

Article

Effect of Selenium Nanoparticles on Carbon Tetrachloride-Induced Hepatotoxicity in the Swiss Albino Rats

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Abstract: Background: This study investigated selenium nanoparticles' protective effects (SE-NPs) against carbon tetrachloride (CCl₄)-induced hepatic injury in rats. Methods: Rats were divided into four groups ($n = 8$). Group 1 rats received the vehicle solution only. Group 2 received a single intraperitoneal injection of 1 mL/kg CCl₄ in liquid paraffin (1:1 *v/v*). Group 3 was treated with SE-NPs (2.5 mg/kg) twice a week for three weeks before receiving CCl₄ challenge. Oxidative stress, liver function, liver histopathology and serum lipid levels were evaluated. Results: Plasma concentrations of aspartate transaminase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), urea, creatinine, malondialdehyde (MDA) and the toxicity marker, lactate dehydrogenase (LDH), were significantly elevated in rats treated with CCl₄ compared to the controls. CCl₄ also caused a significant decline in liver glutathione (GSH) concentration. SE-NP pretreatment significantly improved the level of AST, urea, creatinine, MDA, LDH, and GSH in the CCl₄-injected rats towards the control levels. Conclusions: SE-NPs restored both liver function and hepatic structure in CCl₄ treated rats. SE-NPs exhibit an ability to counter markers of liver injury induced by CCl₄ and restore oxidative stability to lipid profiles and liver structure and function.

Keywords: CCl₄; hepatotoxicity; oxidative stress; liver functions; selenium nanoparticles



Citation: Ebaid, H.; Al-Tamimi, J.; Hassan, I.; Habila, M.A.; Rady, A.M.; Alhazza, I.M.; Ahmed, A.M. Effect of Selenium Nanoparticles on Carbon Tetrachloride-Induced Hepatotoxicity in the Swiss Albino Rats. *Appl. Sci.* **2021**, *11*, 3044. <https://doi.org/10.3390/app11073044>

Received: 14 December 2020

Accepted: 12 March 2021

Published: 29 March 2021

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1. Introduction

Liver damage or hepatotoxicity is one of the most common side effects of any chemical drug or medicine-based treatment. It can be caused by herbal medicines [1], chemicals, metabolic intermediates and viruses. Despite a large body of research, hepatotoxicity still a significant clinical challenge. Chemical-induced liver toxicity is attributed to the classical mechanism, including apoptosis along with an upsurge in cytokine concentration and oxidative stress [2]. Moreover, ROS (Reactive oxygen species)-mediated lipid peroxidation also plays a part in various liver and kidney pathological conditions and injuries; it has caused subsequent liver fibrogenesis in many *in vivo* studies [3]. Carbon tetrachloride (CCl₄) is a well-known hepatotoxicant [4]. This chemical is used in animal models to mimic pathophysiological lesions observed in humans. CCl₄ toxicity is mediated by metabolites that react with antioxidant enzymes [5]. It also raises the extent of inflammatory cytokines, including tumor necrosis factor-alpha (TNF- α), interleukin-1 β (IL-1 β), nuclear factor (NF- κ B). It is well established that CCl₄ elicits free radicals and causes macrophages' activation, leading to the production of inflammatory and profibrogenic mediators [6]. Free radical production is the primary step in the sequence of events leading to membrane lipid peroxidation, apoptosis, and necrosis [7]. Antioxidants provide substantial protection against CCl₄-induced hepatic toxicity [8]. Previous studies show that folic acid and melatonin are potent antioxidants in dealing with oxidative stress *in vivo* [9,10].

Selenium is an essential element for living organisms. It affects the activity of glutathione peroxidase and helps protect cells and tissues from oxidative damage. It is used as a dietary supplement [11] and supports immune responses to prevent various diseases [12,13]. Cancer and immune dysfunction are associated with modest selenium deficiency and altered expression and single nucleotide polymorphisms of some selenoproteins [11,14]. The impact of selenium on health and disease, dietary selenium requirements for support of cognitive and immune functions, and cancer prevention at the molecular level is hot in contemporary research [15]. Cells of the immune system, especially T lymphocytes and macrophages, express most of the 25 genes encoding human selenoproteins, with GPx isoenzymes, GPx1 and GPx4, exhibiting the highest expression levels in both cell types [16]. Selenium derivatives are detoxifying agents toward mercury, cadmium, lead, and many other elements [12]. The study on selenium interacting with other toxic elements *in vivo* is a hot-spot of contemporary research [17]. The suitability of selenium nanoparticles (SE-NPs) in such a study is attributed to their chemical stability, biocompatibility, and low toxicity [18]. Thus, selenium may be useful against hepatotoxicity as demonstrated in this study.

2. Materials and Methods

2.1. Fabrication and Characterization of Stabilized Nano-Selenium

Analytical grade chemicals (Sigma Aldrich, St. Louis, MO, USA), including citric acid, ascorbic acid, and selenium dioxide, were used to prepare the nano-selenium. The selenium dioxide was used as a selenium precursor. In contrast, citric acid and ascorbic acid were employed as stabilizing and reducing agents, respectively, during the nucleation and formation of stabilized nano-selenium particles following the previously described method [19,20]. For this, the selenium oxide and citric acid were mixed in an aqueous medium to form a 200-mL homogenous solution under stirring with a magnetic stirrer at room temperature for two hours. Then 25 mL of ascorbic acid (0.1M) was added to the mixture dropwise in 2 h under vigorous magnetic stirring. The stirring was continued for an additional 2 h at room temperature. In the final stage, the temperature was increased to 80 °C for 15 h. Finally, the mixture was centrifuged, and nano-selenium was collected after drying at 80 °C. The surface morphology of the prepared nano-selenium was examined by SEM, and EDS characterization was applied to identify the surface structural elements. In addition, the size of the nano-selenium particles and the image of the whole formed nano-selenium embedded citric acid were observed by TEM.

