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Research Article

Silymarin and Vanillic Acid Silver Nanoparticles Alleviate the Carbon Tetrachloride-Induced Nephrotoxicity in Male Rats

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Natural copolymer (e.g., chitosan-loaded) and synthetic (e.g., silver nitrate-loaded) nanopolymers have many medical applications in drug delivery research for enhancing the effectuality of traditional medicine. This study aimed to investigate the potential protective activity of vanillic acid, silver nanoparticles (AgNPs) of vanillic acid, and silymarin against carbon tetrachloride (CCl_4)-induced nephrotoxicity in male rats. Rats were divided into five groups; the first group (G1) was a negative control, and the other rats were treated intraperitoneally with CCl_4 to induce kidney toxicity twice weekly, and then divided into four groups, G2 was a positive control and left without treatment, the third group was treated with vanillic acid, the fourth (G4) was treated with vanillic acid-AgNPs, and the fifth (G5) was treated with silymarin. In G2, renal function indices (urea, creatinine, and uric acid) showed elevated levels indicating renal toxicity. Na, K, and Ca ions were decreased, whereas Cl^- was increased. Antioxidants (glutathione S-transferase, glutathione reduced, total antioxidant capacity, superoxide dismutase, and catalase) were decreased, whereas lipid peroxidation was increased in the kidney tissue homogenate. IL1 was increased, whereas CYP-450 was decreased. In the treated group, all biochemical and renal tissue texture were alleviated as a result of treatment with vanillic acid in G3, vanillic acid AgNPs in G4, and silymarin in G5. Vanillic acid AgNPs and silymarin treatment in G4 and G5, respectively, were more efficient than vanillic acid in G5 in protecting the kidneys against CCl_4-induced nephrotoxicity.

1. Introduction

The kidneys balance blood water and electrolyte levels, manage blood pressure, influence blood cell production, adjust the acid–base balance, keep electrolyte concentration, and make the active form of vitamin D; however, when one or both kidneys are acutely or chronically injured they will lose their filtration ability and causes nausea, fatigue, vomiting, and brain fog [1, 2].

Glycerol is commonly used to induce acute kidney disease in model animals [3, 4]; however, other drugs may also induce kidney diseases, such as high doses of acetaminophen (2000 mg/kg body weight) [5], cisplatin at an intravenous dose of 30 mg/m^2 daily for 7 days induced kidney failure in goats [6], adenine at a dose of 0.75% adenine in the diet of rats for 4 weeks [7], and aristolochic acid nephropathy was also used to induce chronic kidney disease in mice [8]. In

addition, a single high dose of cisplatin, which is used for treating solid tumors induced nephrotoxicity [9].

The natural products present are the active constituents of the herbal medicine that were effective in treating kidney disease in humans and experimental animals and in vitro studies [1, 2]. In addition, herbal medicine is commonly used today as an alternative therapy because of its low-cost price compared with traditional medical therapy and its minimal toxicity, which makes medicinal plants occupy a great position [10–12]. Herbal medicine appeared great benefits in nephroprotection to protect kidneys against different types of injuries to avoid dialysis or transplantation, which are the main treatment methods nowadays [13, 14]. On the other hand, incorporating vegetables and fruits rich in antioxidants in the diet improves kidney health and delays chronic kidney failure by reducing inflammation and hampering reactive oxygen species (ROS) formation [15, 16].

The phenolic derivative—vanillic acid (4-hydroxy-3-methoxy benzoic acid)—is an intermediate in the production of vanillin from ferulic acid and showed medicinal activities due to its antioxidant potential as an antibacterial [17], antihypertensive and antioxidant [18], nephroprotective effect [19], anticancer [20], protecting against acetaminophen-induced hepatotoxicity in rats [21], and protecting against carbon tetrachloride (CCl_4)-induced hepatotoxicity [12].

