacts directly with cholesterol.

Blood glucose escapes the pattern, and this is what gives it the value of an internal control. Its 18.2% decrease at the highest dose remained monotonic across the whole range, in agreement with the hypoglycaemic effect reported for the same extract at the same doses (Hammoudi et al., 2020) and with α -glucosidase inhibition by scopoletin (Jang et al., 2018). The hyperglycaemia recorded by Attoui et al. (2021) and Berroukeche et al. (2022) is only apparently contradictory: those authors administered whole root or tuber powder at 15% of the diet, sucrose included (Bousetla et al., 2015), whereas we used an organic extract. The difference therefore lies in the matrix rather than the species. That a metabolic endpoint remains monotonic while all reproductive endpoints reverse establishes that the extract exerts at least two pharmacologically distinct actions, and confines the

hormetic behaviour to the steroidogenic and germinal compartments rather than to the preparation as a whole.

The whole can thus be reduced to a single chain of events. At moderate doses, the coumarins of the extract reinforce antioxidant defences in the testicular interstitial compartment and preserve Leydig cell mitochondrial integrity, hence the output of the StAR/CYP11A1 pair; testosterone rises, spermatogenesis proceeds under better conditions, tubular diameter and epithelial height increase, and the three sperm parameters follow. At 200 mg/kg, the lipid load exceeds the buffering capacity of the tissue: antioxidant enzymes collapse, lipid peroxidation sets in, and inhibition of cholesterol esterase simultaneously restricts cholesterol access to the steroidogenic pathway. Accumulation of circulating cholesterol, the fall in testosterone, withdrawal of the germinal and interstitial compartments, and the preferential impairment of motility and viability then all stem from a common cause. The stability of triglycerides and the monotonic decline in blood glucose delimit the scope of this mechanism, which concerns the gonad and not the whole organism.

Several limitations bound these conclusions. LH and FSH were not assayed, which prevents precise localisation of the level of action along the hypothalamic–pituitary–gonadal axis; intratesticular testosterone and steroidogenic enzyme expression were likewise not measured, so the proposed Leydig cell mechanism remains a coherent interpretation rather than a demonstration. The absence of apoptotic and proliferative markers — Bax/Bcl-2 ratio, caspase-3, Ki-67 — does not allow determination of whether the high-dose regression reflects reversible functional inhibition or early germ cell loss. Finally, the treatment duration, shorter than a complete spermatogenic cycle in the rabbit, restricts the observed changes to late germinal stages and Leydig cell function rather than to spermatogonial renewal.

The practical implication concerns traditional use of this species. The useful window is narrower than empirical tuber consumption would suggest, and the form in which the plant is administered matters as much as the amount: the reversal in the direction of the glycaemic effect between whole powder and extract provides the clearest demonstration. Any reproductive application therefore requires a characterised extract and a capped dose, under the conditions of this protocol around 100 mg/kg/day. Several extensions would test this model: assaying intratesticular testosterone, LH and FSH, together with quantification of StAR, CYP11A1, 3 β -HSD and 17 β -HSD expression, would place the mechanism at molecular level; fractionation of the extract would be the most discriminating test, the coumarin fraction being expected to reproduce the ascending limb without the reversal and the lipid fraction the reversal without the initial benefit; finally, a protocol covering a complete spermatogenic cycle, extended by a recovery period and complemented by a fertility trial, would establish the reversibility of the high-dose withdrawal and give it functional significance.

5. Conclusion

Under the conditions of this study, the organic root extract of *Bunium incrassatum* exerts a biphasic, hormetic effect on male rabbit reproductive function. At 50 and 100 mg/kg/day it reinforces testicular antioxidant defences, lowers lipid peroxidation, raises serum testosterone, and improves seminiferous architecture and sperm quality, the optimum being reached at 100 mg/kg/day for all measured parameters. At 200 mg/kg/day this response reverses: antioxidant enzymes collapse, lipid peroxidation sets in, and the endocrine, histological and sperm gains are not merely abolished but crossed in the opposite direction. Three elements make this threshold a property of the preparation rather than an accident of the protocol: the regularity of the inverted U-shaped curve across methodologically independent parameter families, the existence of a single redox mechanism accounting for it from the interstitial compartment to the spermatozoon, and the coincidence of this optimum with the 100 mg/kg/day safety threshold independently established for the same extract in the same species. The reproductive potential of this plant appears to be significant; however, its effects are strongly influenced by the administered dose and the formulation employed.