2.2. Animals and Experimental Design

Twenty-four male rats (*Rattus norvegicus*) weighing 150–170 g (20 ± 1 weeks) were obtained from the College of Sciences, King Saud University. The rearing and caring of the rats were conducted as per our previously published work [5]. The Animal Ethics Committee approved the study protocol in the Zoology Department in the College of Science at King Saud University (KSU-SE-20-38). Rats were divided into three groups ($n = 8$). Rats from group 1 served as the control and only received the vehicle solution. Group 2 received a single intraperitoneal (IP) injection of 1 mL/kg CCl₄ in liquid paraffin (1:1 *v/v*) [21]. Group 3 was treated with 2.5 mg/kg selenium nanoparticles (SE-NPs) twice a week for three weeks [22] before receiving the CCl₄ challenge.

2.2.1. Blood and Liver Samples

Two days after treatment, animals from all groups were necropsied under light anesthesia. Blood was drawn by puncturing the retro-orbital veins in capillary tubes (heparin-coated) until the animals' death. Plasma was separated after centrifugation at $200 \times g$ for 10 min in Eppendorf tubes and stored at -30 °C. The sample was used to determine levels of liver enzymes and lipid profiling.

The liver was removed, washed with saline, and cut into two parts; one part was used for the histological study and the other for assessing lipid peroxidation (malondialdehyde

(MDA)) and glutathione (GSH). The samples were processed and prepared as homogenates as done earlier [5].

2.2.2. Estimation of Lipid Peroxidation and an Assay of Reduced Glutathione

Lipid peroxidation was evaluated via measurements of the malondialdehyde (MDA) [23]. The homogenized tissue was read at an absorbance of 532 nm. The standard, 1,1,3,3-tetra methoxy propane was applied to determine the concentration of TBARS (Thiobarbituric acid reactive substances) (MDA per mg protein). An amount of 0.5 mL supernatant with 0.5 mL TCA (Trichloroacetic acid) and 0.5 mL TBA were mixed. Samples were incubated for 30 min in boiling water and then immediately cooled. Samples were centrifuged at 4000 rpm for 10 min at 4 °C. Absorbance was measured at the wavelength of 530 nm in 1 mL of the supernatant. According to the manufacturer, the protein concentration was measured at 500 nm in tissue supernatant (Quimica, Clinica Aplicada S.A., Amposta, Spain).

The reduced glutathione was estimated as described previously [24]. A mixture of 1 mL of tissue supernatant and 1 mL of sulfosalicylic acid was incubated for one hour. Then, it was centrifuged for two min at 1200 rpm. The supernatant was diluted with 1.1 mL of PBS and 200 µL of 5,5-dithiobis-2-nitrobenzoic acid (DTNB). The protein concentration was measured in 500 µL of the supernatant for each sample and at a wavelength of 412 nm, according to the reagent manufacturer (Quimica, Clinica Aplicada S.A., Amposta, Spain).

2.2.3. Liver Function Tests and Lipid Profile

Levels of aspartate transaminase (AST), lactate dehydrogenase (LDH), and protein were measured in plasma samples. The measurements were performed with Bio Merieux kits, France, following the manufacturer protocols. Their levels were measured kinetically by the colorimetric method (UV/Visible-Model-80-2106-00 spectrophotometer, Pharmacia Biotech, Cambridge, England).

Lipid profiling, including cholesterol, triglycerides, HDL (High density lipoproteins), and LDL (Low density lipoproteins), were estimated in plasma samples by commercial kits (Salucea Company, Etten-Leur, The Netherlands).

2.2.4. Histological Study

Tissues were fixed firstly in 10% neutral buffered formalin and then embedded in paraffin. Their sections were prepared and stained with hematoxylin–eosin (H&E). Moreover, Mallory trichrome was used for staining collagen deposits in separate slides. The sections were analyzed blind using a Leica DMRB/E light microscope (Heerbrugg, Switzerland).

2.3. Statistical Analysis

MINITAB software (MINITAB, State College, PA, USA, Version 13.1, 2002) was employed for statistical analysis. Data were treated with Anderson–Darling tests and for variance homogeneity. Furthermore, one-way ANOVA and Tukey's method for pairwise comparisons were implemented to determine the overall effects of each treatment. Results were expressed as arithmetic mean (M) ± standard deviation (SD). Only statistically significant differences, with $p < 0.05$, found between a treatment group and the control or between a treatment group and the CCl₄ group were considered.