The biomedical application of silver nanoparticles (AgNPs) has been flourished in the last decades because of their specific biological, physical, and chemical properties; besides, their low cost and high efficiency in drug delivery purposes [12, 21–23]. They raised the efficiency of the pharmacokinetics and pharmacodynamics of drugs by enhancing the action of therapeutic agents and developed their specific and selective delivery system that positively affected human healthcare practice; therefore, researchers advocated developing their design and inventing new systems for drug delivery [24, 25]. They are applied in multifunction drug delivery systems [26]. AgNPs also succeeded in inhibiting vancomycin resistance in *Staphylococcus aureus* [27]. In addition, polymeric nanomaterial therapy using natural polymers, such as chitosan, which is produced by the deacetylation of chitin, and biosynthetic AgNPs have been recommended for nanomedical and biomedical applications [28]. Bee venom polymers like fungal chitosan-loaded nanoparticles conferred significant activity against cervix carcinoma [29].

Silymarin is an antioxidant, anti-inflammatory, and hepatoprotective agent obtained from *Silybum marianum* [12, 30, 31]. Its protection effect is thanks to several activities, such as cell permeability regulator, and membrane stabilizer, antioxidant, anti-inflammatory, inhibiting the deposition of collagen fibers and stimulating liver regeneration [31]. Silymarin has the ability to ameliorate diabetic nephropathy that results from the generation of ROS and the overproduction of superoxide, initiating podocyte injury and leading to the pathogenesis of diabetic kidney disease [32, 33]. Silymarin could protect the kidneys against iron deposition in the kidneys of animal models [34, 35]. It was also effective in protecting mice against renal ischemia–reperfusion injury [36].

The current study aimed to test the probable nephrotoxic effect of CCl_4 as well as the protective effect of vanillic acid, vanillic acid AgNPs, and silymarin against this CCl_4-nephrotoxicity in male rats.

2. Materials and Methods

2.1. Chemicals and Kits. The chemicals of this study are of analytical grades and were purchased from Sigma–Aldrich (Missouri, USA) unless designated to other sources. Olive oil was obtained from a local market and silymarin was purchased from a pharmacy. Kits were purchased from different suppliers and were used according to the instruction of the suppliers.

2.1.1. Synthesis of AgNPs from Vanillic Acid. A photomediated method for reducing silver ions was used in synthesizing silver nitrate nanoparticles of vanillic acid (AgNPs) as described by Zamani and Moradshahi [37] and Alamri et al. [12] as follows: A 2 g vanillic acid quantity was dissolved in a small volume of ethanol and completed to 100 mL by distilled water. Twenty milligrams of silver nitrate was added and mixed with a magnetic stirrer. The pH of the mixture was adjusted to 10.0. A noticeable color change from colorless to yellow to dark brown was encountered that confirm the formation of the AgNPs [12, 37].

2.1.2. Characterization of AgNPs

(1) Examination of the Synthesized Vanillic Acid Nanoparticles under Ultraviolet–Visible Spectroscopy. The AgNPs surface plasmon resonance (SPR) was detected by measuring the UV–Vis spectra of the synthesized particles using a UVS-85 spectrophotometer spanning the range of 300–800 nm [12, 38].

(2) Examination of the Synthesized Nanoparticles under Transmission Electron Microscope. The synthesized vanillic acid AgNPs size, morphology, form, purity, and assembly were examined by transmission electron microscopy (TEM, Jeol JEM-1400, Jeol Ltd., Tokyo, Japan) as follows: a 2–5 μL droplets samples were placed onto a parafilm sheet, the grids (carbon-coated 400-mesh copper grids) of the sample were created, and then swept off by filter paper. Finally, the grids were inserted into the petri dish of the sample [12, 39].

(3) Size Determination and Distribution Using Dynamic Light Scattering. To determine the size and the distribution peak of size within the range of the synthesized vanillic acid AgNPs the AgNPs were dispersed in a liquid sample and then estimated using the NICOMP Nano ZLS (Z3000 zls) particle sizing device (Entegris, Dresden, Germany). The size of the synthesized AgNPs was calculated using dynamic light scattering (DLS). Before analysis, samples of the synthesized vanillic acid AgNPs were diluted ten times in deionized water, a volume of 250 μL was transferred to a cuvette, and then equilibrated at 20°C for 2 minutes [12, 40].

