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Green synthesized selenium nanoparticles attenuate cadmium chloride induced hepatorenal oxidative damage in male Wistar rats

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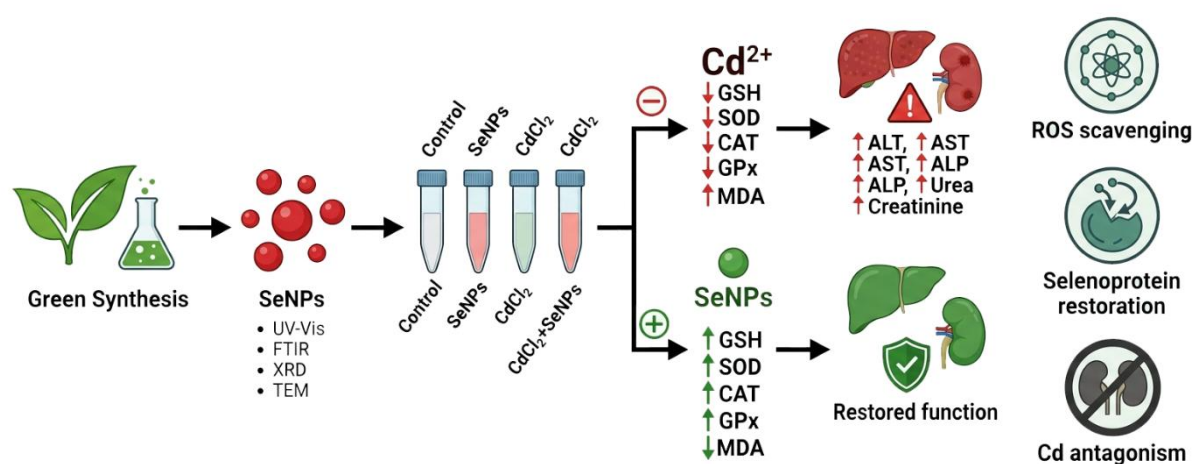
ABSTRACT

Cadmium (Cd) is a persistent environmental and industrial pollutant that accumulates mainly in the liver and the kidneys, where it drives oxidative injury. The present work was designed to find out whether selenium nanoparticles (SeNPs) prepared through an eco-friendly, plant-mediated route could blunt cadmium chloride (CdCl₂) induced hepatorenal damage in male Wistar rats. SeNPs were biosynthesised using a Punica granatum (pomegranate) peel extract that acted as both reducing and capping agent, and were characterised by UV-Vis spectroscopy, FTIR, XRD, FESEM coupled with EDX, TEM and dynamic light scattering. The particles were spherical, reasonably uniform and within the nanometre range, with a characteristic surface-plasmon band and surface functional groups consistent with phytochemical capping. Animals were allocated to four groups (control, SeNPs, CdCl₂, and CdCl₂ + SeNPs) and treated for [duration]. CdCl₂ markedly raised serum ALT, AST, ALP, urea and creatinine, increased malondialdehyde (MDA) in both liver and kidney tissue, and lowered reduced glutathione (GSH) together with the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Histology of the two organs showed degeneration, congestion and inflammatory infiltration. Co-administration of green SeNPs reversed most of these changes: the biochemical markers moved back close to control values, antioxidant capacity was largely restored and tissue architecture was, for the most part, preserved. The protection is best explained by the radical-scavenging behaviour of the nanoparticles, their contribution to selenoprotein-dependent antioxidant defence, and a likely interaction with Cd that limits its pro-oxidant action. Taken together, plant-derived SeNPs appear to be a promising, low-toxicity candidate for mitigating cadmium-related hepatorenal toxicity, although the narrow margin between beneficial and toxic selenium doses means that dose optimisation will remain important.

Highlights

- Selenium nanoparticles were biosynthesised through a simple, Punica granatum (pomegranate) peel-mediated green route.
- CdCl₂ produced clear hepatorenal injury accompanied by oxidative stress in male Wistar rats.
- Green SeNPs lowered serum ALT, AST, ALP, urea and creatinine back toward normal.
- SeNPs restored GSH, SOD, CAT and GPx and curbed lipid peroxidation in both organs.
- Liver and kidney histology was largely preserved by SeNP co-treatment.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Cadmium is among the most hazardous of the non-essential heavy metals, and human exposure to it has crept upward with industrial activity over the past century [1]. People take it in mostly through contaminated food and water, cigarette smoke and a handful of occupational settings such as battery manufacture, smelting and pigment production [2,3]. What makes Cd particularly troublesome is its very long biological half-life on the order of two to three decades so that even small, repeated doses accumulate, largely in the liver and kidneys, the two organs that bear the brunt of its toxicity [1,4,39]. Cd is not a Fenton-active metal at cellular level, but it produces reactive oxygen species (ROS) indirectly by displacing redox-active metals, impairing the mitochondrial electron-transport chain and draining thiol pools, tilting the balance toward oxidative stress [5,6]. Much of the hepatic and renal damage seen after exposure is due to the lipid peroxidation, protein oxidation and antioxidant-enzyme suppression that ensues [7]. Functional consequences include elevated serum aminotransferases and alkaline phosphatase, increased urea and creatinine and reduced tissue antioxidant defences [8].