3. Results

3.1. Characteristics of Fabricated Nano-Selenium

The SEM noticed the morphology and shapes of the prepared nano-selenium particles with a magnification of 6000 (Figure 1A) and 10,000 (Figure 1B), which indicate the formation of the nano-selenium with uniform particles with edges and that the forms are not entirely spherical forms. The EDS analysis (Figure 2 upper) showed the elemental nano-selenium surface composition formed from approximately 64.79% selenium and 35.21% carbon, which is indication of formation of stabilized nano-selenium combination

with an organic matrix. The nano-selenium particles were formed with sizes of about 100–150 nm and embedded in the organic matrix to create structures of sizes between 200 and 300 nm with different shapes, as shown in Figure 2 lower.

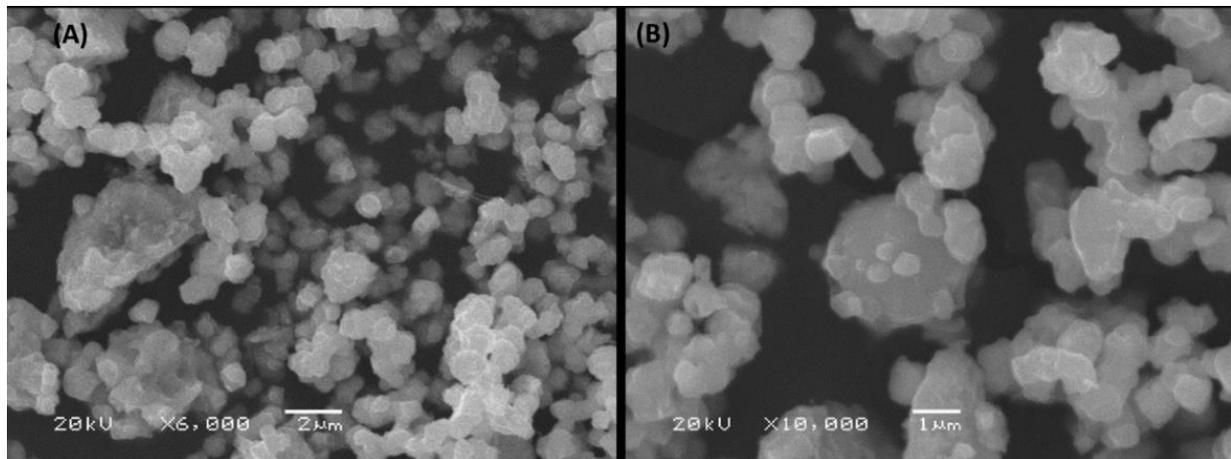


Figure 1. SEM of stabilized nano-selenium with magnifications of 6000 (A) and 10,000 (B).

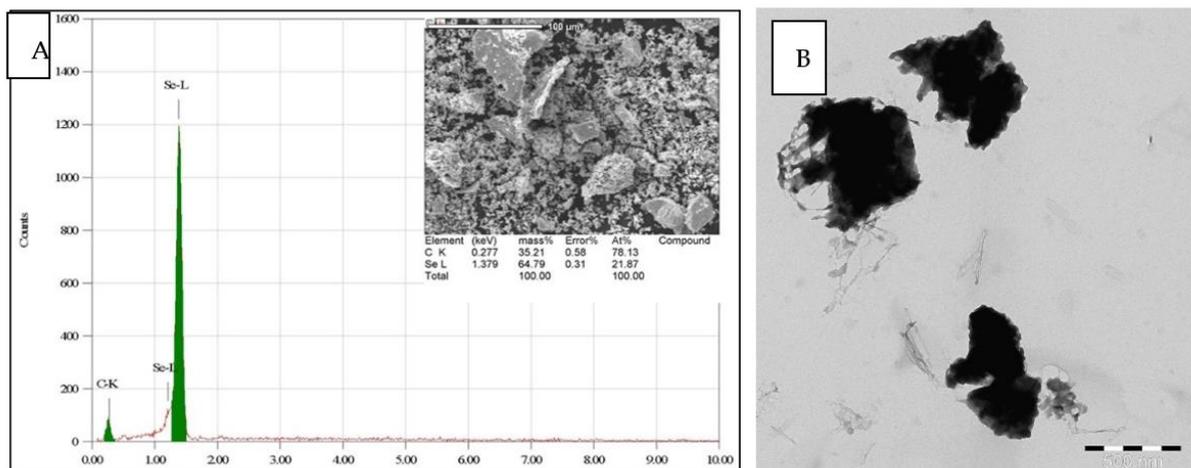


Figure 2. (A) EDS elemental analysis of stabilized nano-selenium. (B) The TEM image of nano-selenium.

3.2. Bioactivity of SE-NPs

3.2.1. Stress Markers

(a) MDA

Free radical production is the primary step in the sequence of events leading to membrane lipid peroxidation, apoptosis as well as necrosis. MDA is an indicative parameter of the lipid peroxidation. Here, the MDA was dramatically increased in the CCl_4 group rats in comparison to the control GSHs. CCl_4 group rats exhibited a 176.92% increase in MDA levels over controls, and CCl_4 + SE-NP group animals showed MDA levels 63.71% lower than CCl_4 group rats (Figure 3). Thus, SE-NPs were found to restore the MDA concentration CCl_4 group in comparison to the controls.

(b) GSHs

Antioxidants provide substantial protection against CCl_4 -induced hepatic toxicity. The GSHs declined in the CCl_4 group rats in comparison to the control GSHs. CCl_4 rats showed a 63.55% reduction in GSHs compared to controls. SE-NPs was found to significantly restore the GSHs in CCl_4 animals in comparison to the control GSHs. The GSHs showed a 73.84% increase in CCl_4 -SE-NPs over levels in the CCl_4 group (Figure 3).

