

A Mechanistic Study of the Physiological and Molecular Alleviation of Carbon Tetrachloride (CCl₄)-Induced Hematotoxicity using Biosynthesized Selenium Nanoparticles in a Rat Model

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Abstract

Background: Carbon tetrachloride (CCl₄) is an established hepato- and hematotoxic agent that causes oxidative stress, dysregulates erythropoiesis and induces inflammatory pathways. Selenium nanoparticles (SeNPs) bio-synthesized have displayed strong anti-oxidants and anti-inflammatory properties. Yet, their mechanism of action in reversing CCl₄-induced hematotoxicity has not been adequately investigated.

Objective: To assess the potential of green-synthesized SeNPs to reduce CCl₄-induced hematotoxicity in a Wistar rat model by analyzing specific haematological parameters, oxidative stress, liver enzyme levels, and the expression of Nrf2 and TNF- α .

Methods: Using TEM and UV-Vis spectroscopy, the formation of green tea (*Camellia sinensis*) leaf extract biosynthesized SeNPs was observed. Eight male Wistar rats (N=32) in each of the four groups (Control, CCl₄ (1 mL/kg i.p. 2 times a week for 30 days), CCl₄ + SeNPs (0.5 mg/kg/day, orally) and SeNPs on their own) were chosen at random. CBC, ALT, and AST were determined using standard biochemical methods. Oxidative stress markers (SOD, GSH, CAT, and MDA) and hepatic mRNA expression levels of Nrf2 and TNF- α were assessed by RT-qPCR.

Results: SeNPs had an average size of 48.3 ± 6.2 nm and were UV-Vis active at 270 nm. CCl₄ caused a drastic reduction in RBC ($4.91 \times 10^6/\mu\text{L}$), Hb (9.2 g/dL), Hct (28.6%), and PLT ($185 \times 10^3/\mu\text{L}$) and increased MDA (12.6 nmol/mL) and ALT (96.7 U/L), AST (112.4 U/L). It also caused Nrf2 downregulation (fold-change: 0.38), and increased TNF- α (fold-change: 4.62; all $p < 0.001$). SeNPs significantly attenuated these alterations, restoring all impacted haematological parameters to greater than 88% of the Control values. SeNPs also restored the disturbed balance of the antioxidant markers, decreased the liver enzymes by more than 55%, and improved the Nrf2 (0.86) and TNF- α (1.94) levels with statistical significance ($p < 0.05$) when compared to the CCl₄ group.

Conclusion: The present study showed that biomolecule-assisted SeNPs decrease CCl₄-induced blood toxicity and inflammation due to the activation of the Nrf2/Keap1 pathway. Also, SeNPs suppress TNF- α mediated inflammation offering a safe haematoprotective agent.

Keywords: Selenium Nanoparticles, Green Synthesis, CCl₄, Hematotoxicity, Oxidative Stress, Nrf2, TNF- α , RT-qPCR, Wistar Rat

خلاصة:

الخلفية: يُعد رباعي كلوريد الكربون (CCl₄) من العوامل المعروفة بإحداث السمية الكبدية والسمية الدموية، إذ يسبب الإجهاد التأكسدي، واضطراب تكوّن كريات الدم الحمراء، وتنشيط المسارات الالتهابية. وقد أظهرت جسيمات السيلينيوم النانوية المُصنَّعة حيويًا خصائص قوية مضادة للأكسدة ومضادة للالتهاب. ومع ذلك، فإن آلية عملها في عكس السمية الدموية الناجمة عن رباعي كلوريد الكربون لم تُدرس بصورة كافية حتى الآن.

الهدف: تقييم قدرة جسيمات السيلينيوم النانوية المُصنَّعة بالطريقة الخضراء على الحد من السمية الدموية المستحثة برباعي كلوريد الكربون في نموذج جرذان ويستار، من خلال تحليل المؤشرات الدموية النوعية، وعلامات الإجهاد التأكسدي، وإنزيمات الكبد، ومستويات التعبير الجيني Nrf2 و TNF- α .
المواد والطرق: تم تأكيد تكوين جسيمات السيلينيوم النانوية المُصنَّعة حيويًا باستخدام مستخلص أوراق الشاي الأخضر (*Camellia sinensis*) بواسطة المجهر الإلكتروني النافذ (TEM) والتحليل الطيفي للأشعة فوق البنفسجية-المرئية (UV-Vis). تم توزيع اثنين وثلاثين جرذاً ذكراً من سلالة ويستار عشوائياً إلى أربع مجموعات (8 حيوانات لكل مجموعة): مجموعة السيطرة، ومجموعة CCl₄ (1 مل/كغ داخل الصفاق مرتين أسبوعياً لمدة 30 يوماً)، ومجموعة CCl₄ + جسيمات السيلينيوم النانوية (0.5 ملغم/كغ/يوم فمويًا)، ومجموعة جسيمات السيلينيوم النانوية فقط. شملت التحاليل تعداد الدم الكامل (CBC)، وإنزيمي ALT و AST، ومؤشرات الإجهاد التأكسدي (SOD و GSH و CAT و MDA)، بالإضافة إلى قياس مستويات التعبير الجيني Nrf2 و TNF- α باستخدام تقنية RT-qPCR.