Selenium (Se), by contrast, is an essential trace element whose biological actions are exerted mostly through selenoproteins such as glutathione peroxidase and thioredoxin reductase, both central to antioxidant defence [9,10]. A long line of work has shown that Se can antagonise Cd toxicity, partly by forming relatively inert Se-Cd complexes and partly by sustaining Se-dependent enzymes [11,12]. Nanoscale selenium is especially attractive here, since SeNPs pair high bioavailability and antioxidant activity with a toxicity that is markedly lower than that of inorganic or organic Se forms [13,14]. Preparing these particles through green, plant-mediated routes adds one more advantage: plant phytochemicals serve as reducing and capping agents, avoiding hazardous solvents and yielding stable, biocompatible nanoparticles [15,16]. Despite the growing

interest, comparatively few studies have looked specifically at green-synthesised SeNPs against Cd-induced hepatorenal injury [17,18]. The present study was therefore set up to prepare SeNPs by a simple *Punica granatum* (pomegranate) peel-mediated method, characterise them properly, and then evaluate their protective effect against CdCl₂-induced hepatorenal oxidative damage in male Wistar rats.

2. MATERIALS AND METHODS

Chemicals and reagents

Sodium selenite (Na₂SeO₃) [or selenious acid, H₂SeO₃] and cadmium chloride (CdCl₂) were of analytical grade and supplied by [manufacturer]. All additional reagents, kits and solvents were of analytical grade and were used as received. Stock and working solutions were newly made in deionised water.

Preparation of the *Punica granatum* peel extract

Fresh *Punica granatum* (pomegranate) fruit peels were harvested from [place], validated by botanist, rinsed completely with tap and distilled water, shade dried and powdered to fine powder. A weighed amount ([X] g) of the powder was extracted in [X] mL deionised water at [≈60-80 °C] for [time] with agitation, chilled and filtered through Whatman No. 1 filter paper. The clear filtrate was stored at 4 °C and used within [time] as the source of the reducing and capping compounds.

Green synthesis of selenium nanoparticles

SeNPs were prepared by adding the plant extract dropwise to an aqueous selenium-salt solution ([X] mM) under continuous magnetic stirring at [temperature] for [time], broadly along the lines of previous plant-mediated techniques [15,19]. The first visual evidence of reduction of Se ions to elemental selenium (Se⁰) and formation of nanoparticles was assumed to be a gradual colour shift of the mixture to brick-red / ruby tone [20]. The colloid was then centrifuged, the pellet washed several times with deionised water and ethanol to remove loosely

attached material and lastly dried. The recovered red powder was stored in the dark until use.

Reaction circumstances were purposefully maintained modest. Mechanistically, the polyphenols and flavonoids in the extract are thought to act as electron donors to reduce selenite (Se⁴⁺) to elemental Se⁰, while these molecules in combination with soluble proteins and sugars are adsorbed on the newly formed nuclei and capped preventing further growth and aggregation [15,21].

The pH of the medium, the extract-to-salt ratio and the temperature all bear on the rate of reduction and on the final particle size, and in our hands a [mildly acidic / near-neutral] pH gave the most stable, evenly coloured colloid with the best yield. No external surfactant or chemical reductant such as sodium borohydride was needed – which is precisely the practical advantage that makes the plant-mediated route attractive in the first place [16].

Characterisation of the nanoparticles

Formation of the particles was first followed by UV–Vis. spectroscopy across 200–600 nm. Surface functional groups contributed by the extract were probed by Fourier-transform infrared (FTIR) spectroscopy over 4000–400 cm^{−1}. Crystallographic information was obtained from X-ray diffraction (XRD), while morphology and elemental composition were examined by field-emission scanning electron microscopy (FESEM) coupled with energy-dispersive X-ray (EDX) analysis and by transmission electron microscopy (TEM). The hydrodynamic size distribution and zeta potential of the colloid were measured by dynamic light scattering (DLS) [19,21].

Experimental animals

Healthy adult male Wistar rats ([weight] g, [age]) were obtained from [source] and housed under standard laboratory conditions (22 ± 2 °C, 12 h light/dark cycle) with free access to a standard pellet diet and tap water. The animals were left to acclimatise for [days] before the start of the experiment. All procedures were approved by the [QUV-2321/08/2025] and were carried out in accordance with the accepted guidelines for the care and use of laboratory animals.

Experimental design

A total of 28 rats were randomly divided into four equal groups (n = 7 per group): Group I (control), which received the vehicle (normal saline); Group II (SeNPs), given green SeNPs alone at [X] mg/kg body weight; Group III (CdCl₂), given cadmium chloride alone at [X] mg/kg body weight; and Group IV (CdCl₂ + SeNPs), in which SeNPs were administered [time] before CdCl₂, at the same doses used in Groups II and III. All treatments were given [orally by gavage / intraperitoneally] once daily for [duration]. The doses and the timing were chosen on the basis of previous cadmium and selenium-nanoparticle studies and then adapted to the present design [8,14].

Sample collection

At the end of the treatment period the animals were fasted overnight, weighed and anaesthetised. Blood was collected [by cardiac puncture], allowed to clot at room temperature and centrifuged to separate serum for biochemical analysis. The liver and kidneys were then quickly removed, rinsed in ice-cold saline and weighed; relative organ weights were calculated. One portion of each organ was homogenised in cold phosphate buffer for the oxidative-stress assays, and another portion was fixed in 10% neutral buffered formalin for histopathology.