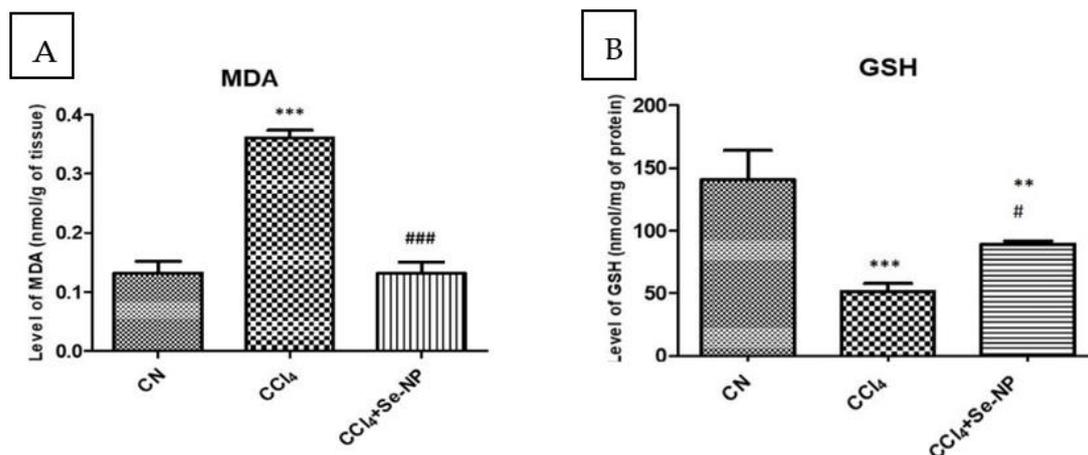


Figure 3. Hepatic oxidative stress parameters (malondialdehyde (A) and reduced glutathione (B)) in rat liver in different treatment groups. * (p -value < 0.05) indicates significant differences in other rat groups in comparison to control (CN) group animals. # (p -value < 0.05) indicates significant differences in different rat groups compared to the CCl₄ group animals. Also ** and ## indicate $p \leq 0.005$ while *** and ### indicate $p \leq 0.001$.

3.2.2. Liver Function Tests

After evaluating oxidative stress, the hepatic function parameters (plasma aspartate aminotransferase and lactate dehydrogenase, gamma-glutamyltransferase and alkaline phosphatase) in different rat groups were estimated.

(a) AST

The AST is one of the strong biomarkers determining the liver function and structure. Here, we found a remarkably very significant (p value < 0.01) increase in the AST concentration of CCl₄ rats in comparison to control rats. Plasma levels of AST in CCl₄ treated rats were 804.40% higher than controls, and Se-NP treatment reduced this increase to 421.43% as compared to controls. Pre-administration of Se-NP treatment before CCl₄ challenge decreased AST activity by 27.29% compared to CCl₄ treatment alone (Figure 4).

(b) Lactate DH

Lactate dehydrogenase (LDH) is expressed extensively in body tissues, in particular liver cells. It is released during hepatic damage, making it a marker of common tissue injuries and diseases involving hepatic toxicity. LDH is a bioindicator for necrotic progression in the living tissue. As expected, LDH was dramatically increased in the CCl₄ rats due to the toxicity compared to the control rats. LDH activity was enhanced by 68.16% in CCl₄ group animals compared to controls, and CCl₄ + Se-NP treated reduced this increase by 19.52% compared to CCl₄ group animals (Figure 4).

(c) Gamma-glutamyltransferase

In the cell membranes of many tissues, gamma-glutamyl transferase (GGT) transfers amino acids and the glutamyl moiety of the glutathione across the cell membrane to various acceptor molecules including water, and peptides, leaving the cysteine product to preserve oxidative stability inside the cell. The concentration of GGT was shown to be significantly (p -value < 0.05) increased due to CCl₄ toxicity in comparison to the control rats. On the contrary, the GGT level was restored approximately to normal levels in the CCl₄ rats treated with Se-NPs in comparison to the control (Figure 4).

(d) Alkaline phosphatase

Alkaline phosphatase (ALP) is of the critical indicators on liver health. Its concentration was found to behave like that of the GGT level in the CCl₄ group. Because of the CCl₄ toxicity, ALP level was significantly (p -value < 0.05) elevated in the CCl₄ rats compared to the control level. Se-NPs were found to partially but not significantly restore the ALP level in CCl₄ rats (Figure 4).