2.2. Animals and Experiment Design. Thirty Sprague Dawley rats were purchased from the Faculty of Pharmacy, Mansoura University, Mansoura, Egypt, and used under the

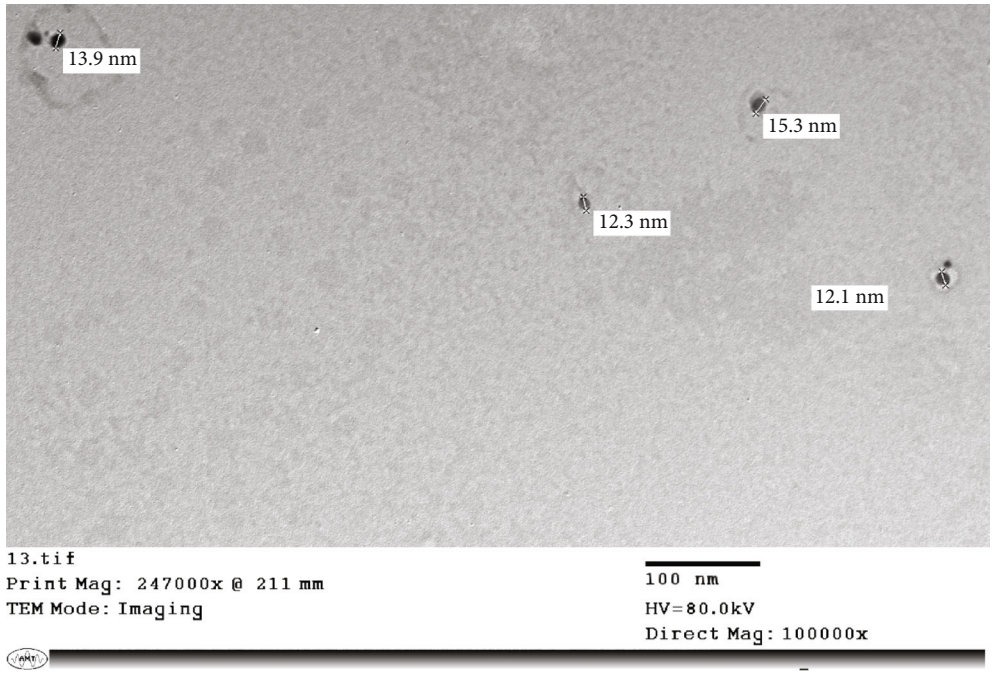


Figure 1: The AgNPs obtained from vanillic acid as seen by TEM. Particle size ranged from 12.1 to 15.3 nm.

approved ethical regulations of Animal houses of Mansoura University (approval code, 0184). The rats were kept under observation for two weeks for acclimatization under standardized laboratory conditions. Food and water were given ad libitum during the experiment span. After acclimatization, the rats were divided into five groups as follows: the first group was the negative control group (G1) received only olive oil in water (1 : 1) intraperitoneally on the first and fourth day every week during the experiment that extended for four weeks, and the other rats were treated intraperitoneally treated with CCl_4 in olive oil (1 : 1) twice weekly on the first and the fourth day to induce kidney toxicity [30], and then divided into four groups, the second group (G2) was a positive control and left without treatment, the third group (G3) was treated with vanillic acid (100 mg/g body weight), the fourth group (G4) was treated with vanillic acid AgNPs [18], and the fifth group (G5) was treated with silymarin (50 mg/kg body weight) [31].

2.3. Blood Collection. By the end of the experiment, the rats were anesthetized by diethyl ether and blood samples were collected, centrifuged for 5 minutes at 3000 rpm, and then the plasma was transferred into new tubes for biochemical analyses.

2.4. Preparation of Kidney Homogenate. One kidney was dissected out, washed with saline solution, kept ice cold and homogenized in ice-cold phosphate buffer (pH 7.4), and then centrifuged at 4000 rpm for 15 minutes. The supernatant was used in the determination of total antioxidant capacity (TAC), antioxidant enzymes, and lipid peroxidation.