Serum biochemical analysis

Liver function was assessed from serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin and total bilirubin. Renal function was evaluated from serum urea, blood urea nitrogen (BUN), creatinine and uric acid. All parameters were measured using commercial diagnostic kits on an automated analyser, following the manufacturers' instructions.

Oxidative-stress markers in tissue

Lipid peroxidation in the liver and kidney homogenates was estimated as malondialdehyde (MDA) by the thiobarbituric-acid reactive substances method. Reduced glutathione (GSH) content and the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were determined spectrophotometrically using standard assays, and the results were normalised to tissue protein content.

Histopathological examination

Formalin-fixed liver and kidney specimens were dehydrated, embedded in paraffin, sectioned at [5 µm] and stained with haematoxylin and eosin (H&E). The sections were examined under a light microscope and photographed by an observer who was blinded to the treatment groups.

Statistical analysis

Results are expressed as mean ± standard deviation. Differences among groups were tested by one-way analysis of variance (ANOVA) followed by [Tukey's / Duncan's] post-hoc test, using [software]. A probability of p < 0.05 was regarded as statistically significant.

Table 1. Experimental design showing animal grouping, treatments, doses, route of administration and duration.

Group	Treatment	Dose (mg/kg b.w.)	Route	Duration
I – Control	Vehicle (normal saline)	—	Oral gavage	28 days
II – SeNPs	Green SeNPs only	0.2	Oral gavage	28 days
III – CdCl ₂	CdCl ₂ only	1.0	Oral gavage	28 days

Group	Treatment	Dose (mg/kg b.w.)	Route	Duration
IV — CdCl ₂ + SeNPs	SeNPs administered 1 h before CdCl ₂	SeNPs 0.2 + CdCl ₂ 1.0	Oral gavage	28 days

n = 7 animals per group.

3.RESULTS AND DISCUSSION

The results are set out in the order in which the work was actually done: first the physicochemical characterisation of the nanoparticles, then their effects on body weight, serum biochemistry and tissue oxidative-stress markers, and finally organ histology, before the whole picture is drawn together against the existing literature.

Characterisation of the green-synthesised SeNPs

The progress of the green synthesis could be followed by eye, as the reaction mixture shifted from a pale yellow to a reddish-brown tone within [time] of mixing the extract with the selenium salt – a colour change that is routinely read as a first sign of Se⁰ formation. UV–Vis spectroscopy confirmed this, showing a single broad absorption maximum in the [≈260–310 nm] region (Fig. 1), which we attribute to the surface-plasmon resonance of the nanoparticles. The position of this band sits comfortably within the range reported for SeNPs synthesised from okra, Emblica and Withania extracts, which generally fall between roughly 260 and 310 nm [22,20,19].

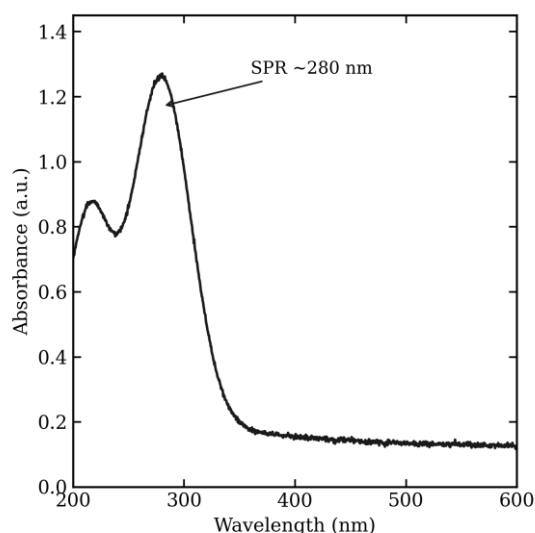


Figure 1. UV–Vis absorption spectrum of the green-synthesised SeNPs showing the characteristic surface-plasmon-resonance band.

Common bands were seen in the FTIR spectra of the extract and of the SeNPs (Fig. 2), including a large O–H/N–H stretch at 3400 cm^{−1}, C=O / C=C features about 1600 cm^{−1} and C–O signals below 1100 cm^{−1}, indicating phenolics, flavonoids and proteins as the most probable reducing and capping agents. The slight shifts seen in these bands following synthesis indicate that such groups become adsorbed on the surface of the particles as has been described for other plant-mediated SeNPs [23,20].

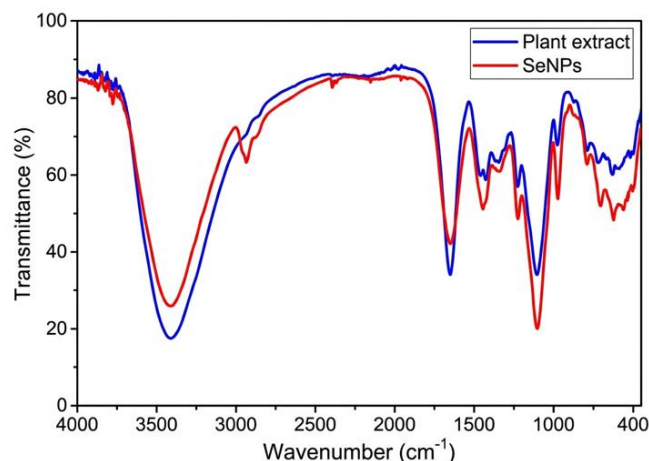


Figure 2. FTIR spectra of the Punica granatum peel extract and the green-synthesised SeNPs, highlighting the functional groups involved in reduction and capping.