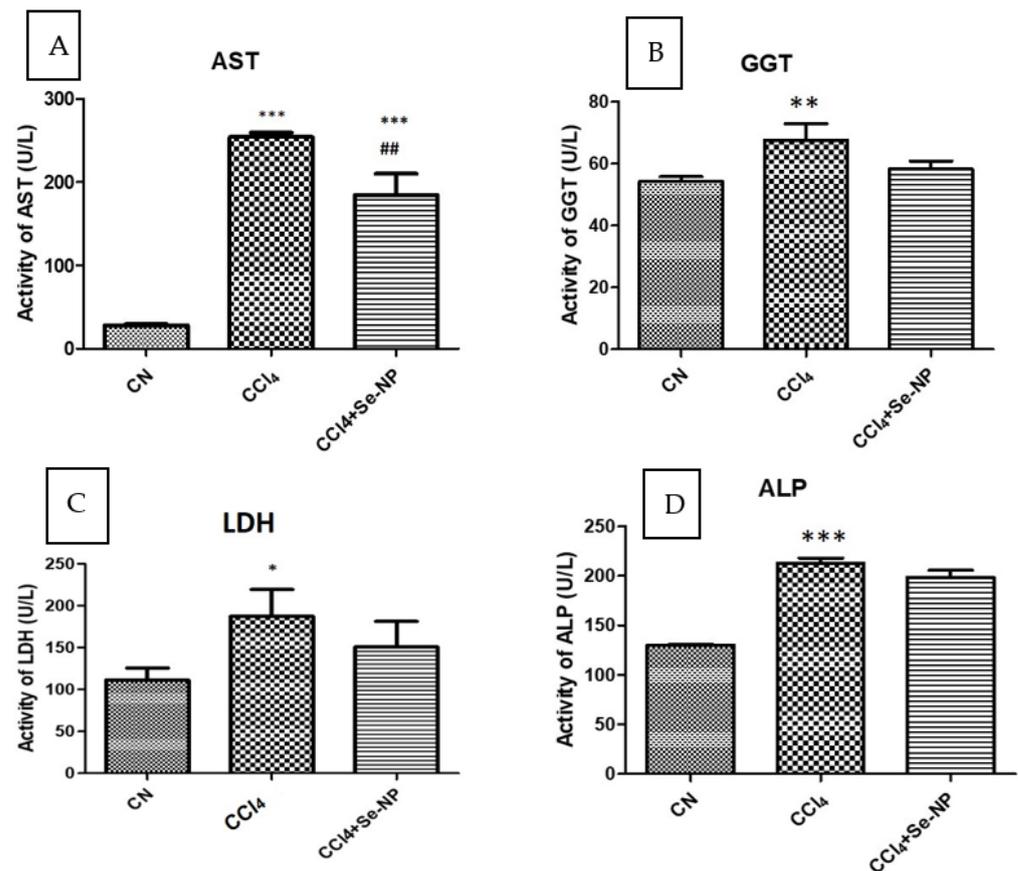


Figure 4. Hepatic function parameters ((plasma aspartate aminotransferase (A) and lactate dehydrogenase (B), gamma-glutamyltransferase (C) and alkaline phosphatase (D)) in different rat groups. * (p -value < 0.05) indicates significant differences in rat groups in comparison to control (CN) group animals. # (p -value < 0.05) reveals significant differences in different rat groups compared to CCl₄ group animals. Also ** and ## indicate $p \leq 0.005$ while *** and ### indicate $p \leq 0.001$.

3.2.3. Lipid Profiling and Glucose Concentration

(a) Cholesterol

CCl₄ group showed a 17.53% increase in cholesterol levels, and CCl₄ + Se-NP treated rats exhibited a decrease of 7.18% (Figure 5).

(b) TAGs

CCl₄ displayed a 6.69% increase in TAG (Triglycerides) level, and CCl₄ + Se-NP treatment produced a decrease of 0.96% compared to the CCl₄ group (Figure 5).

(c) Glucose

CCl₄ treated rats showed a 28.21% increase in glucose levels, and Se-NP treatment reduced this increase by 27.36% compared to controls (Figure 5).

3.3. Liver Histology

Examination of histopathological sections revealed that CCl₄ challenge caused severe hepatic damage. Injuries included disturbed hepatic structure, narrow hepatic sinusoids, infiltration with inflammatory cells, and eosinophilic hepatocytes. Unlike renal tissues, selenium NPs did not produce any apparent improvement in histopathology of hepatic tissues in CCl₄ treated rats (Figure 6).

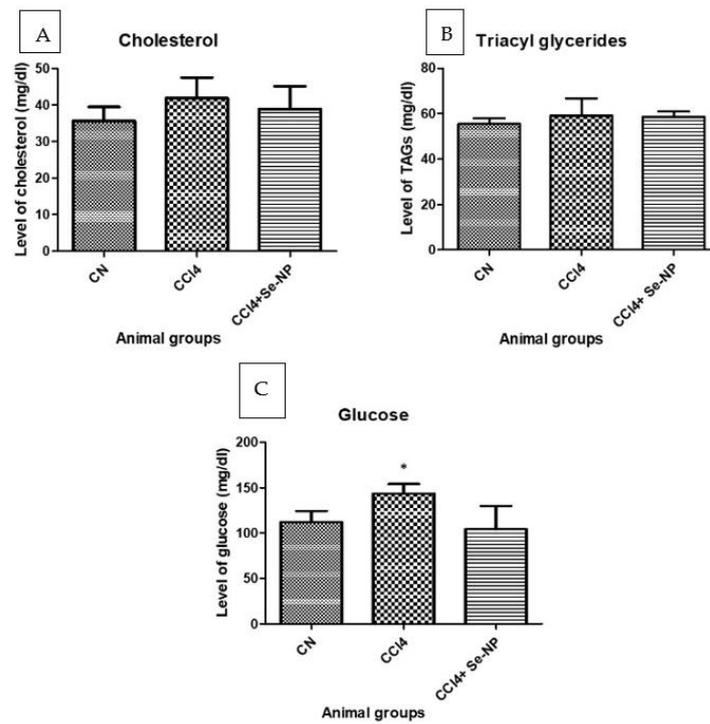


Figure 5. Showing level of total cholesterol (A), triglycerides (B), and glucose concentrations (C) in the plasma from different rat groups. * (p -value < 0.05) indicates significant differences in different rat groups in comparison to control (CN) group animals.