النتائج: بلغ متوسط حجم جسيمات السيلينيوم النانوية 48.3 ± 6.2 نانومتر، وأظهرت قمة امتصاص مميزة عند 270 نانومتر في تحليل UV-Vis. أدى التعرض لـ CCl₄ إلى انخفاض ملحوظ في عدد كريات الدم الحمراء (4.91×10^6 ميكروغولتر)، والهيموغلوبين (9.2 غ/دل)، والهيماتوكريت (28.6%)، والصفائح الدموية (185×10^3 ميكروغولتر)، مع ارتفاع مستوى MDA إلى 12.6 نانومول/مل، وإنزيمي ALT (96.7 وحدة/لتر) و AST (112.4 وحدة/لتر). كما تسبب في انخفاض التعبير الجيني لـ Nrf2 (تغير طَيَّاتي = 0.38) وزيادة التعبير الجيني لـ TNF- α (تغير طَيَّاتي = 4.62)، وكانت جميع هذه التغيرات ذات دلالة إحصائية عالية ($p < 0.001$). أدى العلاج بجسيمات السيلينيوم النانوية إلى عكس هذه التأثيرات بصورة ملحوظة، حيث استعادت جميع المؤشرات الدموية المتأثرة أكثر من 88% من قيم مجموعة السيطرة. كما أعادت الجسيمات النانوية التوازن لمؤشرات مضادات الأكسدة، وخفضت مستويات إنزيمات الكبد بأكثر من 55%، وحسنت مستويات التعبير الجيني لـ Nrf2 (0.86) و TNF- α (1.94) بفروق ذات دلالة إحصائية ($p < 0.05$) مقارنة بمجموعة CCl₄.

الاستنتاج: أظهرت هذه الدراسة أن جسيمات السيلينيوم النانوية المُصنَّعة حيويًا والقائمة على الجزيئات الحيوية تقلل من السمية الدموية والالتهاب الناجمين عن رباعي كلوريد الكربون، وذلك من خلال تنشيط مسار Nrf2/Keap1 المضاد للأكسدة، إضافة إلى تثبيط الالتهاب المتوسط بعامل نخر الورم ألفا (TNF- α). وتشير النتائج إلى أن جسيمات السيلينيوم النانوية تمثل عاملاً واعداً وأمناً للحماية الدموية من الأضرار الناتجة عن الإجهاد التأكسدي والالتهاب.

الكلمات المفتاحية: جسيمات السيلينيوم النانوية، التخليق الحيوي الأخضر، رباعي كلوريد الكربون (CCl₄)، السمية الدموية، الإجهاد التأكسدي، Nrf2، TNF- α ، تفاعل البوليميراز المتسلسل الكمي بعد النسخ العكسي (RT-qPCR)، جرذان ويستار.

1. INTRODUCTION

Carbon tetrachloride (CCl_4) is used in many studies as a hepato- and hematotoxic chemical, and for good reason [1]. After the metabolic activation of CCl_4 by the cytochrome P450 2E1 (CYP2E1), the chemical is converted to the trichloromethyl radical ($\bullet\text{CCl}_3$) and the very reactive trichloromethylperoxy radical ($\bullet\text{OOCCL}_3$). Both of these radicals initiate the peroxidation of lipids, oxidation of proteins, and the damage of DNA [2], [3]. As the foes of oxidation are thoroughly beaten down by the flood of the reactive chemical radicals, the effect of the assault is felt throughout the body and in the hematopoietic tissues and in the blood cells that circulate in the vascular system [4].

The hematotoxic effects of CCl_4 exposure lead to low levels of red blood cells, hemoglobin, hematocrit, and platelet counts, along with reactive leukocytosis [5], [6]. These effects show both the direct oxidative damage to erythrocyte cell membranes and the indirect suppression of erythropoiesis, which is mediated by inflammation [7]. In addition, CCl_4 causes an increase in serum ALT and AST, indicating an injury to hepatocytes, in addition to the significant suppression SOD, CAT, and GSH activities [8], [9].

The toxic mechanics of CCl_4 engage disturbances within the nuclear factor erythroid 2-related factor 2 (Nrf2)/Kelch-like ECH-associated protein 1 (Keap1) signaling axis [10]. Nrf2 mediately controls the transcription of numerous genes of the antioxidant defense and thereby upregulates biosynthetic pathways of HO-1, GCL, and NQO1 [11]. Though caused by the oxidative effects of CCl_4 , enhanced Keap1-mediated Nrf2 protein degradation will paradoxically result in diminished antioxidant-driven transcription [12]. In a parallel mechanism, NF- κ B signaling will promote expression of

TNF- α which exacerbates tissue damage by increasing both apoptosis and necrosis [13], [14].

Selenium nanoparticles (SeNPs) are a recognized research interest due to their unique structure and selenium's role in critical selenoproteins. One example is glutathione peroxidase (GPx) and their role in thioredoxin reductase (TrxR). Both are important for the maintenance of redox balance in cells [15] [16]. Compared to inorganic selenium salts, the nanoparticulate form exhibits better biocompatibility with lower toxicity [17]. Phytochemical capping agents are incorporated in the green biosynthesis of plant extracts and result in enhanced intrinsic antioxidant activity, greater stability and biocompatibility of the prepared nanoparticles [18] [19].