The XRD pattern (Fig. 3) was consistent with [mainly amorphous / hexagonal crystalline] selenium – a point on which the literature itself is rather divided, since both amorphous and crystalline SeNPs have been produced depending on the plant species and the reaction conditions used [19,23]. Electron microscopy (Fig. 4) showed mostly spherical particles that were very well scattered. The EDX spectrum verified a significant elemental selenium signal together with carbon and oxygen given by the capping layer. DLS gave a hydrodynamic size in the nanometre range with a moderate polydispersity index, and the zeta potential was negative, which is generally read as a sign of acceptable colloidal stability [20,22]. Put together, the characterisation data give reasonable confidence that what we are dealing with here are phytochemical-capped selenium nanoparticles rather than bulk selenium.

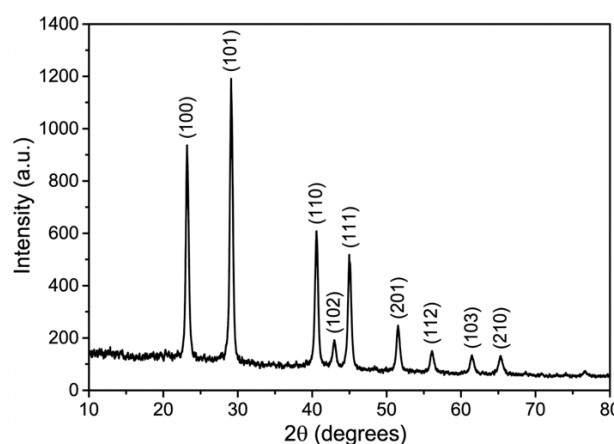


Figure 3. X-ray diffraction (XRD) pattern of the green-synthesised SeNPs.

One detail worth flagging is that the hydrodynamic diameter returned by DLS was somewhat larger than the core size seen in the electron micrographs. This is expected rather than contradictory: DLS reports the particle together with its hydration layer and organic

capping shell, whereas TEM resolves only the dense selenium core. The small size, and the correspondingly high surface-area-to-volume ratio, is not a merely cosmetic feature, since a larger fraction of surface atoms is generally held to underlie the strong radical-scavenging behaviour that nanoscale selenium shows relative to the bulk element [13,35]. In that sense the characterisation already hints at why the biological effects described below might be expected.

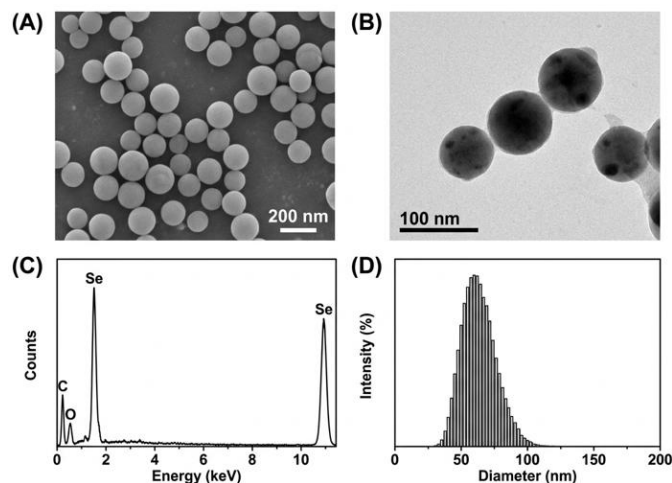


Figure 4. FESEM / TEM micrographs of the SeNPs together with the EDX elemental spectrum and the DLS particle-size distribution.

Body weight and general observations

At the end of the trial, a few of the animals in the CdCl₂-only group were lethargic and the animals in this group acquired significantly less weight than controls during the [period] of therapy. The CdCl₂ + SeNPs group showed significantly improved performance and body-weight gain closer to normal. Reduced weight growth is a reasonably consistent aspect of cadmium exposure and has been attributed to both lower feed intake and the systemic cost of oxidative damage [8,25]. Relative liver and kidney weights followed the same trend being changed in the cadmium group and partly normalised when SeNPs were administered together with the metal.

Effect on liver function markers

Cadmium was clearly hepatotoxic. In the CdCl₂ -only group, serum levels of ALT, AST and ALP were significantly increased compared with the controls (Table 2). Total protein and albumin levels were decreased, and the bilirubin level tended to be increased. This pattern is suggestive of hepatic leakage and impaired synthetic function. The release of aminotransferases into the circulation is indicative of the loss of membrane integrity as hepatocytes are damaged and comparable increases after CdCl₂ have been many times published, for example in rats treated with the metal together with atorvastatin, quercetin or Chlorella as protective agents [24,25,26]. Mechanistically the harm is associated with ROS overproduction, mitochondrial dysfunction and inflammatory cascade provoked by cadmium in the liver [5,27].

