



Contents lists available at <http://qu.edu.iq>
Al-Dalmaj Bulletin of Natural Science
 Journal homepage: <https://dbns.qu.edu.iq/>



Histoprotective Effect of *Lepidium sativum*-Loaded Solid Lipid Nanoparticles Against 6PPD-Quinone-Induced Adrenal Gland Injury in Rats

Reyim Hameid Kamel^{1*}, Rasha A.H. Alathary² and Zainab Falih Dakhl³

¹Department of Biology, Collage of Science Al-Qadisiya University;AL-diwanayah Iraq,.Email: riam.hammed@qu.edu.iq

²Department of Pathological Analysis, College of Science, Al-Qadisiyah University, Iraq, rasha.abdulzahra@qu.edu.iq

³Department of Biology, College of Science, University of Al-Qadisiyah, zainab.falih@qu.edu.iq

ARTICLE INFO

Article history:

Received 28 Dec. 2025

Revised 27 Jan. 2026

Accepted 30 Jan. 2026

Keywords:

6PPD-quinone; adrenal cortex; steroidogenic acute regulatory protein; corticosterone; solid lipid nanoparticles; histomorphometry; tyre wear particles.

ABSTRACT

N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone (6PPD-Q), an ozonation product of the tyre antioxidant 6PPD, is now recognised as a ubiquitous environmental contaminant with demonstrable multi-organ toxicity in mammals, yet its effect on the adrenal cortex has not been characterised. The present work examined whether subchronic 6PPD-Q exposure disturbs adrenocortical structure and glucocorticoid output in adult male albino rats, and whether co-treatment with *Lepidium sativum*-loaded solid lipid nanoparticles (LS-SLN) confers protection. 32 rats were allocated to four groups (n = 8 per group): Control, LS-SLN only, 6PPD-Q only, and 6PPD-Q + LS-SLN, and were treated for duration 28 days. Outcomes comprised body and paired adrenal weights, adrenocortical and zonal thickness by histomorphometry, a six-item semi-quantitative histopathological injury score, zone-resolved immunohistochemical quantification of steroidogenic acute regulatory protein (StAR), and basal and adrenocorticotrophic hormone (ACTH)-stimulated serum corticosterone. 6PPD-Q alone reduced total cortical thickness (698.5 ± 39.8 versus 812.4 ± 45.2 μm ; $p < 0.01$) with the largest losses in the zona glomerulosa and zona fasciculata, produced a composite injury score four-fold above Control (8 [5–10] versus 2 [1–3]; $p < 0.001$), and lowered StAR immunoreactivity in all three cortical zones, most steeply in the zona fasciculata (11.2 ± 1.3 versus 18.6 ± 1.8 ; $p < 0.001$). Functionally, basal corticosterone fell to 62.4 ± 5.8 ng/mL and the ACTH-stimulated concentration to 154.2 ± 14.6 ng/mL (both $p < 0.01$), indicating a reduced adrenal reserve rather than an isolated basal deficit. LS-SLN alone was histologically silent but increased cortical and zonal thickness, StAR expression and both basal and stimulated corticosterone, consistent with a trophic rather than an injurious action. Co-treatment restored every morphometric, immunohistochemical and hormonal end-point to values statistically indistinguishable from Control, although the composite injury score remained modestly but significantly elevated (4 [2–6]; $p < 0.05$), indicating partial rather than complete rescue. Collectively, the adrenal cortex emerges as a previously unrecognised target of 6PPD-Q, with zone-selective structural damage tracking a loss of StAR-dependent steroidogenic capacity, and LS-SLN offers substantial but incomplete protection.

*Corresponding author email:
riam.hammed@qu.edu.iq

1. Introduction

N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is added to rubber formulations, above all to vehicle tyres, as an antiozonant that sacrificially scavenges ozone and so delays cracking of the

rubber matrix (1,2). The chemistry that makes 6PPD useful also makes it environmentally problematic: on contact with atmospheric ozone the parent amine is converted to the corresponding quinone, 6PPD-quinone (6PPD-Q), a more stable and considerably more bioactive transformation product that is shed from tyre and road wear particles into air, road dust, runoff, surface water and soil (2,3). Attention was first drawn to the compound when it was identified as the causative agent of urban runoff mortality syndrome in coho salmon (*Oncorhynchus kisutch*), in which returning adults die within hours of entering storm-affected streams (1). Ingestion and inhalation appear to be the dominant routes, with the digestive and respiratory tracts acting as portals to systemic circulation (2). Population-level work has linked circulating concentrations of para-phenylenediamine quinones to biomarkers of oxidative stress and hepatic injury, which moves the discussion from theoretical exposure toward measurable biological consequence (4).

Mechanistic studies in rodents converge on a small number of recurring themes. Mass spectrometry imaging of exposed mice showed simultaneous perturbation of glutathione and ascorbate metabolism, energy metabolism and amino acid handling across liver, kidney, spleen and testis, identifying redox imbalance as a shared feature of multi-organ injury rather than an organ-specific epiphenomenon (5). Integrated network toxicology and multi-omics analysis of the mouse liver implicated apoptotic signalling, inflammatory activation and lipid metabolic disturbance, with p53, MAPK-family kinases, caspase-8 and tumour necrosis factor emerging as convergent nodes (6). In the central nervous system, prolonged exposure generated reactive oxygen species, neuronal apoptosis, blood–brain barrier disruption and a pro-inflammatory cytokine profile (7,8). Comparable oxidative and metabolic disruption has been reported in invertebrate and fish models, indicating that the underlying mode of action is conserved rather than idiosyncratic (9–12).

Steroidogenic cells operate with a chronically high basal oxidant load and depend on an unusually robust antioxidant apparatus to survive it (13,14). The steroidogenic acute regulatory protein (StAR), a short-lived protein whose activity is exquisitely sensitive to mitochondrial membrane integrity and to the redox state of the organelle (15–17). StAR is expressed across the zona glomerulosa, zona fasciculata and zona reticularis, with the strongest signal in the inner zones, and is acutely inducible by ACTH through cyclic

AMP–protein kinase A signalling (18,19). Any xenobiotic that destabilises adrenocortical mitochondria therefore has a direct route to functional hypoadrenalism, and the adrenal cortex is for that reason among the most frequent targets of toxicant-induced endocrine injury in regulatory toxicology datasets (20–22).

The improve the oral bioavailability and chemical stability of poorly water-soluble compounds, provide sustained release, and are well tolerated in rodents (23,24). SLN-encapsulated phytochemicals have repeatedly outperformed their unencapsulated counterparts in rodent models of inflammatory and oxidative injury, in part by raising and sustaining tissue concentrations of the payload (25). Whether this strategy translates to the adrenal cortex, and whether it can preserve StAR-dependent steroidogenic capacity under contaminant pressure, has not been tested.

The payload selected for that carrier was *Lepidium sativum* L. (garden cress), a member of the Brassicaceae used for centuries as both food and folk remedy across the Middle East, North Africa and South Asia. Its seeds are unusually rich in phenolic acids, flavonoids, glucosinolates, alkaloids, coumarins, sterols and α -linolenic acid, and the crude and solvent-partitioned extracts show strong radical-scavenging activity in vitro (29,30). In rodents this chemistry translates into measurable tissue protection: seed extracts attenuated carbon tetrachloride- and D-galactosamine/lipopolysaccharide-induced hepatic injury, restoring antioxidant enzyme activity and lowering lipid peroxidation, and suppressed the tumour necrosis factor α , interleukin 6, interleukin 1 β and inducible nitric oxide synthase responses that accompany such injury (30–32,34). The relevance of that profile to the present model is direct, since the hepatic, neural and adrenal lesions attributed to 6PPD-Q converge on the same oxidative and pro-inflammatory axis (5–7).