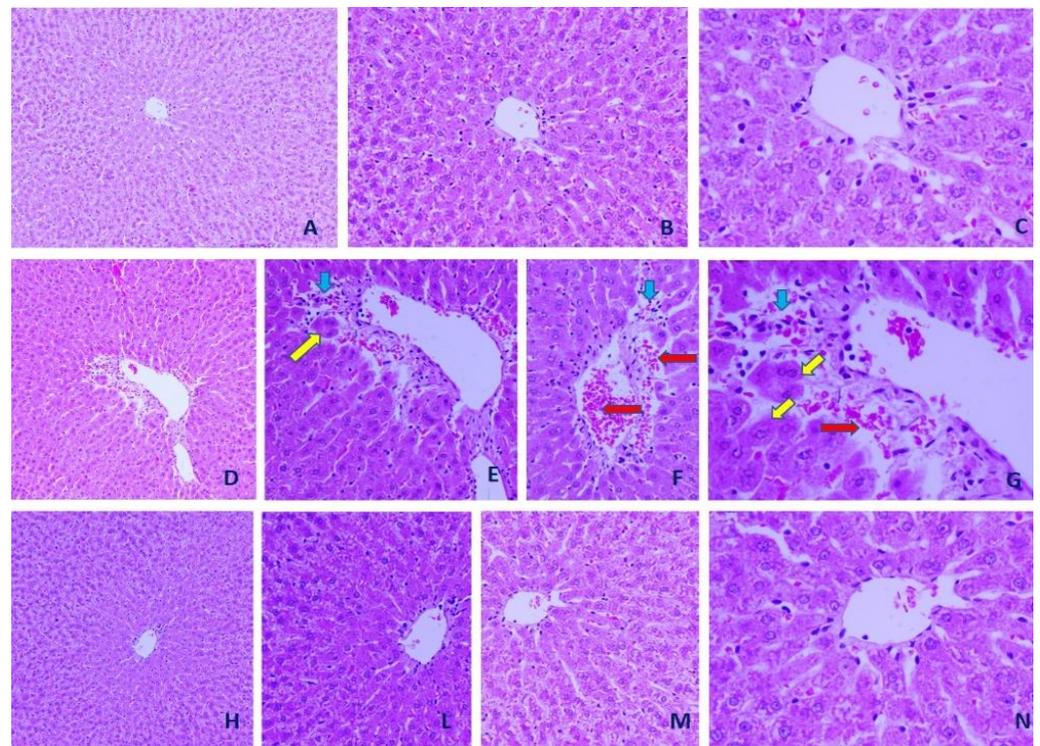


Figure 6. Representative microscopic images from control, CCl₄ and CCl₄ + selenium nanoparticles (SE-NPs) hepatic tissues. (A–C): Control hepatic tissues (×200, ×400, and ×1000, respectively). (D–G) CCl₄ hepatic tissues (×200, ×400, ×400, and ×1000, respectively). (H–N) CCl₄ + SE-NPs hepatic tissues (×200, ×400, ×400, and ×1000, respectively). Hepatocytes (yellow arrows), inflammatory cells (blue arrows) and hemorrhage (red arrow) are shown (hematoxylin–eosin (H&E)).

3.4. Effect of Se-NP on CCl₄-Induced Damage to Nuclear DNA

Comet assay is a reliable method for measuring damage to nuclear DNA in the targeted tissues. DNA in liver tissue in CCl₄ treated rats showed a 133.33% increase in tail-length of nuclear DNA as compared with controls. CCl₄ + Se-NP reduced this increase by 38.29% compared to CCl₄ (Figure 7).