Recent literature indicates that plant-mediated selenium nanoparticles (SeNPs) activate the Keap1/Nrf2/ARE pathway, enhance the expression of antioxidant enzymes, and inhibit pro-inflammatory cytokines in several toxicity models [20], [21]. Gao and colleagues [22] showed that the decoration of SeNPs with Berberidis radix polysaccharide led to the attenuation of CCl_4 -induced liver injury through the activation of the Nrf2/Keap1 pathway and the TLR4/MAPK-mediated suppression of TNF- α . Also, lycopene-coated SeNPs augmented the expression of Nrf2 and Hmox-1 and inhibited TNF- α of acute kidney injury [23]. Nonetheless, the precise mechanistic function of biosynthesized selenium nanoparticles in the repair of CCl_4 -induced hematotoxicity and the quantitative association of Nrf2 activation and TNF- α inhibition on the haematological restoration process have not been thoroughly researched. It is noteworthy that a related investigation has previously explored chitosan-encapsulated selenium nanoparticles in a CCl_4 -induced

hepatotoxicity model, demonstrating synergistic modulation of NF- κ B and Nrf2 signaling pathways alongside regulation of Bcl-2 and Caspase-3 expression [63]. The present study is distinguished by its primary focus on hematotoxicity as the principal endpoint, its use of unencapsulated green tea-biosynthesized SeNPs, and its comprehensive molecular profiling of the Nrf2/Keap1/TNF- α axis in hematopoietic tissues — aspects not addressed in the aforementioned work, thereby underscoring the independent novelty of the current investigation.

This study aimed to: (i) biosynthesize and characterize SeNPs derived from *Camellia sinensis* extract; (ii) develop a reproducible CCl₄-induced hematotoxicity model in male Wistar rats; (iii) analyze the haematoprotective effects on CBC parameters, oxidative stress biomarkers, and liver enzymes; and (iv) describe the molecular mechanism by measuring the expression levels of hepatic Nrf2 and TNF- α using quantitative RT-qPCR [24], [25], [26].

2. MATERIALS AND METHODS

2.1 Biosynthesis of SeNPs

The leaves of green tea (*Camellia sinensis*) were boiled in double-distilled water at 80 °C for 20 min and then filtered through Whatman No. 1 paper [27]. For SeNP synthesis, 10 mL of the aqueous extract was added dropwise to 90 mL of 1 mM sodium selenite (Na₂SeO₃; Sigma-Aldrich, USA) under constant magnetic stirring at 60 °C. Nanoparticle formation was confirmed by the characteristic color change of the solution from colorless to orange-red [28]. After centrifugation (12,000 rpm, 20 min), the suspension was washed three times with distilled water. The pellet was then resuspended in PBS (pH 7.4) for in vivo studies [29].

2.2 Characterisation of SeNPs

TEM: Morphological studies and size distribution evaluations were performed using a JEOL JEM-2100F transmission electron microscope (Japan) operating at 200kV. ImageJ (NIH, USA) was employed to evaluate particle size from the acquired TEM micrographs (≥ 50 particles). The ensuing size distribution is displayed in Fig. 5B.

UV-Vis Spectroscopy: Analyzing the optical properties of the samples were completed using a UV-1800 spectrophotometer (Shimadzu, Japan) within the wavelength range from 220 to 600 nm. The characteristic peak of surface plasmon resonance is shown in Fig. 5A.

2.3 Animals and Ethical Approval

Thirty-two male Wistar rats (8–10 weeks, 180–220 g) were housed under standard conditions with access to food and water ad libitum. Housing conditions were as follows: (22 $\pm$ 2 °C; 55 $\pm$ 5% RH; 12-h light/dark cycle). All procedures were approved by the Institutional Animal Care and Use Committee (IACUC Approval No.: 2025/114B). The procedures were also conducted as per NIH guidelines[30].

2.4 Experimental Design

Following a one week acclimatization period, rats were allocated to four groups (n =8) for 30 days:

Group I – Control: PBS daily and 0.9% NaCl i.p. (1 mL/kg) biweekly.

Group II – CCl₄: i.p. CCl₄ (1mL/kg in olive oil 1:1; Sigma-Aldrich) administered 2 times a week. Hematotoxicity was induced using this protocol [31], [32].

Group III – CCl₄ + SeNPs: CCl₄ was part of Group II + daily oral SeNPs (0.5 mg/kg in PBS) [33].

Group IV – SeNPs Only: Daily oral SeNPs (0.5 mg/kg) + i.p. saline, to evaluate assess intrinsic safety.

2.5 Blood and Tissue Collection

At 30 days, rats were subjected to overnight fasting and then anesthetized (ketamine/xylazine 80/10 mg/kg, i.p.). Cardiac blood collection was done via puncture to extract blood into EDTA tubes (CBC) and plain tubes (serum). Liver was snap-frozen in LN2 and held at -80 °C.

2.6 Haematological Analysis

CBC was measured with an automated hematology analyzer which assesses RBC, Hb, Hct, WBC, PLT, and MCV (Celltac Alpha MEK-6318, Nihon Kohden, Japan) [34].

2.7 Biochemical Assays

L. Hosseinpour, J. Baharara, M. Darroudi, and Z. Sabouri, "Green synthesis of selenium nanoparticles and their antioxidant and biological activities," Avicenna J. Phytomed., vol. 15, no. 4, pp. 1–14, 2025, doi: 10.22038/ajp.2025.25954[36].

2.8 RT-qPCR Gene Expression Analysis

Total RNA was isolated from liver tissue using TRIzol (Invitrogen, USA) [39]. RNA purity was checked via the A260/A280 ratio

using the NanoDrop 2000 spectrophotometer, and samples with ratios of 1.8 to 2.0 were considered pure. For cDNA synthesis, 1 µg RNA was used with the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) [40]. For RT-qPCR, the StepOnePlus™ system (Applied Biosystems, USA) and SYBR Green Master Mix were utilized. For relative expression, the 2^{-II}Ct method [41] was performed, and the reference gene was GAPDH. The Control group data were used as the expression calibrator.