Seed-extract-loaded solid lipid nanoparticles (*L. sativum*-SLN, abbreviated LS-SLN throughout) have been fabricated successfully by both nanoprecipitation and lipid-phase methods, yielding particles of roughly 100–240 nm with entrapment efficiencies above 80%, sustained release over several hours, and demonstrably greater bioefficacy than the free extract; in neuronal cells the nanoparticulate form conferred protection against hydrogen peroxide- and amyloid- β -induced oxidative stress that the unencapsulated extract did not match (29,33). A lipid-based carrier is additionally attractive for an adrenal target, since the cortex is among the most lipid-rich and most densely perfused tissues in the body. Whether LS-SLN can

preserve StAR-dependent steroidogenic capacity under contaminant pressure has nevertheless not been tested.

The present study was therefore designed to determine (i) whether subchronic 6PPD-Q exposure produces measurable structural injury to the adrenal cortex of adult male albino rats, (ii) whether such injury is zone-selective, (iii) whether it is accompanied by a loss of StAR expression and a reduction in basal and ACTH-stimulated corticosterone secretion, and (iv) whether concurrent administration of *Lepidium sativum*-loaded solid lipid nanoparticles attenuates these changes. To our knowledge this is the first evaluation of the adrenal cortex as a target organ for 6PPD-Q and the first attempt to mitigate its endocrine toxicity with a nanoparticulate carrier.

2. Materials and Methods

2.1. Chemicals and reagents

6PPD-quinone (Purity $\geq 98\%$) was obtained from Sigma, USA. Synthetic ACTH (Elabscience, USA), the rat corticosterone enzyme-linked immunosorbent assay (ELISA) kit and the anti-StAR primary antibody (Elabscience, USA).

2.2. Preparation and characterisation of LS-SLN

L. sativum seeds will be cleaned, air-dried, and powdered, then extracted (e.g., methanolic or ethanolic Soxhlet or maceration extraction) following the general approach used in prior LS-SLN work (29,33); the crude extract is concentrated under reduced pressure and stored at $-20\text{ }^{\circ}\text{C}$ protected from light until formulation. LS-SLN will be prepared by a solvent-emulsification/homogenisation or hot-melt nanoprecipitation method, using the seed extract as the aqueous/active phase and a physiologically compatible lipid as the solid matrix, consistent with previously reported LS-SLN fabrication protocols (29,33).

2.3. Animals and ethical approval

Adult male albino rats (Wistar) weighing 180–190 g and aged 10 weeks were obtained from animal. Animals were housed 8 per cage under controlled conditions ($22 \pm 2\text{ }^{\circ}\text{C}$, $50 \pm 10\%$ relative humidity, 12 h

light/12 h dark cycle) with free access to standard pelleted chow and tap water, and were acclimatised for 7–14 days before the start of treatment. Only males were used, in order to avoid the confounding influence of the oestrous cycle on hypothalamic–pituitary adrenal activity (26).

2.4. Experimental design

Rats were randomised and divided into four groups of $n = 8$ animals each:

Group I (Control): received 8 rats, gavage water daily for duration.

Group II (LS-SLN only): received LS-SLN at 150 mg/kg body weight gavage daily for duration.

Group III (6PPD-Q only): received 6PPD-Q at 10 mg/kg body weight gavage daily for duration.

Group IV (6PPD-Q + LS-SLN): received 6PPD-Q as in Group III together with LS-SLN as in Group II.

Body weight was recorded on day 1 and at the end of the treatment period, and body weight gain was calculated as the difference between the two. Animals were inspected daily for clinical signs and mortality; no deaths occurred during the study.

2.5. Blood sampling, ACTH stimulation test and corticosterone assay

To minimise circadian variation, all sampling was performed between 08:00 and 10:00 h. For basal corticosterone, blood was collected from the retro-orbital plexus / tail vein within ≤ 3 min of removing the animal from its cage, since handling itself raises circulating glucocorticoids. The adrenal reserve was then probed with an ACTH stimulation test: synthetic ACTH was injected at a dose of 0.5 $\mu\text{g/kg}$ body weight, administered by the intraperitoneal (ip) route, and a second blood sample was taken 30 or 60 min later (21,22). Samples were allowed to clot at room temperature, centrifuged at $3000 \times g$ for 15 min, and the serum stored at -80°C until analysis. Corticosterone was measured by ELISA.

2.6. Necropsy and adrenal weights

At the end of the treatment period animals were weighed, anaesthetised with (ketamine and xylocaine), ip and euthanised by injection. Both adrenal glands were rapidly excised, cleared of adherent

periadrenal fat under a dissecting microscope, blotted and weighed together on an analytical balance to the nearest 0.1 mg. The adrenal-to-body-weight ratio was expressed as mg per 100 g of final body weight, so that absolute organ mass could be interpreted independently of treatment-related differences in somatic growth. The left gland was processed for histology and immunohistochemistry and the right gland was snap-frozen and stored at -80°C for ancillary analyses.

2.7. Histological processing and histomorphometry

Adrenal glands were immersion-fixed in 10% neutral buffered formalin for 24 h, dehydrated through graded ethanol, cleared in xylene and embedded in paraffin. Sections of 5 μm were cut from the mid-transverse plane of each gland, so that every section contained capsule, all three cortical zones and medulla, and were stained with haematoxylin and eosin (27).

Histomorphometric measurements were made on digitised images captured at magnification 10&40 X with a camera and microscope and analysed in ImageJ (2010, USA) after calibration against a stage micrometer. Total cortical thickness was measured as the radial distance from the inner aspect of the capsule to the corticomedullary junction. The zona glomerulosa, zona fasciculata and zona reticularis were delineated on the basis of their characteristic cellular arrangement arched clusters, radial cords, and anastomosing cords respectively and each was measured along the same radial axis.

2.8. Semi-quantitative histopathological scoring

Five histopathological features were graded on a 0–3 ordinal scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe): cellular degeneration, cytoplasmic vacuolation, necrosis, vascular congestion, and cytoarchitectural or zonal disorganisation. A composite injury score was obtained by summing the five individual scores, giving a theoretical range of 0–15. Scoring was performed independently by two observers blinded to group allocation on coded slides, with disagreements resolved by joint review at a multi-headed

microscope. The scoring system and blinding procedure follow established recommendations for valid semi-quantitative histopathology in experimental studies (28).

2.9. StAR immunohistochemistry and zone-resolved quantification

Sections were dewaxed, rehydrated and subjected to heat-induced epitope retrieval in 10 mM sodium citrate buffer (pH 6.0) for 10 minutes. Endogenous peroxidase was quenched with 3% hydrogen peroxide for 10 min and non-specific binding blocked with blocking serum for 20 minutes. Sections were incubated with the anti-StAR primary antibody at a dilution of 1:200 for 24 hours at 4 °C, followed by a biotinylated secondary / polymer-based detection system, visualised with 3,3'-diaminobenzidine and counterstained with Mayer's haematoxylin. Negative controls were processed in parallel with the primary antibody omitted from the incubation step; a section of tissue known to express StAR was stained alongside as a positive control.