2.5. Biochemical Analysis. Kidney function indices, such as creatinine, urea, and uric acid were determined in serum using Human Diagnostic Kit (Wiesbaden, Germany). Serum electrolytes, such as sodium, potassium, calcium, and chlorine ions were measured using Human Diagnostic Kit. Interleukin-1 (IL-1) was determined using MyBioSource Kit (San Diego, CA, USA), whereas cytochrome P-450 (CYP-450) was determined using Abbexa Kit from Cambridge, UK. All analyses were done according to the instructions of the suppliers. Biodiagnostic Kit (Cairo, Egypt) was used for the estimation of antioxidants [superoxide dismutase (SOD), catalase (CAT), glutathione reduced (GSH), and glutathione S-transferase (GST)] and TAC in the kidney tissue homogenate. Biodiagnostic Kit was also used in the estimation of lipid peroxidation as revealed by malondialdehyde (MDA) in the kidney tissue homogenate. All analyses were achieved according to the method described by the supplier.

2.6. Histological Study. The kidney tissue was immediately fixed in 10% formalin, dehydrated in ethanol (70%, 80%, and 90%), cleared in xylene, embedded in paraffin, and then sectioned by microtome. The sections were then stained with hematoxylin–eosin (H&E) according as described by Bancroft and Stevens [41].

2.7. Statistical Analysis. Data were analyzed using the statistical package (SPSS) program, version 17.0 [42] and presented as mean ± standard error, and reanalyzed by the one-way analysis of variance (ANOVA statistical measure) using Duncan's test for comparison of significance between groups.