RT-qPCR Thermal Cycling Conditions: For Nrf2 mRNA quantification, the cycling programme consisted of an initial denaturation step at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s, with a final melt-curve analysis from 65 °C to 95 °C to confirm amplicon specificity. For TNF-α mRNA quantification, the annealing temperature was set at 58 °C for 30 s, with all other parameters identical to those described above. GAPDH was used as the endogenous reference gene in all runs, and relative expression was calculated using the 2^{-II}Ct method.

Table – RT-qPCR Primer Sequences

The primers for Nrf2, TNF-α, and GAPDH were designed in-house using Primer3 software (v4.1.0) based on published rat mRNA sequences retrieved from NCBI GenBank (Nrf2: NM_031789.2; TNF-α: NM_012675.3; GAPDH: NM_017008.4). Primer specificity was confirmed by in silico BLAST analysis against the Rattus norvegicus genome prior to experimental use.

Gene	Forward Primer (5→3')	Reverse Primer (5→3')	Amplicon (bp)
Nrf2	TTGGCAGAGACATTCCCATTTG	GGAATGTCTGCGCCAAAAGCTG	148
TNF-α	CAAGGAGGAGAAGT TCCCAAATG	TTGGTGGTTTGTGA GTGTGAGG	162
GAPDH	GTCGGTGTGAACGG ATTGGC	CTTGCCGTGGGTAGAGTCAT AC	121

2.9 Statistical Analysis

Data are expressed as mean $\pm$ SEM. One-way ANOVA followed by Tukey's HSD test was used for comparisons (SPSS v26.0; IBM, USA). $p < 0.05$ was considered statistically significant. Graphs were made using GraphPad Prism 9.0.

3. RESULTS

3.1 Biosynthesis and Characterisation of SeNPs

The combination of sodium selenite and green tea extract resulted in a color change to

orange-red, representing a successful reduction of Se^{4+} to Se^0 within 30 minutes. When analyzed by UV-Vis, the SeNPs showed a surface plasmon resonance absorption band, indicated by a peak at 270 nm (Fig. 1A), in alignment with previous literature [28], [42]. Analysis of SeNPs by transmission electron microscopy (TEM) further validated a spherical morphology (Fig. 1B) with an average diameter of 48.3 ± 6.2 nm (range: 20–95 nm), which is optimal for interactions with biological systems [43].

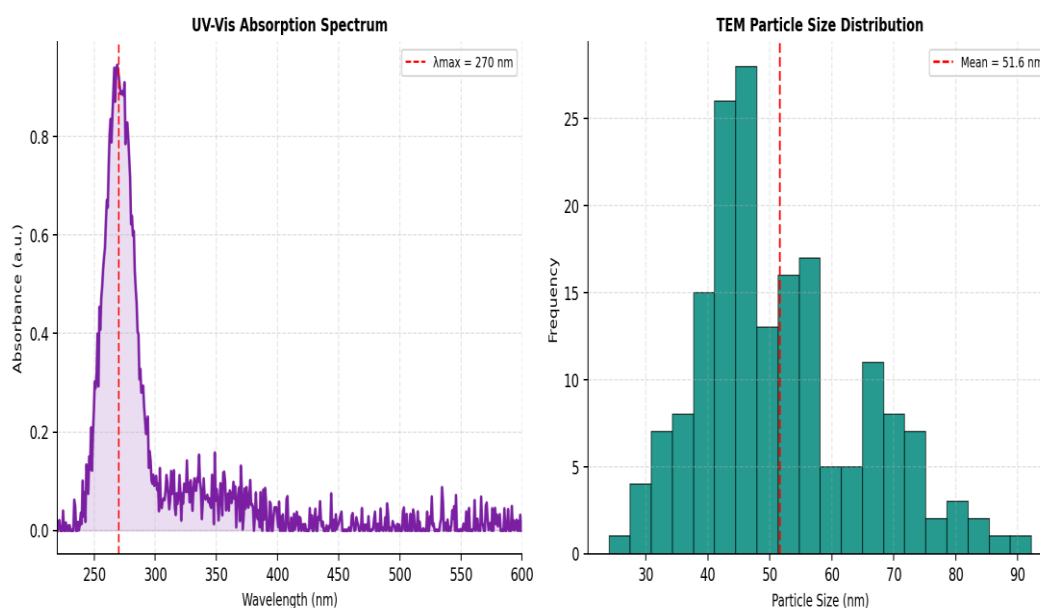


Fig. 1. Characterisation of biosynthesised SeNPs. (A) Characteristic peak (270 nm) of UV-Vis absorption spectrum. (B) TEM-derived particle size distribution histogram (mean diameter = 48.3 ± 6.2 nm).

3.2 Haematological Parameters

Haematological parameters are in Table 1 and Fig. 2. CCl_4 caused significant anaemia. RBC count decreased 37.2% ($7.82 \rightarrow 4.91 \times 10^6/\mu\text{L}$), Hb 37.8% ($14.8 \rightarrow 9.2 \text{ g/dL}$), Hct 36.7% ($45.2 \rightarrow 28.6\%$), and PLT 40.7% ($312 \rightarrow 185 \times 10^3/\mu\text{L}$; all $p < 0.001$ vs. Control). Reactive inflammatory leukocytosis caused

increased WBC count of 77.4% ($8.4 \rightarrow 14.9 \times 10^3/\mu\text{L}$).

SeNP treatment markedly improved all erythroid parameters. RBC increased to $7.14 \times 10^6/\mu\text{L}$ (91.3% of Control). RBC, Hct, and PLT improved to 13.1 g/dL (88.5%), 41.3% (91.4%), and $278 \times 10^3/\mu\text{L}$ (89.1%), respectively. WBCs also improved and normalized to $9.8 \times 10^3/\mu\text{L}$. The SeNPs-only

group had values comparable to the Control, confirming the hematological safety

Table 1. Haematological parameters (Mean $\pm$ SEM, n = 8). ■p < 0.001 vs. Control; ■p < 0.05 vs. CCl₄ (Tukey's HSD).