StAR immunoreactivity was quantified separately in the zona glomerulosa, zona fasciculata and zona reticularis. 8 non-overlapping fields per zone per animal were captured at magnification 10&40x and analysed in ImageJ using colour deconvolution to separate the diaminobenzidine chromogen from the haematoxylin counterstain; the result is expressed as the percentage immunopositive area / mean optical density $\times$ area fraction state the exact metric and its units. Image capture settings were held constant across all slides and analysis was performed blind to group allocation. Zone-resolved quantification was adopted because StAR expression is normally non-uniform across the cortex, being weakest in the outer glomerulosa and strongest in the inner zones, so that a whole-cortex measurement would obscure zone-specific effects (18,19).

2.10. Statistical analysis

Analyses were performed in SPSS, 2018. Continuous variables body and adrenal weights, cortical and zonal thickness, StAR immunoreactivity and serum corticosterone were tested for normality with the Shapiro–Wilk test and for homogeneity of variance with Levene's test, then compared across the four groups by one-way analysis of variance followed by Tukey's HSD post hoc test; results are expressed as mean $\pm$ standard deviation. Ordinal histopathological scores were analysed by the Kruskal–Wallis test with Dunn's

post hoc test and are reported as median with interquartile or full range. ACTH-stimulated corticosterone was additionally examined by two-way repeated-measures ANOVA with sampling time as the within-subject factor, to test the group $\times$ stimulation interaction. A two-sided p value below 0.05 was regarded as significant, and significance is annotated as *p < 0.05, **p < 0.01 and ***p < 0.001 against the Control group, with "ns" denoting a non-significant difference.

3. Results

3.1. *Body weight and adrenal gland weight*

Initial body weight did not differ significantly among the Control (185.21 ± 0.56 g), LS-SLN only (184.55 ± 0.66 g), 6PPD-Q only (187.01 ± 0.69 g) and 6PPD-Q + LS-SLN (183.55 ± 0.62 g) groups, confirming that randomisation produced comparable starting cohorts. Final body weight and body weight gain, in contrast, were significantly affected by treatment. The highest final body weight and weight gain were recorded in the LS-SLN only group (325.11 ± 0.84 g and 140.56 ± 0.73 g), followed by the 6PPD-Q + LS-SLN group (295.33 ± 0.74 g and 111.78 ± 0.36 g). The Control (259.63 ± 0.23 g and 74.42 ± 0.67 g) and 6PPD-Q only (255.98 ± 0.27 g and 68.97 ± 0.81 g) groups showed lower values, the 6PPD-Q only group falling slightly below Control.

Paired adrenal weight also differed significantly among groups, being highest in the LS-SLN only group (123.56 ± 0.54 mg) and lowest in the 6PPD-Q only group (95.25 ± 0.21 mg), with Control (112.25 ± 0.29 mg) and 6PPD-Q + LS-SLN (102.52 ± 0.63 mg) intermediate. After normalisation to final body weight, the adrenal-to-body-weight ratio was highest in the Control group (43.23 ± 0.12 mg/100 g) and significantly reduced in the LS-SLN only (38.01 ± 0.19), 6PPD-Q only (37.21 ± 0.09) and 6PPD-Q + LS-SLN (34.71 ± 0.23 mg/100 g) groups, the lowest ratio occurring in the combined-treatment group. Overall, LS-SLN alone increased final body weight, weight gain and absolute adrenal mass, whereas 6PPD-Q alone did not increase weight gain relative to Control; the combined treatment produced intermediate somatic effects and the lowest relative adrenal weight (Table 1).

Table 1. Body weight and adrenal gland weight parameters.

Parameter	Control	LS-SLN only	6PPD-Q only	6PPD-Q + LS-SLN
Initial body weight (g)	185.21 ± 0.56	184.55 ± 0.66	187.01 ± 0.69	183.55 ± 0.62
Final body weight (g)	259.63 ± 0.23	325.11 ± 0.84	255.98 ± 0.27	295.33 ± 0.74
Body weight gain (g)	74.42 ± 0.67	140.56 ± 0.73	68.97 ± 0.81	111.78 ± 0.36
Paired adrenal weight (mg)	112.25 ± 0.29	123.56 ± 0.54	95.25 ± 0.21	102.52 ± 0.63
Adrenal-to-body-weight ratio (mg/100 g)	43.23 ± 0.12	38.01 ± 0.19	37.21 ± 0.09	34.71 ± 0.23

Values are mean ± SD, $n = 8$ per group. Initial body weight did not differ significantly among groups; all other parameters showed significant treatment-related differences ($p < 0.05$).

3.2. Adrenal cortical and zonal thickness

Total cortical thickness was significantly increased in the LS-SLN only group ($904.7 \pm 51.3 \mu\text{m}$, $p < 0.05$) and significantly decreased in the 6PPD-Q only group ($698.5 \pm 39.8 \mu\text{m}$, $p < 0.01$) compared with Control ($812.4 \pm 45.2 \mu\text{m}$), while the 6PPD-Q + LS-SLN group was intermediate and not significantly different from Control ($795.2 \pm 43.6 \mu\text{m}$). Zona glomerulosa thickness rose in the LS-SLN only group ($112.5 \pm 8.4 \mu\text{m}$, $p < 0.05$) and fell in the 6PPD-Q only group ($85.2 \pm 6.3 \mu\text{m}$, $p < 0.05$) relative to Control ($98.3 \pm 7.1 \mu\text{m}$), with the combined group unchanged ($95.7 \pm 7.0 \mu\text{m}$). Zona fasciculata thickness followed the same pattern, being higher after LS-SLN alone ($635.2 \pm 36.7 \mu\text{m}$, $p < 0.05$) and lower after 6PPD-Q alone ($478.4 \pm 28.9 \mu\text{m}$, $p < 0.01$) than in Control ($562.8 \pm 31.5 \mu\text{m}$), and unchanged in the combined group ($548.6 \pm 32.1 \mu\text{m}$). The zona reticularis was unaffected by LS-SLN alone ($157.0 \pm 10.2 \mu\text{m}$) but significantly thinner after 6PPD-Q alone ($134.9 \pm 8.7 \mu\text{m}$, $p < 0.05$) than in Control ($151.3 \pm 9.8 \mu\text{m}$), with the combined group again indistinguishable from Control ($150.9 \pm 9.5 \mu\text{m}$). These measurements indicate that 6PPD-Q thins the cortex in a zone-selective manner, affecting the glomerulosa and fasciculata most, and that co-treatment abolishes the statistical difference from Control (Table 2).

Table 2. Adrenal cortical and zonal thickness (histomorphometry).

Parameter	Control	LS-SLN only	6PPD-Q only	6PPD-Q + LS-SLN
Total cortical thickness (μm)	812.4 ± 45.2	904.7 ± 51.3*	698.5 ± 39.8**	795.2 ± 43.6 ns
Zona glomerulosa thickness (μm)	98.3 ± 7.1	112.5 ± 8.4*	85.2 ± 6.3*	95.7 ± 7.0 ns
Zona fasciculata thickness (μm)	562.8 ± 31.5	635.2 ± 36.7*	478.4 ± 28.9**	548.6 ± 32.1 ns
Zona reticularis thickness (μm)	151.3 ± 9.8	157.0 ± 10.2 ns	134.9 ± 8.7*	150.9 ± 9.5 ns

Values are mean ± SD. * $p < 0.05$, ** $p < 0.01$ versus Control; ns = not significant versus Control.