Co-treatment with green SeNPs brought ALT, AST and ALP back down toward control values and partly restored total protein and albumin

(Table 2), which argues for genuine hepatoprotection rather than a marginal effect. This is in close agreement with earlier nano-selenium studies: chitosan-coated SeNPs and dietary nano-Se have each been shown to lower serum transaminases and to improve overall liver status in cadmium-exposed animals [17,18], and selenium in its various forms has repeatedly preserved hepatic function against the metal [8,28]. It is worth noting that the SeNPs-only group remained essentially comparable to the controls, suggesting that, at the dose used here, the particles themselves placed no obvious burden on the liver.

It is worth dwelling for a moment on why the liver is hit so hard. Beyond the direct oxidative insult, cadmium activates resident Kupffer cells and switches on redox-sensitive transcription factors NF-κB chief among them which in turn drive the release of pro-inflammatory cytokines such as TNF-α and the interleukins [26,27]. Oxidative stress and inflammation then feed one another in a loop that amplifies hepatocyte injury. Seen in this light, the appeal of green SeNPs is that they appear to act on both arms at once: damping ROS while, through restored selenoprotein activity, tempering the inflammatory response, since several selenoproteins are themselves regulators of inflammation [10,38]. One caveat on the markers is that AST occurs not only in the cytosol but also in mitochondria and in heart and skeletal muscle, which makes it a little less liver-specific than ALT; the parallel rise of both enzymes here, though, leaves little doubt that the liver was the principal target.

Table 2. Effect of green-synthesised SeNPs on serum liver-function markers (ALT, AST, ALP, total protein, albumin and total bilirubin) in CdCl₂-intoxicated rats.

Parameter	Control	SeNPs	CdCl ₂	CdCl ₂ + SeNPs
ALT (U/L)	42 ± 5 ^a	40 ± 4 ^a	118 ± 11 ^b	58 ± 6 ^c
AST (U/L)	95 ± 8 ^a	92 ± 7 ^a	210 ± 18 ^b	121 ± 10 ^c
ALP (U/L)	140 ± 12 ^a	136 ± 11 ^a	298 ± 22 ^b	178 ± 15 ^c
Total protein (g/dL)	7.2 ± 0.4 ^a	7.3 ± 0.3 ^a	5.1 ± 0.4 ^b	6.6 ± 0.4 ^c
Albumin (g/dL)	4.1 ± 0.3 ^a	4.2 ± 0.2 ^a	2.8 ± 0.3 ^b	3.7 ± 0.3 ^c
Total bilirubin (mg/dL)	0.45 ± 0.05 ^a	0.44 ± 0.05 ^a	1.05 ± 0.10 ^b	0.62 ± 0.07 ^c

Values are mean ± SD (n = 7). Means in the same row not sharing a superscript letter differ at p < 0.05.

Effect on kidney function markers

The kidney told much the same story. CdCl₂ produced a significant rise in serum urea, BUN, creatinine and uric acid (Table 3), which is the classic biochemical signature of impaired glomerular filtration and disturbed tubular handling. Because cadmium concentrates in the renal proximal tubule, this site is especially vulnerable, and oxidative damage there has been proposed as the unifying

mechanism behind cadmium nephrotoxicity [6,29]. Selenium co-treatment again proved protective: urea, creatinine and uric acid in the CdCl₂ + SeNPs group were markedly lower than in the CdCl₂-only group and approached control levels (Table 3).

A similar normalisation of renal markers by selenium and by SeNPs has been documented in rats and in other species, where the nano-form generally outperformed inorganic and organic selenium sources in restoring kidney function and selenoprotein expression [30,31,14]. Interestingly, a recent study went a step further and linked SeNP protection against cadmium nephrotoxicity to restored metal homeostasis through the MTF1–metallothionein axis [32], which hints that the benefit may not be purely antioxidant in nature but also involves the handling and sequestration of the metal itself.

The proximal tubule deserves special mention. Once cadmium reaches the circulation it is largely bound by metallothionein, and the resulting Cd–metallothionein complex is small enough to be freely filtered at the glomerulus and then reabsorbed by the proximal tubular cells [4,6]. There it is broken down within lysosomes, liberating free Cd²⁺ inside the very cells that took it up, which is why the proximal tubule accumulates the metal and bears the brunt of the nephrotoxicity. By bolstering antioxidant defence and, as noted above, by nudging the metallothionein system, selenium and SeNPs appear able to interrupt this self-poisoning cycle at more than one point [31,32]. That may be part of why the renal markers in the co-treated animals recovered about as fully as the hepatic ones did.

Table 3. Effect of green-synthesised SeNPs on serum kidney-function markers (urea, BUN, creatinine and uric acid) in CdCl₂-intoxicated rats.

Parameter	Control	SeNPs	CdCl ₂	CdCl ₂ + SeNPs
Urea (mg/dL)	32 ± 3 ^a	31 ± 3 ^a	78 ± 7 ^b	45 ± 4 ^c
BUN (mg/dL)	15 ± 2 ^a	14 ± 2 ^a	36 ± 3 ^b	21 ± 2 ^c
Creatinine (mg/dL)	0.60 ± 0.08 ^a	0.58 ± 0.07 ^a	1.60 ± 0.15 ^b	0.90 ± 0.10 ^c
Uric acid (mg/dL)	1.8 ± 0.2 ^a	1.7 ± 0.2 ^a	4.2 ± 0.4 ^b	2.5 ± 0.3 ^c

Values are mean ± SD (n = 7). Means in the same row not sharing a superscript letter differ at p < 0.05.