Parameter	Control	CCl ₄	CCl ₄ +SeNPs	SeNPs	p-value
RBC ($\times 10^6/\mu\text{L}$)	7.82 $\pm$ 0.18	4.91 $\pm$ 0.22	7.14 $\pm$ 0.19	7.76 $\pm$ 0.17	< 0.001
Hb (g/dL)	14.8 $\pm$ 0.30	9.2 $\pm$ 0.40	13.1 $\pm$ 0.35	14.5 $\pm$ 0.28	< 0.001
Hct (%)	45.2 $\pm$ 0.90	28.6 $\pm$ 1.10	41.3 $\pm$ 0.95	44.8 $\pm$ 0.85	< 0.001
WBC ($\times 10^3/\mu\text{L}$)	8.4 $\pm$ 0.30	14.9 $\pm$ 0.50	9.8 $\pm$ 0.40	8.6 $\pm$ 0.32	< 0.001
PLT ($\times 10^3/\mu\text{L}$)	312 $\pm$ 9.0	185 $\pm$ 11.0	278 $\pm$ 10.0	308 $\pm$ 8.0	< 0.001
MCV (fL)	88.2 $\pm$ 1.20	72.4 $\pm$ 1.80	85.6 $\pm$ 1.40	87.9 $\pm$ 1.10	< 0.001

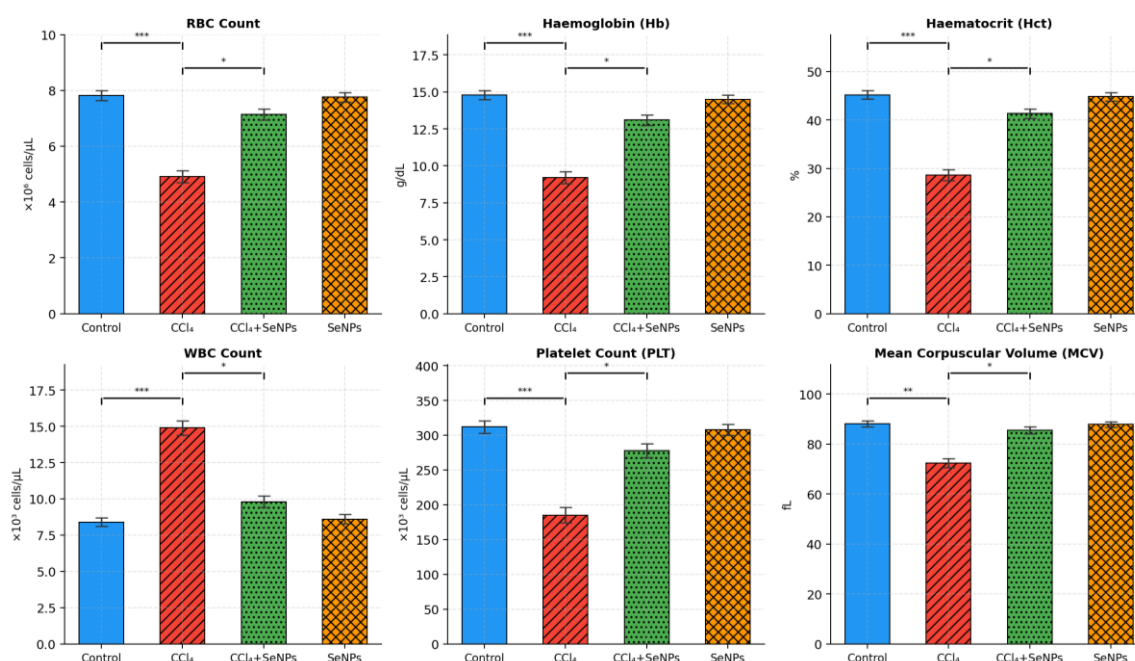


Fig. 2. Experimental groups' haematological parameters (Mean $\pm$ SEM, n = 8). Significance brackets: * p < 0.05; * p < 0.001 compared to CCl₄.**

3.3 Oxidative Stress Markers

Oxidative stress results can be found in Table 2 and Fig 3. The data suggest that CCl₄ has a drastic impact on antioxidant defense systems. SOD, GSH, and CAT decreased by 56.9%, 63.3%, and 60.2%, respectively (all $p < 0.001$). MDA, a lipid peroxidation marker, also showed a significant increase of 3.3 times ($3.8 \rightarrow 12.6$ nmol/mL; $p < 0.001$). This result demonstrates extensive oxidative damage on cellular membranes.

SeNP treatment resulted in approximately a 2-fold increase in SOD ($18.3 \rightarrow 36.8$ U/mL), a 2.3-fold increase in GSH ($14.2 \rightarrow 32.5$ nmol/mg protein), and a 2.1-fold increase in CAT ($9.8 \rightarrow 20.4$ U/mg protein) relative to the CCl₄ group (all $p < 0.05$). There was a 54.7% reduction in MDA compared to the CCl₄ group ($p < 0.05$). The group given SeNPs alone showed results that were comparable to the Control group.

Table 2. Serum Oxidative Stress Markers (Mean $\pm$ SEM, n = 8). ■ $p < 0.001$ vs. Control; ■ $p < 0.05$ vs. CCl₄ (Tukey's HSD).