3.3. Semi-quantitative histopathological injury scores

In the LS-SLN only group no individual feature and no composite score differed from Control, indicating that the formulation alone caused no detectable adrenal injury. The 6PPD-Q only group, by contrast, showed significantly higher scores for cellular degeneration (2 [1–2], $p < 0.01$), cytoplasmic vacuolation (2 [1–3], $p < 0.01$), vascular congestion (2 [1–2], $p < 0.01$) and cytoarchitectural or zonal disorganisation (2 [1–3], $p < 0.01$), together with a significant increase in necrosis (1 [0–2], $p < 0.05$). The composite injury score was markedly elevated (8 [5–10] versus 2 [1–3] in Control, $p < 0.001$). The 6PPD-Q + LS-SLN group showed intermediate values, with every individual feature — cellular degeneration (1 [0–1]), vacuolation (1 [0–2]), necrosis (0 [0–1]), vascular congestion (1 [0–1]) and zonal disorganisation (1 [0–1]) statistically indistinguishable from Control. The composite score, however, remained significantly above Control (4 [2–6], $p < 0.05$), indicating partial rather than complete attenuation of 6PPD-Q-induced injury (Table 3).

Table 3. Semi-quantitative histopathological injury scores.

Histopathological feature	Control	LS-SLN only	6PPD-Q only	6PPD-Q + LS-SLN
Cellular degeneration	0 (0–1)	0 (0–0) ns	2 (1–2)**	1 (0–1) ns
Cytoplasmic vacuolation	1 (0–1)	0 (0–1) ns	2 (1–3)**	1 (0–2) ns
Necrosis	0 (0–0)	0 (0–0) ns	1 (0–2)*	0 (0–1) ns
Vascular congestion	1 (0–1)	0 (0–1) ns	2 (1–2)**	1 (0–1) ns
Cytoarchitectural / zonal disorganisation	0 (0–1)	0 (0–0) ns	2 (1–3)**	1 (0–1) ns
Composite injury score	2 (1–3)	1 (0–2) ns	8 (5–10)***	4 (2–6)*

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus Control (Kruskal–Wallis with Dunn’s post hoc test); ns = not significant versus Control.

3.4. StAR immunohistochemical quantification by cortical zone

StAR immunoreactivity was significantly altered by treatment in all three cortical zones. In the zona glomerulosa it increased after LS-SLN alone (15.8 ± 1.5 , $p < 0.05$) and decreased after 6PPD-Q alone (8.7 ± 0.9 , $p < 0.01$) relative to Control (12.4 ± 1.2), with the combined group intermediate and not significantly different from Control (11.9 ± 1.1). The largest effects were seen in the zona fasciculata, where LS-SLN alone raised StAR expression to 24.3 ± 2.1 ($p < 0.01$) and 6PPD-Q alone reduced it to 11.2 ± 1.3 ($p < 0.001$)

against a Control value of 18.6 ± 1.8 ; the combined group returned to 17.5 ± 1.6 (ns). In the zona reticularis, LS-SLN alone had no significant effect (11.2 ± 1.0) whereas 6PPD-Q alone lowered StAR expression to 6.5 ± 0.7 ($p < 0.01$) from a Control value of 9.8 ± 0.9 , and the combined group again did not differ from Control (9.4 ± 0.8). The magnitude of the 6PPD-Q-induced loss therefore paralleled the zonal pattern of structural thinning, being greatest in the zona fasciculata (Table 4).

Table 4. StAR immunohistochemical quantification by cortical zone.

Marker	Zone	Control	LS-SLN only	6PPD-Q only	6PPD-Q + LS-SLN
StAR	ZG	12.4 ± 1.2	$15.8 \pm 1.5^*$	$8.7 \pm 0.9^{**}$	11.9 ± 1.1 ns
StAR	ZF	18.6 ± 1.8	$24.3 \pm 2.1^{**}$	$11.2 \pm 1.3^{***}$	17.5 ± 1.6 ns
StAR	ZR	9.8 ± 0.9	11.2 ± 1.0 ns	$6.5 \pm 0.7^{**}$	9.4 ± 0.8 ns

Values are mean $\pm$ SD; ZG, zona glomerulosa; ZF, zona fasciculata; ZR, zona reticularis. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ versus Control; ns = not significant versus Control.

3.5. Serum corticosterone

Basal serum corticosterone was significantly increased in the LS-SLN only group (112.6 ± 9.4 ng/mL, $p < 0.01$) and significantly decreased in the 6PPD-Q only group (62.4 ± 5.8 ng/mL, $p < 0.01$) compared with Control (85.3 ± 7.2 ng/mL), while the combined group was intermediate and unchanged from Control (81.7 ± 7.0 ng/mL). The same rank order was preserved after ACTH stimulation: concentrations rose to 268.4 ± 22.1 ng/mL after LS-SLN alone ($p < 0.01$) and reached only 154.2 ± 14.6 ng/mL after 6PPD-Q alone ($p < 0.01$), against 215.8 ± 18.3 ng/mL in Control, with the combined group at 205.3 ± 17.5 ng/mL (ns). The failure of the 6PPD-Q group to match the Control response to a maximal trophic stimulus indicates a reduction in secretory reserve rather than a simple shift in basal tone (Table 5).

Table 5. Basal and ACTH-stimulated serum corticosterone.

Parameter	Control	LS-SLN only	6PPD-Q only	6PPD-Q + LS-SLN
Basal serum corticosterone (ng/mL)	85.3 ± 7.2	$112.6 \pm 9.4^{**}$	$62.4 \pm 5.8^{**}$	81.7 ± 7.0 ns
ACTH-stimulated corticosterone (ng/mL)	215.8 ± 18.3	$268.4 \pm 22.1^{**}$	$154.2 \pm 14.6^{**}$	205.3 ± 17.5 ns

*Values are mean $\pm$ SD. ** $p < 0.01$ versus Control; ns = not significant versus Control.*

3.6. Qualitative histological findings

Sections from Control animals (Fig. 1A, B) showed an adrenal cortex invested by a connective tissue capsule with normal architecture of the zona glomerulosa and zona fasciculata. The LS-SLN only group (Fig. 1C, D) likewise showed a normally organised cortex, with anastomosing cords of zona reticularis separated by blood capillaries, and a medulla containing chromaffin cells, intervening venous sinusoids and ganglion cells.

The 6PPD-Q only group (Fig. 1E, F) showed pyknotic nuclei in the zona glomerulosa and zona fasciculata, focal haemorrhage between the cells, and zona fasciculata cells with a less vacuolated, more acidophilic cytoplasm consistent with lipid depletion. The zona reticularis contained pyknotic nuclei and interstitial haemorrhage, while the medulla showed pyknosis of connective tissue and endothelial nuclei, severely congested and dilated venous sinusoids, and disarrangement of the chromaffin cells. In the 6PPD-Q + LS-SLN group (Fig. 1G, H), the cortex retained its normal capsular and zonal organisation, with only slight interstitial haemorrhage, residual cytoplasmic acidophilia in the zona fasciculata, and occasional pyknotic nuclei in the zona reticularis.