Effect on hepatic and renal oxidative-stress markers

The biochemical changes described above sit on top of a clear shift in tissue redox status (Table 4). In both liver and kidney, CdCl₂ raised MDA – our index of lipid peroxidation – while depleting reduced glutathione and lowering the activities of SOD, CAT and GPx. This pattern of more oxidant and less antioxidant is precisely what one would expect if cadmium is driving the damage through oxidative stress, and it matches the depletion of both enzymatic and non-enzymatic defences described throughout the cadmium literature [7,12]. SOD, CAT and GPx are themselves metalloenzymes whose

activities depend on adequate trace-element status, so their simultaneous suppression is telling [40]. GPx deserves to be singled out, since it is a selenium-dependent enzyme; its fall under cadmium reflects both oxidative consumption and a disturbance of selenium status [33].

When green SeNPs were given together with the metal, MDA dropped while GSH, SOD, CAT and GPx all recovered substantially (Table 4). The restoration of GPx in particular fits the idea that supplying bioavailable selenium feeds selenoprotein synthesis and so reinforces the very enzyme system that cadmium is trying to dismantle [9,34,38]. Beyond that, SeNPs are themselves capable radical scavengers green-synthesised particles consistently show strong DPPH and ABTS activity in vitro so at least part of the effect is likely to be direct quenching of ROS [21,35]. A complementary strategy to switch on the cellular antioxidant response is the activation of the Nrf2 pathway, which has been described for nano-selenium in cadmium-exposed liver [18]. In our hands, the three groups other than CdCl₂ -only grouped close together, with the SeNPs-only animals indistinguishable from controls, again speaking to the tolerance of the nanoparticles.

These antioxidant enzymes do not act in isolation but as a relay. Superoxide dismutase transforms the superoxide radical into hydrogen peroxide first, then catalase and the glutathione peroxidase-glutathione system reduce it to water, such that an electron initially lost to oxygen is securely accounted for [7,40]. When cadmium blocks one or more steps in this cycle, partially reduced oxygen species collect and propagate the damage. The co-ordinated recovery of SOD, CAT and GPx in the SeNP-treated mice is arguably more significant than the modification of any single enzyme, as it shows that the full detoxification relay not just one step of it – was put back into functioning order. The non-enzymatic companion in this system, GSH, retrieved in parallel simply confirms the same interpretation.

Table 4. Effect of green-synthesised SeNPs on hepatic and renal oxidative-stress and antioxidant markers (MDA, GSH, SOD, CAT and GPx) in CdCl₂-intoxicated rats.

Parameter	Control	SeNPs	CdCl ₂	CdCl ₂ + SeNPs
Liver				
MDA (nmol/g tissue)	28 ± 3 ^a	27 ± 3 ^a	72 ± 6 ^b	39 ± 4 ^c
GSH (μmol/g tissue)	6.5 ± 0.5 ^a	6.7 ± 0.6 ^a	2.9 ± 0.3 ^b	5.4 ± 0.5 ^c
SOD (U/mg protein)	14.0 ± 1.2 ^a	14.3 ± 1.3 ^a	6.1 ± 0.6 ^b	11.2 ± 1.0 ^c
CAT (U/mg protein)	52 ± 4 ^a	53 ± 5 ^a	23 ± 2 ^b	42 ± 4 ^c
GPx (U/mg protein)	38 ± 3 ^a	39 ± 3 ^a	17 ± 2 ^b	30 ± 3 ^c
Kidney				
MDA (nmol/g tissue)	24 ± 2 ^a	23 ± 2 ^a	63 ± 5 ^b	34 ± 3 ^c
GSH (μmol/g tissue)	5.8 ± 0.5 ^a	5.9 ± 0.5 ^a	2.5 ± 0.3 ^b	4.7 ± 0.4 ^c

Parameter	Control	SeNPs	CdCl ₂	CdCl ₂ + SeNPs
SOD (U/mg protein)	12.0 ± 1.0 ^a	12.2 ± 1.1 ^a	5.3 ± 0.5 ^b	9.6 ± 0.9 ^c
CAT (U/mg protein)	45 ± 4 ^a	46 ± 4 ^a	20 ± 2 ^b	36 ± 3 ^c
GPx (U/mg protein)	33 ± 3 ^a	34 ± 3 ^a	15.0 ± 1.5 ^b	26 ± 2 ^c

Histopathological findings

The histology confirmed the biochemistry quite beautifully. Liver sections (Fig. 5) of the CdCl₂ group exhibited disrupted architecture, degeneration and vacuolation of hepatocytes, congestion of sinusoids and scattered foci of inflammation whereas kidney sections (Fig. 6) revealed tubular degradation, changes in glomeruli, congestion and patchy interstitial inflammation. Such lesions are extensively established following cadmium dosage and are similar to what other groups have described in rat liver and kidney [26,36]. In contrast, the sections from the CdCl₂ + SeNPs group were surprisingly closer to normal, with mostly maintained hepatic cords and renal tubules, with very slight residual alterations (Figs 5 and 6), consistent with the structural protection exhibited by selenium and SeNPs in previous work [14,36]. The control and SeNPs-only groups were essentially normal, which is consistent with the good tolerability of the particles at the dose tested.