6. Declarations

6.1 Author Contributions

Conceptualization: HD and SB; **Data curation:** HD, SB and SC; **Formal analysis:** HD, NH and NC; **Funding acquisition:** HD and SB; **Methodology:** HD and SB; **Supervision:** SB; **Writing – original draft:** HD and SB; **Writing – review and editing:** HD, NH and SB. All authors have read and agreed to the published version of the manuscript.

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6.3 Conflict of Interests

The authors declare that they have no competing interests related to this work.

Ethical approval. The protocol was approved by the Animal Care Committee of the Biotechnology Research Centre (CRBt, Constantine, Algeria).

7. References

1. Agarwal, A., Virk, G., Ong, C., & du Plessis, S. S. (2014). Effect of oxidative stress on male reproduction. *World Journal of Men's Health*, 32(1), 1–17. <https://doi.org/10.5534/wjmh.2014.32.1.1>
2. Aiouaz, N., & Bitam, A. (2022). Nutritional and therapeutic effects of dried tuber powder of *Bunium incrassatum* in hypo- and hyperthyroid female rats. *Journal of Food and Pharmaceutical Sciences*, 2(1), 92–108.

3. Amraoui, W., Bouteafaha, I., Belattar, N., & Arrar, L. (2024). Antioxidant, analgesic and anti-inflammatory activities of the aqueous root extract of *Bunium incrassatum*. *Archives of Biological Sciences*, 76(1), 15–25.
4. Attoui, A., Berroukeche, F., Guedri, A., & Toul, F. (2021). Effect of the roots of Algerian *Bunium incrassatum* on biological, biochemical and histological parameters of mature female rats. *Plant Archives*, 21, 201–209.
5. Berroukeche, F., Attoui, A., Toul, F., Ziane, L., Mokhtari Soulimane, N., & Merzouk, H. (2022). Antioxidant and anti-hemolytic efficacy of acetonetic and ethanolic extracts of *Bunium incrassatum* tubers and effect of powder supplementation in Wistar rats. *Asian Journal of Agriculture and Biology*, 2022(3), 202112379.
6. Bousetla, A., Zellagui, A., Derouiche, K., & Rhouati, S. (2015). Chemical constituents of the roots of Algerian *Bunium incrassatum* and evaluation of its antimicrobial activity. *Arabian Journal of Chemistry*, 8(3), 313–316. <https://doi.org/10.1016/j.arabjc.2011.01.022>
7. Bouzid, A., Baali, N., Rahmoune, B., Benali, M., Benmehdi, H., & Berroukeche, F. (2024). Antioxidant and hepatoprotective activities of *Bunium incrassatum* root extract against sodium fluoride toxicity in rats. *Fluoride*, 57(3), e262, 1–9.
8. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1–2), 248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
9. Buț, M.-G., Tero-Vescan, A., Pușcaș, A., Jîtcă, G., & Marc, G. (2024). Exploring the inhibitory potential of phytosterols β -sitosterol, stigmasterol, and campesterol on 5- α reductase activity in the human prostate: An in vitro and in silico approach. *Plants*, 13(22), 3146. <https://doi.org/10.3390/plants13223146>
10. Calabrese, E. J., & Mattson, M. P. (2017). How does hormesis impact biology, toxicology, and medicine? *NPJ Aging and Mechanisms of Disease*, 3, 13. <https://doi.org/10.1038/s41514-017-0013-z>
11. Chentouh, S., Boulahbel, S., Ouldjaoui, A., Hammoudi, N., Djebaili, H., & Adjal, E. (2017). Effect of organic extracts from roots of *Bunium incrassatum* on hematological parameters and histological changes in the genital tract in mature female rabbits. *Journal of Fundamental and Applied Sciences*, 9(3), 1618–1633.