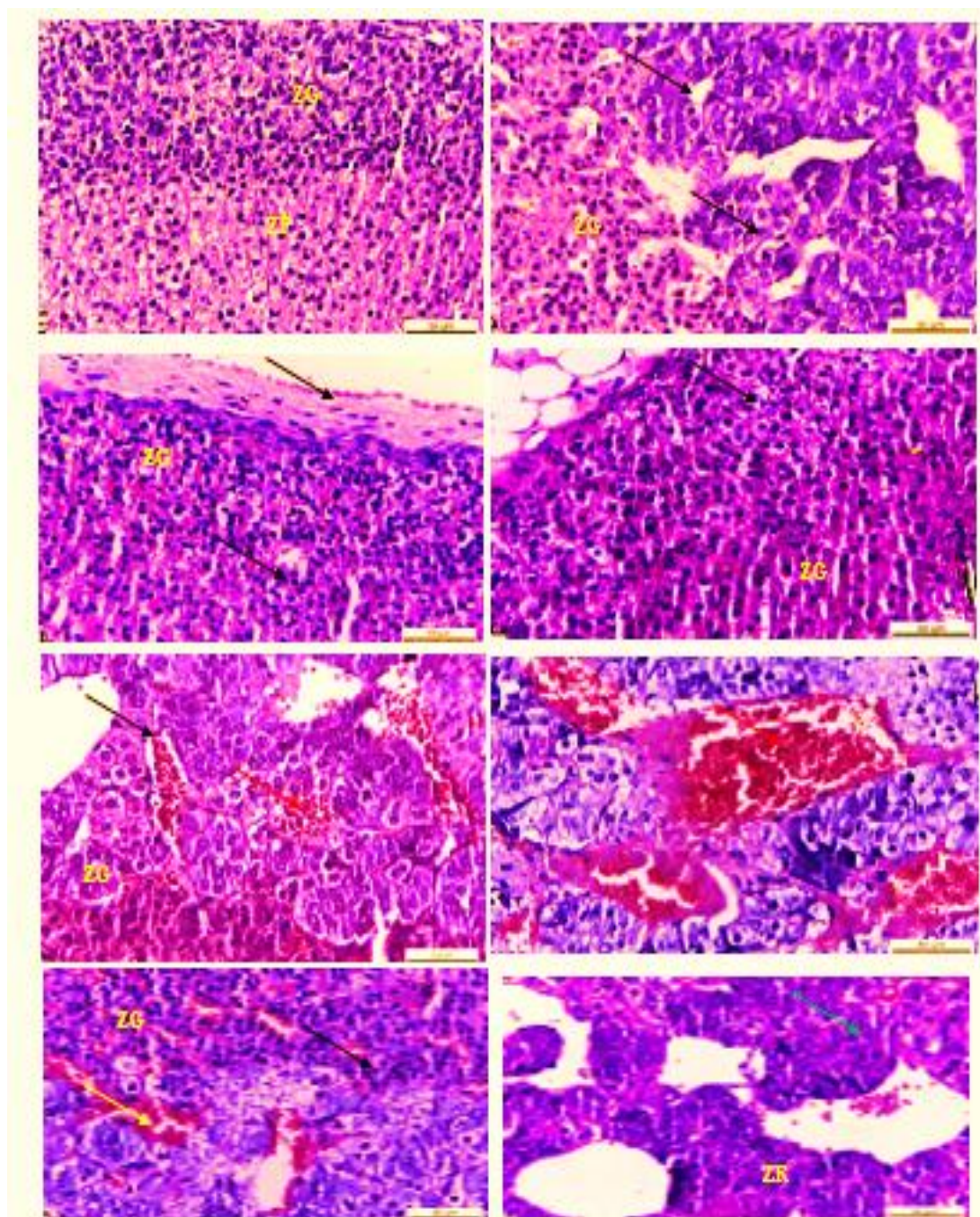


Figure 1.

Representative photomicrographs of haematoxylin and eosin (H&E)-stained sections of the rat adrenal gland. (A, B) Control; (C, D) LS-SLN only; (E, F) 6PPD-Q only; (G, H) 6PPD-Q + LS-SLN. Panels A, C, E and G are low-magnification views ($\times 10$; scale bar = [25] μm) showing the capsule, zona glomerulosa (ZG), zona fasciculata (ZF), zona reticularis (ZR) and medulla (M); panels B, D, F, H are high-magnification views ($\times 40$; scale bar = [50] μm). Arrows indicate pyknotic nuclei ([BLACK ARROW]), congestion and haemorrhage ([RED ARROW]), cytoarchitectural disorganisation ([YELLOW ARROW]) and altered zona fasciculata cells

with less vacuolated, more acidophilic cytoplasm ([GREEN ARROW]).