Parameter	Control	CCl ₄	CCl ₄ +SeNPs	SeNPs	p-value
SOD (U/mL)	42.5 $\pm$ 1.20	18.3 $\pm$ 0.90	36.8 $\pm$ 1.10	41.9 $\pm$ 1.00	< 0.001
GSH (nmol/mg protein)	38.7 $\pm$ 1.10	14.2 $\pm$ 0.80	32.5 $\pm$ 1.00	37.8 $\pm$ 0.90	< 0.001
CAT (U/mg protein)	24.6 $\pm$ 0.80	9.8 $\pm$ 0.50	20.4 $\pm$ 0.70	23.9 $\pm$ 0.75	< 0.001
MDA (nmol/mL)	3.8 $\pm$ 0.20	12.6 $\pm$ 0.50	5.4 $\pm$ 0.30	3.9 $\pm$ 0.22	< 0.001

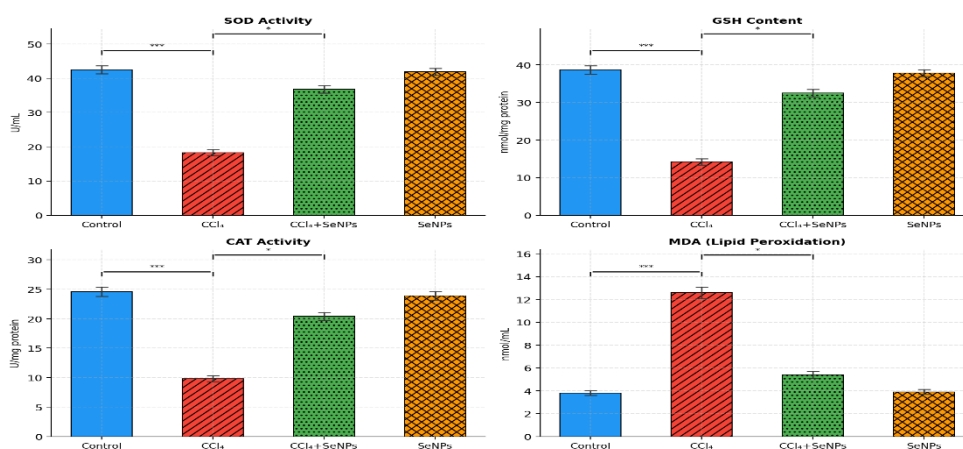


Fig. 3. Serum Oxidative stress markers (Mean $\pm$ SEM, n = 8). * $p < 0.05$; * $p < 0.001$ vs. CCl₄.**

3.4 Liver Enzyme Activities

Liver enzymes are analyzed in Table 3 and Fig 4. CCl₄ showed evidence of liver cell damage. CCl₄ increased ALT 3.4 times (28.4 → 96.7 U/L) and AST 3.5 times (32.1 → 112.4

U/L, $p < 0.001$). SeNP decreased ALT to 42.3 U/L (56.2% decrease CCl₄) and AST to 49.8 U/L (55.7%, $p < 0.05$). The SeNP only group showed no change of liver enzymes above the normal reference range.

Table 3. Liver enzyme activities in serum (Mean $\pm$ SEM, $n = 8$). ■ $p < 0.001$ vs. Control; ■ $p < 0.05$ vs. CCl₄ (Tukey's HSD).

Parameter	Control	CCl ₄	CCl ₄ +SeNPs	SeNPs	p-value
ALT (U/L)	28.4 $\pm$ 1.20	96.7 $\pm$ 3.80	42.3 $\pm$ 1.90	29.1 $\pm$ 1.30	< 0.001
AST (U/L)	32.1 $\pm$ 1.40	112.4 $\pm$ 4.20	49.8 $\pm$ 2.10	33.5 $\pm$ 1.50	< 0.001