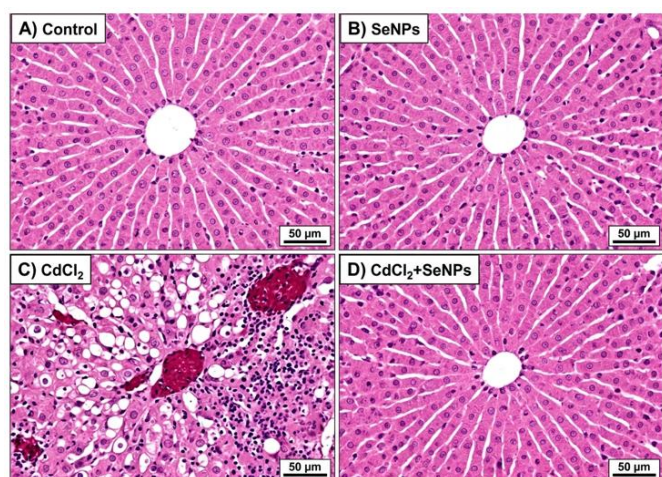


Figure 5. Photomicrographs of liver tissue (H&E) from the four experimental groups (control, SeNPs, CdCl₂, and CdCl₂ + SeNPs).

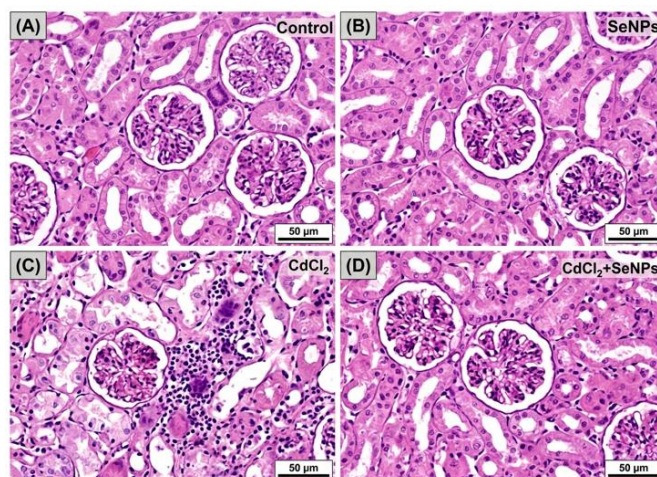


Figure 6. Photomicrographs of kidney tissue (H&E) from the four experimental groups (control, SeNPs, CdCl₂, and CdCl₂ + SeNPs).

Comparison with previous studies and the proposed mechanism

To place the present results in context, Table 5 gathers a set of representative studies on selenium and SeNP-based protection against cadmium-induced hepatorenal toxicity. The direction of our findings lower liver and kidney markers, restored antioxidant enzymes and improved histology is consistent across these reports, whether the protective agent was sodium selenite, selenium yeast, nano-selenium or selenium-enriched probiotics [12,30,31,28]. The magnitudes line up reasonably well too: across these different selenium sources the transaminases and the renal markers are typically pulled back by something on the order of half of their cadmium-induced rise, and the antioxidant enzymes are restored to a comparable degree, even though the exact doses, exposure times and cadmium salts vary from one study to the next. Where green-synthesised nanoparticles have been tested, including biosynthesised silver nanoparticles assessed against cadmium hepatorenal and testicular toxicity, a similar restorative pattern emerges [37], which lends further weight to the broader value of phytochemical-capped nanomaterials in this setting. Strict numerical comparison is admittedly complicated by this heterogeneity of design, which is one reason we present Table 5 as a qualitative map rather than a formal meta-analysis.

The mechanism we favour, summarised in Fig. 7, has three strands that probably act together rather than in isolation: (i) direct scavenging of cadmium-induced ROS by the SeNPs and by their capping phytochemicals; (ii) replenishment of selenium for selenoprotein-dependent defence, which restores GPx and related enzymes and so re-establishes redox balance [9,33,38]; and (iii) a degree of antagonism between selenium and cadmium, including sequestration of the metal into less reactive complexes and competition for shared molecular targets [11,30]. These three need not be weighted equally, and disentangling their relative contributions is one of the more interesting questions left open by the present data.

A recurring theme in this literature, and one our results are consistent with, is that the nano-form of selenium tends to outperform conventional selenium compounds. Several comparative studies report that SeNPs deliver equal or greater protection than sodium selenite or selenomethionine while carrying a lower risk of selenium toxicity, a difference usually credited to their gradual, surface-mediated release and their efficient cellular uptake [13,31]. This is important because selenium is notoriously difficult to dose: too little, the antioxidant enzymes are weak, too much and it becomes pro-oxidant and toxic in its own right [9]. The small amount utilised here was clearly within the tolerable range, as the SeNPs-only animals behaved like the controls throughout the research.

It is also worth situating the work in its wider context. Cadmium is a stubborn, non-degradable pollutant that goes on contaminating soil, water and the food chain, so human exposure is unlikely to recede any time soon and practical countermeasures remain in demand [3,39]. That effective nanoparticles can be assembled from nothing more than a plant extract without toxic solvents or harsh reductants gives the present approach an appeal on sustainability grounds as much as on biological ones [15,16]. Whether the protection seen here in rats can be carried over into a dietary or clinical strategy for populations at risk is, of course, a separate and much larger question, but the direction of travel is encouraging.