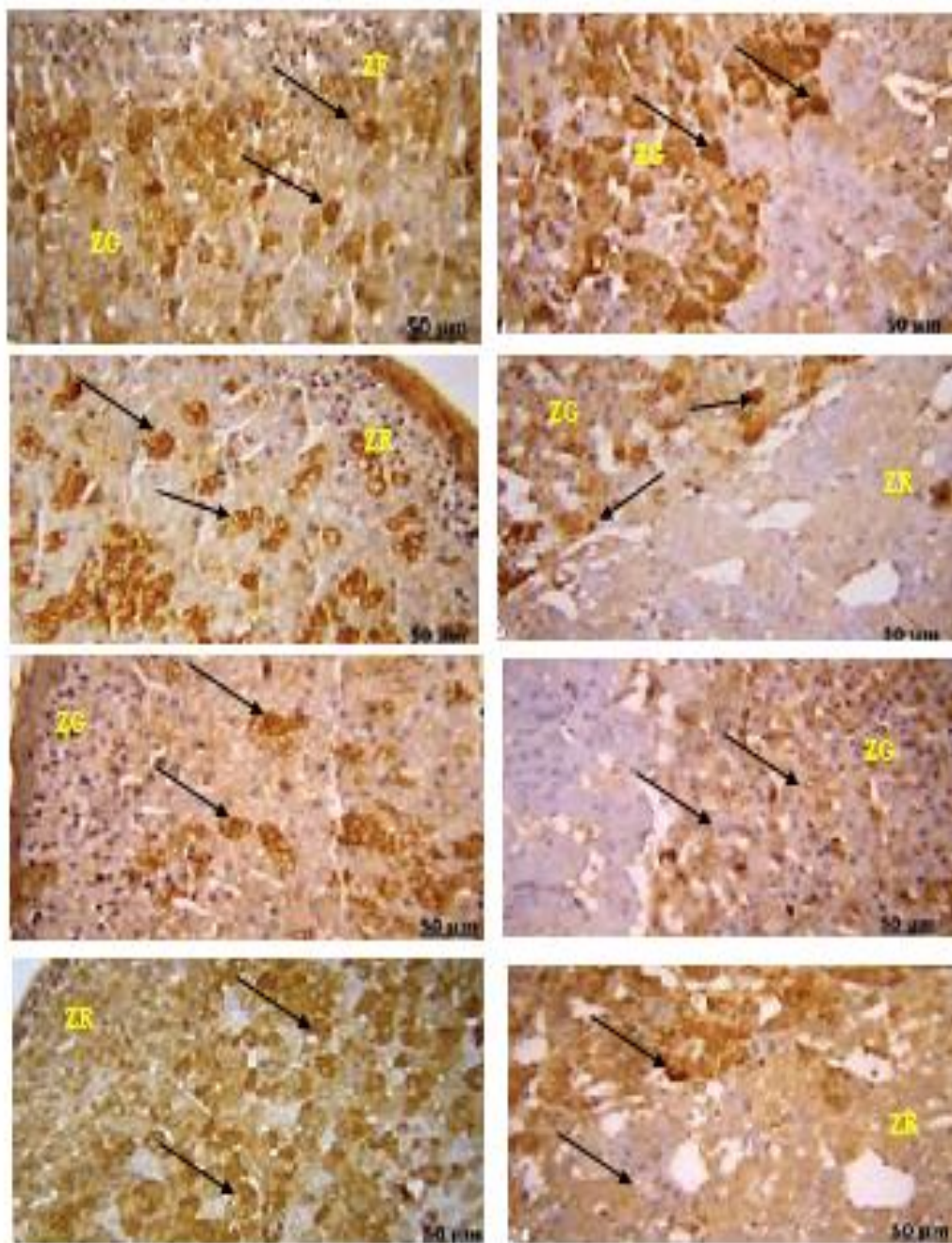


Figure 2. Representative photomicrographs of steroidogenic acute regulatory protein (StAR) immunohistochemistry (brown DAB reaction product; Mayer's haematoxylin counterstain) in the rat adrenal cortex. (A, B) Control; (C, D) LS-SLN only; (E, F) 6PPD-Q only; (G, H) 6PPD-Q + LS-SLN. The cortical zones in which StAR expression was quantified are labelled ZG (zona glomerulosa), ZF (zona fasciculata) and ZR (zona reticularis). Magnification $\times[40]$; scale bar = $[25\&50]$ μm .

Immunohistochemically (Fig. 2), Control sections showed the expected StAR reaction in the cells of the adrenal cortex. The LS-SLN only group showed strongly stained cells, including in the medulla. The 6PPD-Q only group showed only a weak cytoplasmic reaction in both cortical and medullary cells, whereas the

6PPD-Q + LS-SLN group showed a mild to moderate reaction in cortex and medulla, intermediate between the two.

4. Discussion

The principal finding of this study is that the adrenal cortex is a target of 6PPD-quinone. Subchronic exposure thinned the cortex, disorganised its zonal architecture, depleted StAR protein in all three zones, and reduced both resting and ACTH-stimulated corticosterone secretion. These four observations are internally consistent: a gland with fewer and more damaged steroidogenic cells, each expressing less of the rate-limiting cholesterol transport protein, should secrete less glucocorticoid at rest and should fail to reach a normal ceiling when maximally driven. Given the accumulating evidence that 6PPD-Q reaches human tissues through both ingestion and inhalation (2,4), the demonstration of an endocrine target with no established biomarker in routine toxicological screening is of some consequence.

The zonal pattern of injury is informative rather than incidental. The zona fasciculata sustained the largest absolute and proportional losses in both thickness and StAR immunoreactivity, followed by the zona glomerulosa, with the zona reticularis affected but least so. The fasciculata is the principal glucocorticoid-producing zone and carries the highest steroidogenic flux; its cells are packed with cholesterol-rich lipid droplets and with mitochondria whose P450 side-chain cleavage system generates superoxide as an unavoidable by-product of catalysis (13,14). A zone operating at that metabolic intensity has the least spare antioxidant capacity and is therefore predicted to fail first under an additional oxidant load. That the gradient of damage follows the gradient of steroidogenic activity supports an oxidative, mitochondrially centred mechanism rather than a non-specific cytotoxic one.

Such a mechanism is consistent with what is known about 6PPD-Q in other mammalian tissues. Spatial metabolomic work in exposed mice identified disturbance of glutathione and ascorbate metabolism alongside energy metabolism across several organs, positioning redox imbalance as the shared substrate of multi-organ toxicity (5). Hepatic studies implicate apoptotic and inflammatory signalling with p53, MAPK-family kinases and caspase-8 as convergent nodes (6), and neural studies report reactive oxygen species

accumulation with downstream apoptosis (7). Human hepatocyte lines respond to 6PPD-Q with excess reactive oxygen species and reduced viability (8). The histological picture seen here pyknosis, cytoplasmic acidophilia consistent with lipid depletion, focal necrosis and vascular congestion fits the same template. The loss of the normal vacuolated appearance of fasciculata cells is particularly telling, since it points to depletion of the cholesteryl ester stores that feed steroidogenesis and would by itself limit substrate availability independently of any effect on StAR.

The StAR data provide the mechanistic bridge between structure and function. StAR mediates the rate-limiting delivery of cholesterol to the inner mitochondrial membrane and has a half-life measured in minutes, so its steady-state concentration reports on the current biosynthetic activity and mitochondrial integrity of the cell rather than on its history (15–17). Its expression is normally strongest in the inner cortical zones and is acutely inducible by ACTH through cAMP–PKA signalling (18,19). A coordinated fall across all three zones, steepest where steroidogenic demand is highest, therefore indicates a generalised impairment of steroidogenic competence at the mitochondrial level. Since StAR function depends on the integrity of mitochondria-associated membranes and on the local redox environment (16,17), oxidative damage to those membranes offers a parsimonious explanation for simultaneous structural injury and steroidogenic failure.

The hormonal data allow that impairment to be graded. Basal corticosterone fell by roughly a quarter, but the more diagnostic result is the blunted response to exogenous ACTH: the 6PPD-Q group reached only about seventy per cent of the Control stimulated concentration. Basal glucocorticoid measurement alone is a weak index of adrenal competence, because rising endogenous ACTH can sustain resting output from a gland that has lost much of its reserve; provocative testing is the accepted way to reveal that loss (21,22). The concurrent presence of low basal and low stimulated values argues that the lesion is primarily adrenal rather than a downregulation of central drive, and it identifies the functional deficit as a reduced ceiling, which would be expected to manifest clinically only under stress.

Organ weight required careful interpretation here. Absolute paired adrenal weight was lowest in the 6PPD-Q group, in keeping with cortical thinning, but the adrenal-to-body-weight ratio was lowest in the combined-treatment group. The discrepancy arises because body weight itself was a treatment variable: LS-

SLN markedly increased somatic growth, so a normal adrenal expressed relative to a heavier body yields a lower ratio. This is a familiar pitfall in adrenal toxicology, where relative organ weight is a poor index whenever a treatment changes body mass, and it is the reason both absolute and relative values are reported and interpreted alongside morphometry (20,22). Read together with the thickness measurements, the data indicate that the low ratio in the combined group reflects somatic growth rather than adrenal atrophy.