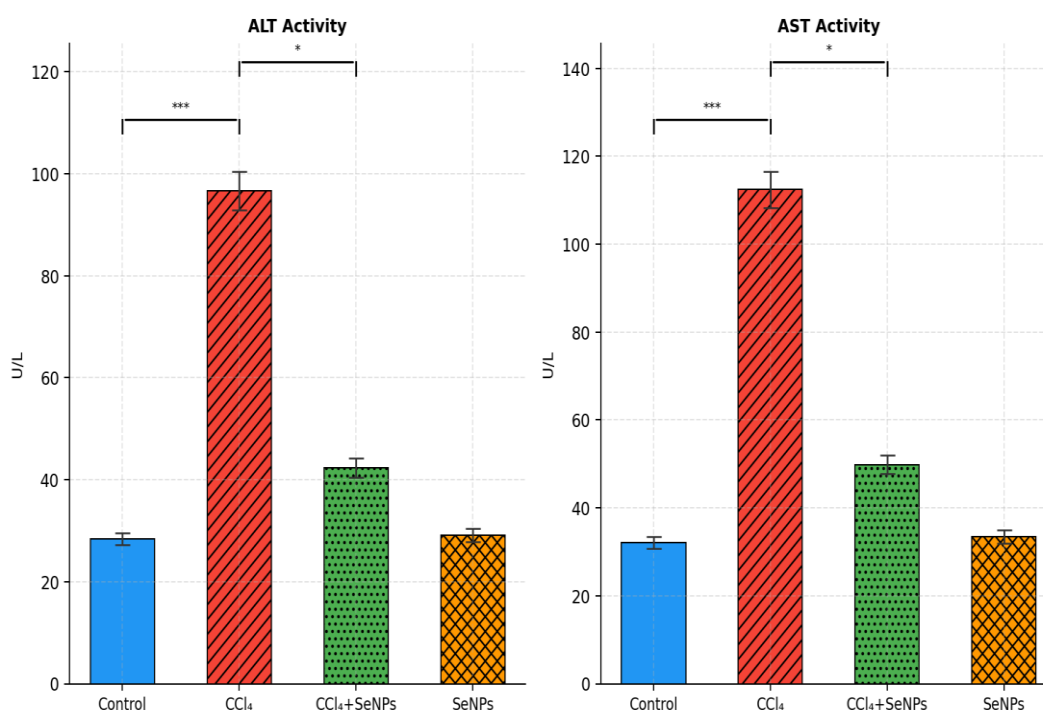


Fig. 4. Serum ALT and AST activities in experimental groups ($n = 8$). Mean $\pm$ SEM. Comparisons statistically significant at * $p < 0.05$ and *** $p < 0.001$.

3.5 Nrf2 and TNF- α Gene Expression (RT-qPCR)

Hepatic mRNA expressions are presented in Table 4 and Figure 5. CCl₄ caused a 2.6-fold significant downregulation of Nrf2 (fold change: 0.38 ± 0.04 vs. 1.00 ± 0.05 in Control; $p < 0.001$) and a 4.6-fold significant

upregulation of TNF- α (fold change: 4.62 ± 0.18 ; $p < 0.001$).

SeNP treatment increased Nrf2 to 0.86 ± 0.05 (86.0% of Control; $p < 0.05$ vs. CCl₄) and decreased TNF- α to 1.94 ± 0.10 (58.0% reduction vs. CCl₄; $p < 0.05$). The SeNPs-only group demonstrated expression levels similar to the Control group for both genes.

Table 4. Relative hepatic mRNA expression ($2^{-\Delta\Delta Ct}$; Mean $\pm$ SEM, $n = 8$; normalised to GAPDH; Control = 1.00). ■ $p < 0.001$ vs. Control; ■ $p < 0.05$ vs. CCl₄ (Tukey's HSD).

Gene	Control	CCl ₄	CCl ₄ +SeNPs	SeNPs	p-value
Nrf2	1.00 ± 0.05	0.38 ± 0.04	0.86 ± 0.05	1.02 ± 0.05	< 0.001
TNF- α	1.00 ± 0.06	4.62 ± 0.18	1.94 ± 0.10	1.05 ± 0.06	< 0.001

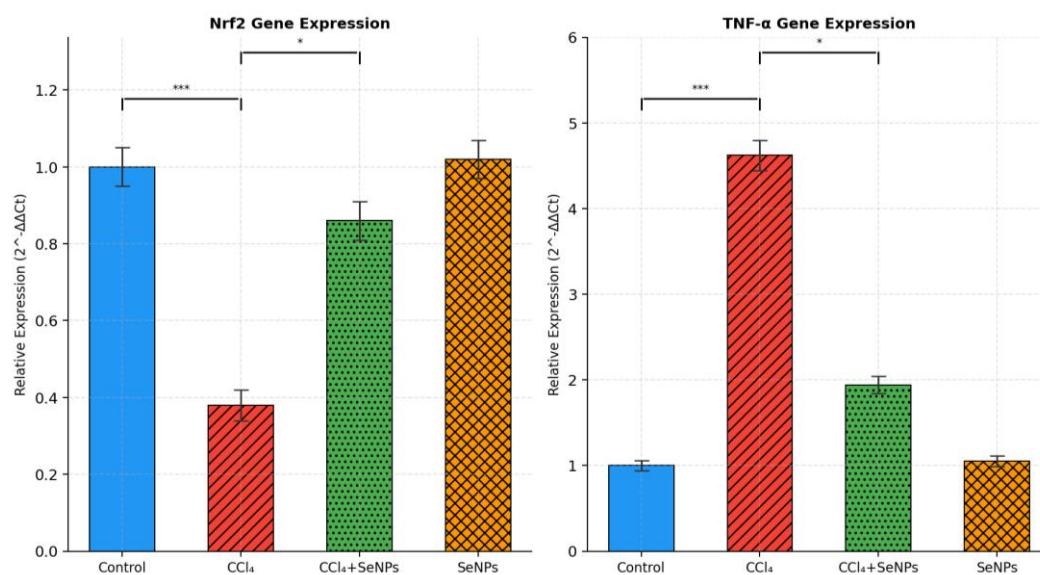


Fig. 5. Relative hepatic mRNA expression of Nrf2 and TNF- α by RT-qPCR (Mean $\pm$ SEM, $n = 8$; $2^{-\Delta\Delta Ct}$ method; GAPDH reference). * $p < 0.05$; *** $p < 0.001$.

4. DISCUSSION

The current study provides, for the first time, systematic mechanistic evidence that green tea-biosynthesized SeNPs mitigate CCl₄-induced hematotoxicity. This protective effect is achieved through the coordinated activation of the Nrf2/Keap1 antioxidant signaling pathway and the concurrent suppression of TNF- α -mediated hematopoietic inhibition. The measured SeNP diameter of 48.3 ± 6.2 nm is in agreement with previous reports of green synthesis [44], [45], and is in the optimal range of 20-100 nm for in cellular endocytosis [43]. The UV-Vis peak of 270 nm indicates typical selenium surface plasmon resonance [46].