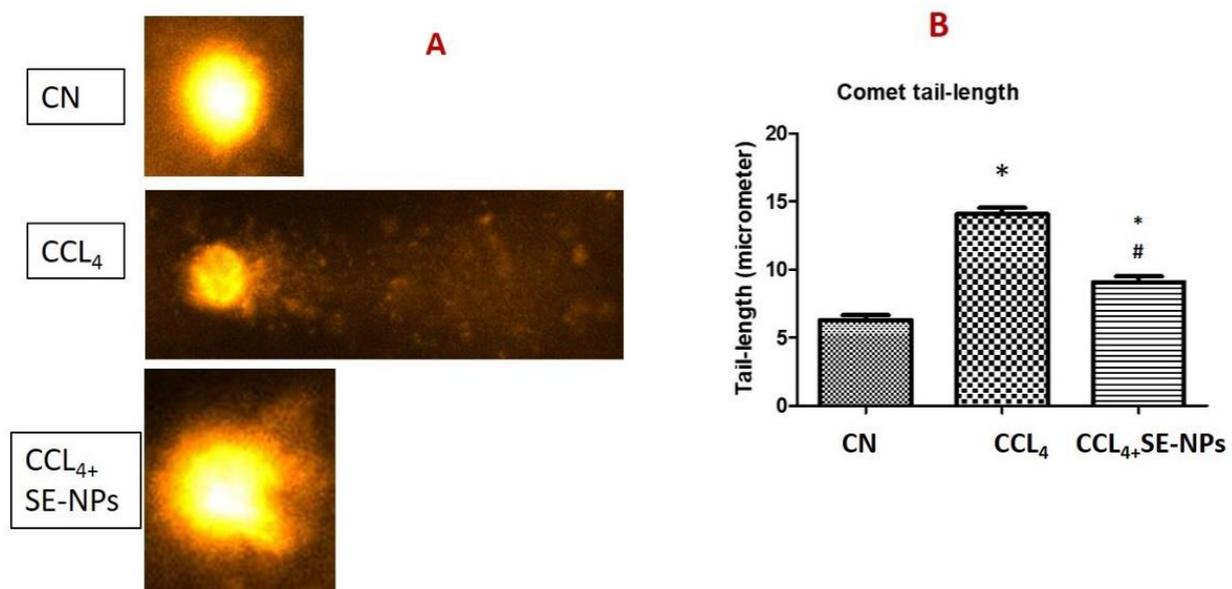


Figure 7. Representative photos (A) and data analysis (B) of the Comet tail length from different rat groups. * (p -value < 0.05) indicates significant differences in different rat groups in comparison to control (CN) group animals. # (p -value < 0.05) indicates significant differences in different rat groups in comparison to the CCl₄ group animals.

4. Discussion

This study is to investigate the effectiveness of SE-NPs in protection against CCl₄-induced hepatotoxicity. CCl₄, an established hepatotoxicant, is converted to various metabolites, including radicals like CCl₃. They are involved in liver disease, cirrhosis, and hepatic carcinoma [25]. Its exposure to rats causes hepatic injuries due to the generated free radicals. It also significantly enhances the release of ALT (alanine transaminase) and AST (aspartate transaminase) to plasma and prominent alteration in lipid profiles [26]. Selenium, as an antioxidant, is a necessary trace element in humans and is suggested as a dietary supplement for its health benefits [27]. Nanotechnology is developed as an amalgamation of chemistry, engineering, biology, and medicine. Nanoparticles have proven useful for the early detection of tumors and the development of novel treatments [28]. We have previously found that Se-NPs have strong anti-diabetic and anti-oxidant properties in gestational diabetic mothers in rat models [29]. The Se-NPs regulated inflammation-mediated complications in diabetic mothers and pups. Ag-NPs showed a potential role in preventing the deleterious effects on DNA in nephrotoxicity [30].

Our findings show that administration of SE-NPs significantly counters hepatotoxicity caused by CCl₄, which might be due to antioxidant activity. SE-NPs might scavenge free radicals as reported in other investigations, which in turn lowers serum cholesterol, triglycerides and lipid peroxide [31]. The CCl₄ rats exhibited a profound elevation in total cholesterol and triglycerides levels. Hyperlipidemia was detected due to the hepatotoxic effects of CCl₄. Hyperlipidemia is a common feature in patients with liver disease. Thus, the cardiovascular risk indices may also be increased. It has been observed that hypercholesterolemia is associated with disturbed angiogenesis, which delayed the tissue healing process [32]. SE-NPs treatment of CCl₄ rats produced marked amelioration of the tested

lipids. Thus, it can be suggested that the alleviation of lipids may be involved, at least in part, in the enhanced tissue healing observed in CCl₄ rats treated with SE-NPs.

Hyperlipidemia interferes with liver functions. Oxidative stress damages the stability and integrity of biological membranes and increases permeability, resulting in the outflow of cytoplasmic enzymes such as ALT, ALP, and AST into the blood [33]. Thus, the ALT, ALP and AST activities in the serum are essential indices for evaluating liver injury. In the current experiment, serum ALT activity, ALP, and AST in the CCl₄ group were significantly higher than that in the normal rats, suggesting that CCl₄ exerted a toxic effect on CCl₄-injected rats. Recent results indicated that oxidative stress and lipid peroxidation elevated inflammation and enzyme and GGT activity [34].

On the other hand, breaking down extracellular glutathione and making GGT component amino acids available to the cells has an essential physiological role in counteracting oxidative stress [35]. Indeed, conditions that increase serum GGT lead to increased free radical production and the threat of glutathione depletion [35]. It is more likely that CCl₄ increased GGT in a similar mechanism in the CCl₄-injected rats. The antioxidant treatment may inhibit disease progression. Therefore, treatment of CCl₄-injected rats with SE-NPs restored the liver function-enzyme concentration with the GGT level.

The hepatotoxicity of CCl₄ is attributed to the generation of free radicals. They activate the Kupffer cells and macrophages that further elicit inflammatory mediators causing fibrosis. The heightened release of free radicals is the first step in a chain of events leading to lipid peroxidation of membranous cell organelles, resulting in apoptosis and necrosis [6]. The elevation in cytokine concentrations and oxidative stress might be the possible chemically-induced liver apoptosis mechanisms [7]. In addition, ROS-mediated lipid peroxidation is the main reason behind the pathogenesis of various liver toxicity and liver fibrosis followed by histological distortion and DNA damage in the target tissues in the experimental animals [8].