LS-SLN alone produced a coherent trophic profile: greater cortical and zonal thickness, higher StAR expression, higher basal and stimulated corticosterone, and larger absolute adrenal mass, all without any histopathological penalty, since injury scores were indistinguishable from Control. Solid lipid nanoparticles are designed to raise and sustain tissue concentrations of poorly soluble lipophilic agents, and SLN-encapsulated phytochemicals have repeatedly outperformed their free counterparts in rodent models of oxidative and inflammatory injury (23–25). The adrenal cortex, being lipid-rich and highly perfused, is a plausible site of preferential accumulation for a lipid-based carrier. The composition of the payload offers a plausible mechanistic account of the effect. Garden cress seed extract is a mixture of phenolics, flavonoids, glucosinolate-derived isothiocyanates and α -linolenic acid, and its documented actions direct radical scavenging, restoration of superoxide dismutase and catalase activity, suppression of lipid peroxidation, and downregulation of tumour necrosis factor α , interleukin 6 and inducible nitric oxide synthase target precisely the processes implicated in 6PPD-Q toxicity (30–32). Encapsulation raises and prolongs tissue exposure to these constituents relative to the free extract, which is the most likely reason the nanoparticulate form is effective at doses at which crude extracts are usually not (29,33). The enhancement should nonetheless be treated with caution: a chronic increase in glucocorticoid secretion is not self-evidently beneficial, and the concurrent gain in body weight raises the question of whether the observed adrenal changes are a direct pharmacological effect of *Lepidium sativum*, an effect of the lipid carrier itself, or a secondary consequence of altered growth and feed efficiency. A vehicle-only nanoparticle group, absent from the present design, would have separated these possibilities.

The protective effect in the combined group was substantial but not complete, and the distinction matters. Every continuous end-point cortical and zonal thickness, zone-resolved StAR expression, basal and

stimulated corticosterone returned to values statistically indistinguishable from Control, and each individual histopathological feature likewise lost significance. Yet the composite injury score remained significantly elevated. Composite scores aggregate sub-threshold changes across several features, so the pattern indicates residual low-grade damage distributed across multiple lesion types rather than persistence of any single one. Functionally, the gland appears rescued; structurally, a measurable footprint of exposure remains. Whether that footprint resolves after a recovery period, or predisposes to failure under prolonged exposure or a second stressor, cannot be determined from a single terminal time point.

Several limitations should be acknowledged. The study used only adult males, so the findings cannot be extrapolated to females, in whom hypothalamic–pituitary–adrenal reactivity differs, nor to developing animals, in whom the adrenal cortex is undergoing zonal remodelling. A single dose level of 6PPD-Q was tested, which precludes the construction of a dose–response relationship or the identification of a no-observed-adverse-effect level. Mechanistic inference rests on morphology, a single steroidogenic protein and hormone output; direct measurement of tissue oxidative stress markers, of mitochondrial function, of the downstream steroidogenic enzymes CYP11A1, 3 β -HSD and CYP11B1, and of apoptotic indices would be needed to confirm the proposed pathway. Plasma ACTH was not measured, so the contribution of altered central drive cannot be excluded with certainty. Neither 6PPD-Q nor its metabolites were quantified in adrenal tissue, leaving internal dose unverified. Finally, the absence of an empty-carrier control limits interpretation of the LS-SLN-only effects. Future work should address dose–response, both sexes, a recovery phase, and direct assessment of adrenal mitochondrial and redox status, ideally with quantification of 6PPD-Q in the target organ.

Despite these constraints, the practical implication is straightforward. Regulatory and research screening of emerging contaminants routinely examines liver, kidney, lung and brain, and increasingly the gonads, while the adrenal cortex is seldom examined beyond organ weight and a single haematoxylin and eosin section. The present data suggest that for 6PPD-Q this is an oversight: a reduced glucocorticoid ceiling would be invisible in an unstressed animal and undetectable by basal hormone measurement alone, yet it is exactly the kind of deficit that matters under physiological challenge.

5. Conclusion

Subchronic exposure to 6PPD-quinone caused zone-selective adrenocortical injury in adult male albino rats, with thinning of the cortex that was most pronounced in the zona fasciculata and zona glomerulosa, significant histopathological damage across degeneration, vacuolation, necrosis, vascular congestion and zonal disorganisation, depletion of StAR immunoreactivity in all three cortical zones, and reduction of both basal and ACTH-stimulated serum corticosterone. The parallel between the zonal gradient of structural loss and the zonal gradient of StAR depletion points to an oxidative, mitochondrially centred impairment of the rate-limiting step of steroidogenesis. Co-administration of *Lepidium sativum*-loaded solid lipid nanoparticles restored every morphometric, immunohistochemical and hormonal end-point to values statistically indistinguishable from Control, while a residual elevation of the composite injury score showed the protection to be partial rather than absolute; the formulation given alone was histologically silent but trophic. The adrenal cortex should therefore be regarded as a target organ in the risk assessment of this widespread tyre-derived contaminant, and lipid-based nanocarriers merit further evaluation as a mitigation strategy. Confirmation will require dose–response studies in both sexes with direct measurement of adrenal redox and mitochondrial end-points.

References

1. Tian Z, Zhao H, Peter KT, Gonzalez M, Wetzel J, Wu C, et al. A ubiquitous tire rubber-derived chemical induces acute mortality in coho salmon. *Science*. 2021;371(6525):185–9. doi:10.1126/science.abd6951
2. Dong P, Liu M, Wang H, Chen J, Xu X, Su H, et al. Not just a fish killer: multi-organ toxicity and mechanisms of 6PPD-quinone. *Toxics*. 2026;14(4):288. doi:10.3390/toxics14040288
3. Li Y, Zeng J, Liang Y, Zhao Q, Wang Z, Zhu L, et al. A review of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) and its derivative 6PPD-quinone in the environment. *Toxics*. 2024;12(6):394. doi:10.3390/toxics12060394
4. Song S, Gao Y, Feng S, Cheng Z, Huang H, Xue J, et al. Widespread occurrence of two typical N,N'-substituted p-phenylenediamines and their quinones in humans: association with oxidative stress and liver damage. *J Hazard Mater*. 2024;468:133835. doi:10.1016/j.jhazmat.2024.133835

5. Jia K, Sun J, Du Q, Qu Y, Han J, Liu H, et al. Mass spectrometry imaging unveils the metabolic effect of 6PPD-quinone in exposed mice. *Environ Sci Technol.* 2025;59(9):4282–91. doi:10.1021/acs.est.4c11156
6. Li B, Xu C, Zhang D, Wang S, Xu J, Xiao B, et al. Combined analysis of network toxicology and multiomics revealed the potential mechanism of 6PPDQ-induced hepatotoxicity in mice. *Environ Sci Technol.* 2025;59(21):10204–14. doi:10.1021/acs.est.5c03906
7. [Chang-Sheng Ma](#); [Yu-Xi Liu](#); [Bo Han](#); [Min Bai](#); [Dong-Lun Li](#); [Shu-Chen Meng](#); [Li-Ying Zhang](#); [Meng-Yuan Duan](#); [Mao-Tao He](#). Long-term exposure to tire-derived 6-PPD quinone causes neurotoxicity and neuroinflammation via inhibition of HTR2A in C57BL/6 mice. *Environ Sci Technol.* 2025;59(3):1542–52. doi:10.1021/acs.est.4c09276
8. Qi Y, Qiu A, Wei X, Huang Y, Huang Q, Huang W. Effects of 6PPD-quinone on human liver cell lines as revealed with cell viability assay and metabolomics analysis. *Toxics.* 2024;12(6):389. doi:10.3390/toxics12060389
9. Shi R, Bao Y, Liu W, Liu J, Wang X, Shi X, et al. Tire rubber antioxidant 6PPD and 6PPD-quinone disrupt the energy supply and lipid metabolism of earthworms. *Environ Sci Technol.* 2025;59(18):9426–36. doi:10.1021/acs.est.4c13570
10. Zhang X, Shi R, Shi X, Du J, He Y, Liu W. Ecotoxicity of 6PPD and 6PPD-Q in aquatic ecosystems: mechanisms, influencing factors, and mitigation strategies. *J Hazard Mater.* 2026;501:140856. doi:10.1016/j.jhazmat.2025.140856
11. Feng Y, Xian H, Bai R, Wang X, Chen J, Zhang Y, et al. Multi-omics insights into 6PPD- and 6PPDQ-induced gut–liver axis disruption and non-alcoholic fatty liver disease progression in zebrafish (*Danio rerio*). *J Hazard Mater.* 2025;495:138822. doi:10.1016/j.jhazmat.2025.138822
12. Rao C, Zuo Y, Xiang D, Zhang W, Yang Y, Chen L, et al. Exploring the fate of 6PPD in zebrafish (*Danio rerio*): understanding toxicokinetics, biotransformation mechanisms, and metabolomic profiling at environmentally relevant levels. *J Hazard Mater.* 2025;489:137687. doi:10.1016/j.jhazmat.2025.137687
13. Hanukoglu I. Antioxidant protective mechanisms against reactive oxygen species (ROS) generated by mitochondrial P450 systems in steroidogenic cells. *Drug Metab Rev.* 2006;38(1–2):171–96. doi:10.1080/03602530600570040
14. Midzak A, Papadopoulos V. Adrenal mitochondria and steroidogenesis: from individual proteins to functional protein assemblies. *Front Endocrinol (Lausanne).* 2016;7:106. doi:10.3389/fendo.2016.00106