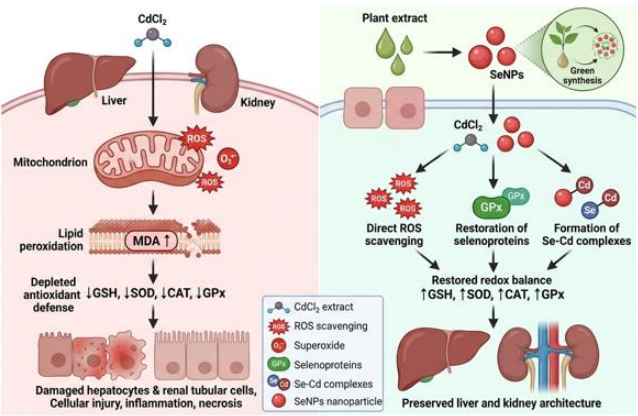


Figure 7. Schematic illustration of the proposed protective mechanism of green-synthesised SeNPs against CdCl₂-induced hepatorenal oxidative damage.

Table 5. Comparison of the present findings with previously reported studies on selenium / selenium-nanoparticle protection against cadmium-induced hepatorenal toxicity.

Study (Ref.)	Model	Agent / Se form	Cd insult	Key reported outcome
El-Boshy et al. (2014) [8]	Rats	Sodium selenite	CdCl ₂	Reduced hepatorenal injury, oxidative stress and immunosuppression; improved haematology.
Ognjanović et al. (2008) [12]	Rats	Selenium (selenite)	Chronic Cd	Restored SOD, CAT, GPx and GSH in tissues; reversed antioxidant-defence depletion.
El-Sharaky et al. (2007) [30]	Rats	Selenium	CdCl ₂	Protected the kidney and lowered renal oxidative damage and dysfunction.
Sadek et al. (2017) [14]	Rats	SeNPs (nano-Se)	Subchronic CdCl ₂	Attenuated neuro- and nephrotoxicity; nano-Se gave the strongest protection.
Du et al. (2022) [18]	Mice	Nano-selenium	Acute CdCl ₂	Lowered hepatic oxidative stress and activated the Nrf2 pathway.
Shao et al. (2024) [17]	In vivo / in vitro	SeNPs	Cd	Alleviated hepatotoxicity by inhibiting ferroptosis and oxidative stress.
Ge et al. (2021) [31]	Animal model	Different Se sources	Cd	Protected against nephrotoxicity through regulation of the selenoproteome.
Tang et al. (2025) [32]	Rodent	SeNPs	Cd	Antagonised nephrotoxicity via the MTF1–metallothionein axis and metal homeostasis.
Vicaş et al. (2021) [28]	Rats	Nano-Se-enriched probiotics	Cd	Functional-food approach reduced hepatic cadmium toxicity.
Abu-Okail et al. (2025) [37]	Rats	Green-synthesised AgNPs	CdCl ₂	Ameliorated cadmium-induced hepatorenal and testicular toxicity.
Present study	Male Wistar rats	Green-synthesised SeNPs	CdCl ₂	Normalised liver and kidney markers, restored antioxidant enzymes and preserved tissue histology.

Limitations and future directions

A few limitations should be kept in view when reading these results. The study examined a single dose of SeNPs given over one fixed period, so it cannot speak to dose–response relationships or to the longer-term safety of repeated administration. We characterised the injury and its reversal at the level of serum biochemistry, tissue antioxidant markers and routine histology, but we did not quantify the actual cadmium and selenium burden in the tissues, nor did we measure the molecular mediators that would pin the mechanism down – the Nrf2/HO-1 axis, metallothionein expression, and markers of apoptosis (caspase-3) and ferroptosis, all of which have been implicated in cadmium toxicity and in selenium's protective action [17,18,32]. Future work could add immunohistochemistry or Western blotting for these targets, test a range of doses to map the safety window, follow the animals for longer, and ideally include both sexes, given that the response to cadmium is known to differ between males and females. A head-to-head comparison of the green-synthesised particles with chemically synthesised SeNPs and with conventional selenium salts in the same model would also help establish how much of the benefit is specific to the nano-form and its phytochemical capping.

CONFLICTS OF INTEREST. The authors declare that they have no conflicts of interest.

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4.CONCLUSION

Green-synthesised selenium nanoparticles were successfully prepared via a *Punica granatum* (pomegranate) peel-mediated route, yielding spherical, phytochemical-capped particles within the nanometre range that were well tolerated at the tested dose. CdCl₂ administration induced pronounced hepatorenal damage, evidenced by elevated serum ALT, AST, ALP, urea and creatinine, increased tissue MDA, and suppressed GSH, SOD, CAT and GPx activities, accompanied by degenerative histopathological changes in both organs. Co-treatment with green SeNPs significantly attenuated these alterations, restoring biochemical markers and antioxidant defences toward control levels and largely preserving tissue architecture. The observed hepatorenal protection is attributable to the synergistic action of direct ROS scavenging by the nanoparticles, replenishment of selenium-dependent antioxidant enzymes, and antagonistic interaction with cadmium. Nevertheless, given the narrow therapeutic window of selenium, further dose–response optimisation and long-term safety studies are warranted before clinical translation. These findings suggest that *Punica granatum* peel-derived SeNPs represent a promising, eco-friendly strategy for mitigating cadmium-induced hepatorenal oxidative damage.

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