12. Derbak, H., Moussaoui, A., Benberkane, A., & Ayad, A. (2021). In vitro effect of total alkaloid extract of *Peganum harmala* seeds on epididymal ram spermatozoa. *Asian Pacific Journal of Reproduction*, 10(5), 232–238.
13. Ehigiator, O. B., & Adikwu, E. (2020). Reproductive toxicity of co-administration of aspirin and metronidazole in male Wistar rats. *World Journal of*
 Journal of Animal Environment, Vol. 18, No. 2, Spring 2026
 Pharmaceutical Research, 9(5), 117–131.
14. El-Ratel, I. T., Wafa, W. M., El-Nagar, H. A., Aboelmagd, A. M., & El-Kholy, K. H. (2021). Beneficial effects of curcumin and phytogetic extracts with garlic on semen quality, reproductive hormones, thyroid activity, immunity, hepatic antioxidant activity and fertility in male rabbits. *Animal Science Journal*, 92, e13560. <https://doi.org/10.1111/asj.13560>
15. Flohé, L., & Günzler, W. A. (1984). Assays of glutathione peroxidase. *Methods in Enzymology*, 105, 114–121. [https://doi.org/10.1016/S0076-6879\(84\)05015-1](https://doi.org/10.1016/S0076-6879(84)05015-1)
16. Gao, X.-Y., Li, X.-Y., Zhang, C.-Y., & Bai, C.-Y. (2024). Scopoletin: A review of its pharmacology, pharmacokinetics and toxicity. *Frontiers in Pharmacology*, 15, 1268464. <https://doi.org/10.3389/fphar.2024.1268464>
17. Hammoudi, N., Hafid, N., Moumen, Y., Chentouh, S., Djebaili, H., & Boulahbel, S. (2020). Effect of treatment with organic matter extract of *Bunium incrassatum* roots on hematological parameters and histology of adrenal glands in pregnant rabbits. *Algerian Journal of Environmental Science and Technology*, 6(2), 1368–1376.
18. Iczkowski, K. A., Sun, E. L., & Gondos, B. (1991). Morphometric study of the prepubertal rabbit testis: Germ cell numbers and seminiferous tubule dimensions. *American Journal of Anatomy*, 190(3), 266–272. <https://doi.org/10.1002/aja.1001900306>
19. Jang, J.-H., Park, J.-E., & Han, J.-S. (2018). Scopoletin inhibits α -glucosidase in vitro and alleviates postprandial hyperglycemia in mice with diabetes. *European Journal of Pharmacology*, 834, 152–156. <https://doi.org/10.1016/j.ejphar.2018.07.032>
20. Johnsen, S. G. (1970). Testicular biopsy score count — a method for registration of spermatogenesis in human testes: Normal values and results in 335 hypogonadal males. *Hormones*, 1(1), 2–25. <https://doi.org/10.1159/000178170>
21. Khalaf, R. R., Khazaal, S., Abouzeinab, N. S., & Khalil, M. I. (2024). Evaluation of selected folk herbs on the fertility of Sprague Dawley male rats: Biochemical, histological, and molecular investigations. *Life*, 14(12), 1620. <https://doi.org/10.3390/life14121620>
22. Kooti, W., Mansouri, E., Ghasemiboroon, M., Harizi, M., Ashtary-Larky, D., & Afrisham, R. (2014). The effects of hydroalcoholic extract of *Apium graveolens* leaf on the number of sexual cells and testicular structure in rat. *Jundishapur Journal of Natural Pharmaceutical Products*, 9(4), e17532. <https://doi.org/10.17795/jjnpp-17532>
23. Luna, L. G. (1968). *Manual of histologic staining methods of the Armed Forces Institute of Pathology* (3rd ed.). McGraw-Hill.
24. Marklund, S., & Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide

- dismutase. *European Journal of Biochemistry*, 47(3), 469–474. <https://doi.org/10.1111/j.1432-1033.1974.tb03714.x>
25. Meikle, A. W., Stringham, J. D., Woodward, M. G., & Bishop, D. T. (1996). Oleic acid inhibits cholesteryl ester hydrolase and reduces testosterone synthesis in Leydig cells. *Journal of Andrology*, 17(2), 150–157.
 26. Mekraf, M., Boufadi, M. Y., Keddari, S., Abbassene, F., & Farah, A. (2025). Phytochemical identification by HPLC/UV, antioxidant efficacy and litholytic potential of *Bunium incrassatum* extracts. *Tropical Journal of Natural Product Research*, 9(3), 1211–1219.
 27. Monageng, E., Offor, U., Takalani, N. B., Mohlala, K., & Opuwari, C. S. (2023). A review on the impact of oxidative stress and medicinal plants on Leydig cells. *Antioxidants*, 12(8), 1559. <https://doi.org/10.3390/antiox12081559>
 28. Nasiri Boroujeni, S., Ansari Malamiri, F., Bossaghzadeh, F., Esmaili, A., & Moudi, E. (2022). The most important medicinal plants affecting sperm and testosterone production: A systematic review. *JBRA Assisted Reproduction*, 26(3), 522–530. <https://doi.org/10.5935/1518-0557.20220007>
 29. Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95(2), 351–358. [https://doi.org/10.1016/0003-2697\(79\)90738-3](https://doi.org/10.1016/0003-2697(79)90738-3)
 30. Olawuyi, T. S., Ukwenya, V. O., Jimoh, A. G., & Akinola, O. A. (2018). Cytoprotective effect of aqueous leaf extract of *Lawsonia inermis* on aluminium-induced oxidative stress: Seminiferous tubule histomorphometry and germ cell stereology in adult Wistar rats. *JBRA Assisted Reproduction*, 22(4), 330–338. <https://doi.org/10.5935/1518-0557.20180058>
 31. Perrone, P., & D'Angelo, S. (2025). Hormesis and health: Molecular mechanisms and the key role of polyphenols. *Food Chemistry Advances*, 7, 101030. <https://doi.org/10.1016/j.focha.2025.101030>
 32. Pourentezari, M., Talebi, A., Abbasi, A., Khalili, M. A., Mangoli, E., & Anvari, M. (2014). Effects of acrylamide on sperm parameters, chromatin quality, and the level of blood testosterone in mice. *Iranian Journal of Reproductive Medicine*, 12(5), 335–342.
 33. Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7), 671–675. <https://doi.org/10.1038/nmeth.2089>
 34. Shaat, A. R., Sadek, K. M., Mahmoud, S. F., Saleh, H., Sayed, S., Shukry, M., Ghamry, H. I., & Zeweil, M. M. (2025). Assessing the impact of ghee, olive oil and margarine on male rabbit fertility and reproductive hormones. *Journal of Animal Physiology and Animal Nutrition*, 109(1), 249–261. <https://doi.org/10.1111/jpn.14071>
 35. Shokoohi, M., Shoorei, H., Soltani, M., Abtahi-Eivari, S. H., Salimnejad, R., & Moghimian, M. (2018). Journal of Animal Environment, Vol. 18, No. 2, Spring 2026 Protective effects of the hydroalcoholic extract of *Fumaria parviflora* on testicular injury induced by torsion/detorsion in adult rats. *Andrologia*, 50(8), e13047. <https://doi.org/10.1111/and.13047>
 36. Singh, J., Srivastava, S., Zehra, A., Prajapati, P., Agarwal, V., Kumar, A., Mishra, V., & Kushwaha, S. (2025). Beta(β)-sitosterol attenuates chronic unpredictable stress (CUS) induced testicular damage in the experimental rat model. *Reproductive Sciences*, 32(4), 1312–1330. <https://doi.org/10.1007/s43032-025-01825-7>
 37. Thang, P. Q., Thuy, N. T. T., Van Anh, L. T., Hung, P. H., & Hai, N. V. (2020). Effects of herbal mixture supplementation on reproductive performance and antioxidant status in male rabbits. *World Rabbit Science*, 28(4), 207–217. <https://doi.org/10.4995/wrs.2020.13547>
 38. Toul, F., & Djendar, N. (2023). RP-HPLC phenolic profile and antioxidant activity of *Bunium incrassatum* umbels. *Journal of the Turkish Chemical Society Section A: Chemistry*, 10(2), 505–512.
 39. Toul, F., Djendar, A., Seladji, M., & Berroukeche, F. (2022). Phenolic profile and antioxidant activity of *Bunium incrassatum* seeds. *Journal of the Turkish Chemical Society Section A: Chemistry*, 9(4), 1231–1240.
 40. Tufarelli, V., Tsiplakou, E., & Laudadio, V. (2018). Quercetin supplementation and its effects on semen quality of rabbit bucks under heat stress conditions. *Animal Science Journal*, 89(9), 1273–1279.
 41. Uwejigho, O. E., Nwangwa, E. K., Dike, A. D., & Ohwin, E. P. (2025). Kolaviron ameliorates testicular dysfunction and oxidative stress in male Wistar rats exposed to sodium valproate. *Journal of Experimental and Clinical Anatomy*, 24(1), 45–54.
 42. Wing, T.-Y., & Christensen, A. K. (1982). Morphometric studies on rat seminiferous tubules. *American Journal of Anatomy*, 165(1), 13–25. <https://doi.org/10.1002/aja.1001650103>
 43. World Health Organization. (1992). *WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction* (3rd ed.). Cambridge University Press.
 44. Xiong, Z., Li, X., Yang, Y., Ding, H., Ma, H., Zhou, X., & Jiang, M. (2022). Chronic stress inhibits testosterone synthesis in Leydig cells through mitochondrial damage via Atp5a1. *Journal of Cellular and Molecular Medicine*, 26(3), 902–912. <https://doi.org/10.1111/jcmm.17085>
 45. Yang, D., Wang, T., Long, M., & Li, P. (2020). Quercetin: Its main pharmacological activity and potential application in clinical medicine. *Oxidative Medicine and Cellular Longevity*, 2020, 8825387. <https://doi.org/10.1155/2020/8825387>
 46. Zeweil, H. S., & El-Gindy, Y. M. (2017). Parsley seeds serve as a protective agent for sperm quality, blood lipid profile and antioxidant status in young and old male rabbits. *World Rabbit Science*, 25(3), 215–223. <https://doi.org/10.4995/wrs.2017.4773>