CCl₄ causes ROS-mediated biotransformation leading to moderate-to-severe toxicity that disturbs cellular redox balance and compromises structural settings in the target tissues [36]. ROS increases lipid peroxidation (MDA levels) and compromises levels of GSH [9] in the target tissues. The histological evaluation of kidney sections further agrees with these conclusions. Results are consistent with the nephrotoxic effect of potassium bromate [37]. Furthermore, CCl₄ is converted to highly reactive metabolites that can affect the cellular components (proteins, enzymes, lipids, and nucleic acids) and cellular structures. Moreover, free radicals generated during metabolism and biotransformation of CCl₄ cause chromosomal aberrations, DNA-base transversions, DNA adduct formation, and 8-OH dG. Administration of CCl₄ caused genotoxicity and DNA fragmentation in hepatic tissues in the male Sprague–Dawley rats [38]. This activity can explain the present study results, in which comet assays show a noticeable increase in DNA tail length in CCl₄-treated rats. Hence, CCl₄ damages the biological functions of critical biomolecules that disrupt cellular structure and function [39]. The elevated concentration of MDA is a strong index of oxidative stress in CCl₄-challenged rats. However, its decreased concentration was observed in the CCl₄ administered rats treated with SE-NPs. Thus, the SE-NPs can effectively protect against lipid peroxidation.

SE-NPs have shown antioxidant protection in vitro and in vivo studies [29,31]. All the organisms have effective defense mechanisms that prevent and neutralize damage incurred by free radicals. The NPs restore the activity of GSH and catalase significantly. GSH acts as a non-enzymatic antioxidant that reduces H₂O₂, hydroperoxides, and xenobiotic toxicity [40]. In contrast, a significant decrease in MDA in hepatic tissues is confirmed after pretreatment with SE-NPs and hence protects against hepatic lipid peroxidation incurred by CCl₄. Antioxidant activity and antilipidemic effects of SE-NPs have been reported to modulate blood pressure and alleviate the damage to target organs [41]. GSH is an essential determinant for cell survival or death during disturbed oxidative stress [42].

Inflammatory macrophages and dendritic cells are a physiological defense mechanism for injury and toxicity that are still inadequately addressed by the immune system [43].

Infiltration of inflammatory cells, if prolonged, can exacerbate fibrosis [44]. We previously found that carcinogens trigger all critical pro-inflammatory cytokines (IL-1, 2, 6, and 7) as well as transcription factors (NF- κ B and TNF- α) in target organs [45]. Immune cells then become aggressive, causing extensive inflammation and fibrosis in the liver, as evidenced in histopathological findings. SE-NPs were found to remarkably ameliorate inflammatory infiltration with the immune cells in the hepatic tissues (Figure 6).

NF- κ B might be downregulated by SE-NPs that induce oxidative stability, although this activity was not addressed in the present study. This amelioration of hepatic seems to be mediated by the ceasing of oxidative stress and suppression of NF- κ B, the critical regulator of inflammatory response. Downregulation of NF- κ B would exert diminishing production of pro-inflammatory cytokines.

Hepatic injury markers are significantly low in the animals treated with any antioxidant treatment. SE-NPs also noticeably attenuated the plasma liver enzymes induced by CCl₄, which led to the restoration of the key enzymes' activities to normal activity. The effect of SE-NPs was further confirmed through histopathological examinations. Hence, pretreatment with SE-NPs has broad anti-inflammatory effects that can attenuate the allergies and inflammation in CCl₄-challenged rats.

This study, hence, confirms the hypolipidemic effect of SE-NPs in CCl₄-treated rats. It is evident from the low concentrations of cholesterol and triglycerides after the NPs treatment. Similar findings have been reported in many animal model studies [5]. This effect of NPs may be attributed to the enhanced catabolism of cholesterol to bile acids [46] and suppressing cholesterol synthesis and LDL receptor activity.

5. Conclusions

Use of nanotechnology may allow the development of novel therapeutic strategies. Our findings provide evidence of possible antioxidant and anti-inflammatory effects of SE-NPs. This combination restored normal oxidative stress balance, perhaps by inducing oxidative stability and reducing tissue damage. SE-NPs treatment produced significant improvement in liver function and histological architecture after CCl₄ challenge. The size of NPs, only several hundred nanometers, may allow close and frequent interaction with biomolecules present both on cell surfaces and inside cells. Currently, no data exist to determine if SE-NPs' activity toward CCl₄-induced hepatic injury might be clinically useful. The present study establishes a possible use of SE-NPs to treat CCl₄-induced hepatic toxicity *in vivo*. However, further research is warranted to elucidate the in-depth mechanisms.

Author Contributions: H.E. designed this study, analyzed the data, prepared the figures, and prepared and finalized the manuscript. M.A.H. Prepared and characterized the nanoparticles. J.A.-T. and I.H. performed the animal handling, treatment, sample preparation and conducted all biochemical studies. A.M.R. and I.M.A. revised the manuscript. Writing–review and editing, A.M.A. All authors have read and agreed to the published version of the manuscript.

Funding: Researchers Supporting Project number (RSP-2020/225), King Saud University, Riyadh, Saudi Arabia.

Institutional Review Board Statement: All procedures were conducted in accordance with guidelines for the care and use of experimental animals by the Committee for the Purpose of Control and Supervision of Experiments on Animals and the National Institutes of Health. The present work was approved by the KSU ethical committee under approval number KSU-SE-20-38.

Data Availability Statement: The data used to support the findings of this study are included in the article.

Acknowledgments: This work was supported by Researchers Supporting Project number (RSP-2020/225), King Saud University, Riyadh, Saudi Arabia.

Conflicts of Interest: The authors declare no conflict of interest.

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