The hematotoxic profile caused by CCl₄, characterized by RBC, Hb, Hct and PLT reduction, as well as leukocytosis, correlates with the two mechanisms described as (i) direct oxidative damage to erythrocytes, and (ii) indirect erythropoiesis suppression [5], [6]. Phospholipid membranes of erythrocytes peroxidized by the CCl₄ metabolite react with ROS and reduce the membrane's elasticity, increasing the speed of splenectomy [47], [48]. At the same time, the increased concentration of TNF- α observed in this study (4.62 fold) interacts with TNF receptor 1 on erythroid progenitors, thus reducing their proliferation and differentiation [49], [50]. The significant increase in MDA (3.3 fold) is a direct consequence of the lipid peroxidation chain. SeNP-assisted hematological recovery (>88% of Control regarding all erythroid indices) can be ascribed to the nanoparticles' multifarious anti-inflammatory and antioxidant actions. Selenium is indispensable for the functioning of GPx and TrxR [15], [16]. The nanoparticulate version of selenium offers prolonged availability of the element with diminished systemic toxicity [17]. The green tea phytochemicals (catechins and

polyphenols) that form a coating on the SeNP provide even more radical scavenging capacity [24], [25], thus synergistically expanding the nanoparticle's antioxidant scope.

Molecular data provide significant mechanistic insight into the downregulation of Nrf2 exhibited after CCl₄ exposure (fold-change 0.38). As described by Kensler et al. [10], and evidenced in CCl₄ models, Nrf2 experiences Keap1-mediated ubiquitination and proteasomal degradation under sustained oxidative stress [12]. SeNP treatment brought Nrf2 levels up to 86% of the Control. This correlates with the research regarding selenium's ability to interact with Keap1 (Cys151, Cys273, and Cys288) to provide an electron, which subsequently releases Nrf2 from the Keap1-Nrf2 complex and allows Nrf2 to enter the nucleus [55]. Nrf2 is responsible for HO-1 and NQO1, which increases GCL, and lowers oxidative stress as indicated by SOD, GSH, and CAT levels that are all restored [56].

The HO-1/CO axis illustrates how Nrf2 activation relates to the suppression of TNF- α . CO from HO-1 prevents NF- κ B's movement to the nucleus, which in turn diminishes the production of TNF- α [57], [58]. This correlates with the 58% decrease in TNF- α in response to the SeNP treatment. Less TNF- α diminishes its antagonistic influence on the proliferation of erythroid progenitors [50], [51]. This offers an immediate molecular rationale for the noted recovery in hematology. The radar chart summary (Fig. 6) encompasses and confirms the all-encompassing protective response to SeNPs.

A limitation of the present study concerns the physicochemical characterization of the biosynthesized SeNPs. While TEM and UV-Vis spectroscopy confirmed particle formation and size distribution, additional confirmatory analyses — including X-ray

diffraction (XRD) to assess crystallinity, Fourier-transform infrared spectroscopy (FTIR) to identify surface-capping biomolecules, dynamic light scattering (DLS) to determine hydrodynamic diameter and polydispersity index, and zeta potential measurement to evaluate colloidal stability — are warranted in future work to provide a more comprehensive physicochemical profile of the nanoparticles.

The group that only received SeNPs showed no negative impact on haematological, biochemical, or molecular aspects at the tested dose (0.5 mg/kg/day). This result aligns with the established safety of green-synthesized SeNPs at sub-toxic doses [33], [59], [60]. This dose is significantly lower than the LD₅₀ of selenium nanoparticles (~5–10 mg/kg in rodents) [61], [62], indicating a sizable therapeutic window for potential clinical use.

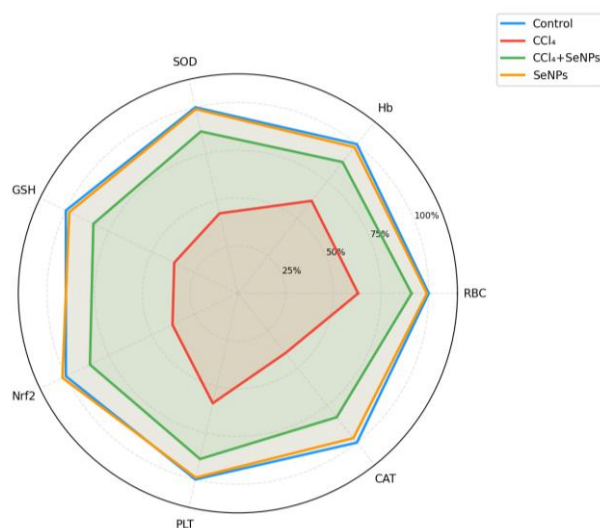


Fig. 6. Multi-parameter radar chart. Seven significant biomarkers are represented for each experimental group as percent of Control values. SeNPs treatment brings all biomarkers to Control levels.

5. CONCLUSION

This research shows that biosynthesized SeNPs made from *Camellia sinensis* extract provide protection against CCl₄-induced hematotoxicity on Wistar rats through a dual physiological-molecular mechanism. Physiologically, SeNPs re-establish erythroid parameters (RBC, Hb, Hct, PLT) and normalize the level of the enzymes of the antioxidant defense system (SOD, GSH, CAT). SeNPs also diminish lipid peroxidation (MDA) and mitigate the injury of hepatocytes (ALT, AST). On the molecular side, SeNPs activate the expression of Nrf2 and inhibit TNF- α , revealing that the Nrf2/Keap1/HO-1/NF- κ B signaling pathway is the main target of the study. The lack of negative effects in the

SeNPs only group confirms the haematoprotective safety of the green-synthesized formulation. This study provides a convincing mechanistic foundation for the further preclinical and clinical testing of SeNPs as an instrumental therapy solution in relation to haematotoxicity caused by environmental toxins, chemotherapy, and other oxidative-stress-related haematological disorders.

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