15. Stocco DM, Clark BJ. Regulation of the acute production of steroids in steroidogenic cells. *Endocr Rev.* 1996;17(3):221–44. doi:10.1210/edrv-17-3-221
16. Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr Rev.* 2011;32(1):81–151. doi:10.1210/er.2010-0013
17. Prasad M, Kaur J, Pawlak KJ, Bose M, Whittall RM, Bose HS. Mitochondria-associated endoplasmic reticulum membrane (MAM) regulates steroidogenic activity via steroidogenic acute regulatory protein (StAR)–voltage-dependent anion channel 2 (VDAC2) interaction. *J Biol Chem.* 2015;290(4):2604–16. doi:10.1074/jbc.M114.605808
18. Peters B, Clausmeyer S, Obermüller N, Woyth A, Kränzlin B, Gretz N, et al. Specific regulation of StAR expression in the rat adrenal zona glomerulosa: an in situ hybridization study. *J Histochem Cytochem.* 1998;46(11):1215–21. doi:10.1177/002215549804601101
19. Hattangady NG, Olala LO, Bollag WB, Rainey WE. Acute and chronic regulation of aldosterone production. *Mol Cell Endocrinol.* 2012;350(2):151–62. doi:10.1016/j.mce.2011.07.034
20. Rosol TJ, Yarrington JT, Latendresse J, Capen CC. Adrenal gland: structure, function, and mechanisms of toxicity. *Toxicol Pathol.* 2001;29(1):41–8. doi:10.1080/019262301301418847
21. Harvey PW, Everett DJ, Springall CJ. Adrenal toxicology: a strategy for assessment of functional toxicity to the adrenal cortex and steroidogenesis. *J Appl Toxicol.* 2007;27(2):103–15. doi:10.1002/jat.1221
22. Hinson JP, Raven PW. Effects of endocrine-disrupting chemicals on adrenal function. *Best Pract Res Clin Endocrinol Metab.* 2006;20(1):111–20. doi:10.1016/j.beem.2005.09.006
23. Mishra V, Bansal KK, Verma A, Yadav N, Thakur S, Sudhakar K, et al. Solid lipid nanoparticles: emerging colloidal nano drug delivery systems. *Pharmaceutics.* 2018;10(4):191. doi:10.3390/pharmaceutics10040191
24. Duan Y, Dhar A, Patel C, Khimani M, Neogi S, Sharma P, et al. A brief review on solid lipid nanoparticles: part and parcel of contemporary drug delivery systems. *RSC Adv.* 2020;10(45):26777–91. doi:10.1039/D0RA03491F
25. Zhang F, Liu Z, He X, Li Z, Shi B, Cai F. β -Sitosterol-loaded solid lipid nanoparticles ameliorate complete Freund's adjuvant-induced arthritis in rats: involvement of NF- κ B and HO-1/Nrf-2 pathway. *Drug Deliv.* 2020;27(1):1329–41. doi:10.1080/10717544.2020.1818883
26. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *PLoS Biol.* 2020;18(7):e3000410. doi:10.1371/journal.pbio.3000410

27. Layton C, Bancroft JD, Suvarna SK. Fixation of tissues and the hematoxylin and eosin stain. In: Suvarna SK, Layton C, Bancroft JD, editors. Bancroft's theory and practice of histological techniques. 8th ed. Amsterdam: Elsevier; 2019. p. 40–75, 126–38.
28. Gibson-Corley KN, Olivier AK, Meyerholz DK. Principles for valid histopathologic scoring in research. Vet Pathol. 2013;50(6):1007–15. doi:10.1177/0300985813485099
29. Al-Saran N, Subash-Babu P, Al-Harbi LN, Alrfaei BM, Alshatwi AA. Neuroprotective effect of solid lipid nanoparticles loaded with *Lepidium sativum* (L.) seed bioactive components enhance bioavailability and Wnt/ β -catenin/CaMK-II signaling cascade in SH-SY5Y neuroblastoma cells. Nanomaterials. 2024;14(2):199. doi:10.3390/nano14020199
30. [Saeid Vazifeh¹](#), [Parya Kananpour¹](#), [Mahna Khalilpour¹](#), [Sajjad Vazifeh Eaisalou¹](#), [Michael R Hamblin²](#). Anti-inflammatory and immunomodulatory properties of *Lepidium sativum*. Biomed Res Int. 2022;2022:3645038. doi:10.1155/2022/3645038
31. Raish M, Ahmad A, Alkharfy KM, Ahamad SR, Mohsin K, Al-Jenoobi FI, et al. Hepatoprotective activity of *Lepidium sativum* seeds against D-galactosamine/lipopolysaccharide induced hepatotoxicity in animal model. BMC Complement Altern Med. 2016;16(1):501. doi:10.1186/s12906-016-1483-4
32. Al-Asmari AK, Athar MT, Al-Shahrani HM, Al-Dakheel SI, Al-Ghamdi MA. Efficacy of *Lepidium sativum* against carbon tetrachloride induced hepatotoxicity and determination of its bioactive compounds by GC-MS. Toxicol Rep. 2015;2:1319–26. doi:10.1016/j.toxrep.2015.09.006
33. [Asad Ahmad¹](#), [Heena Tabassum²](#), [Rabia Nabi³](#), [Anuradha Mishra¹](#), [Iffat Zareen Ahmad²](#). Solid lipid nanoparticles of *Lepidium sativum* L. seed extract: formulation, optimization and in vitro cytotoxicity studies. Drug Res (Stuttg). 2022. doi:10.1055/a-1800-5956
34. Zamzami MA, Baothman OAS, Samy F, Abo-Golayel MK. Amelioration of CCl₄-induced hepatotoxicity in rabbits by *Lepidium sativum* seeds. Evid Based Complement Alternat Med. 2019;2019:5947234. doi:10.1155/2019/5947234