3. Results and Discussion

3.1. Synthesis and Characterization of AgNPs. The emergence of dark brown color in the vanillic acid solution

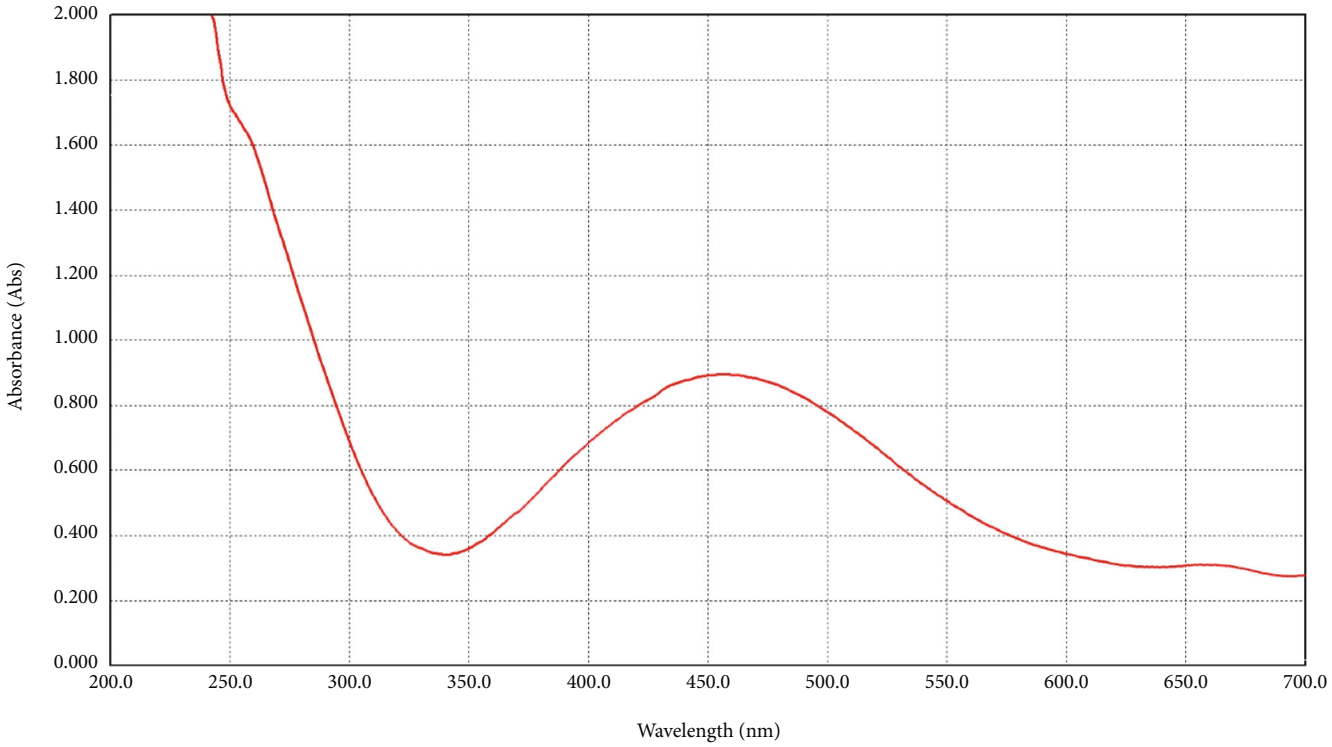


Figure 2: The plasmon resonance band peak of AgNPs surface at 450 nm as seen under UV–Visible spectra.

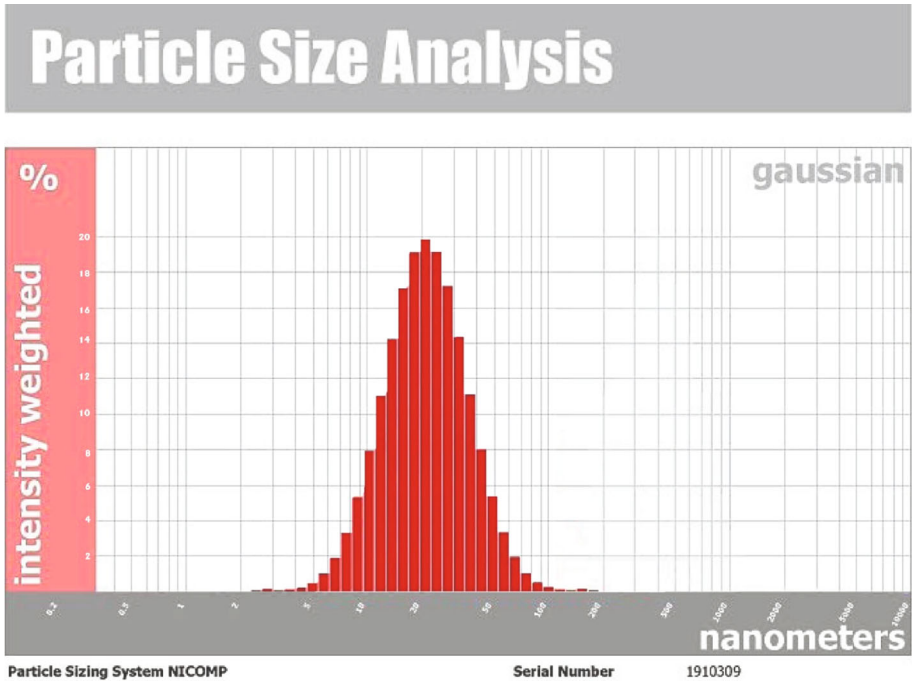


Figure 3: The average particle size of the synthesized AgNPs of vanillic acid was 21.6 nm as appeared by DLS spectrum analysis.

following the addition of silver nitrate confirmed AgNP synthesis [37, 43]. The proper synthesis of the reduced AgNPs of vanillic acid in the solution was visualized under UV–Vis spectrum, which is one of the most important ways for confirming the synthesis of AgNPs shows that the synthesized metal nanoparticles exhibited SPR and their characteristic SPR band occurred at 450 nm as seen in Figure 1 [44, 45]. This result is consistent with that of Ashraf et al. [46], they stated that the AgNPs exhibit a maximum UV–Visible absorption in the range of 400–500 nm thanks to the SPR.

Table 1: Effect of treating CCl_4-induced nephrotoxicity in male rats vanillic acid, vanillic acid AgNPs, and silymarin on kidney function indices.

Parameters (mg/dl)	Statistics	G1 (negative control)	G2 (positive control)	G3 (treated with vanillic acid)	G4 (treated with vanillic acid AgNPs)	G5 (treated with silymarin)
Urea	Mean ± SE LSD 0.05 = 3.878	16.00 ± 1.31^{e}	58.00 ± 2.03^{a}	43.66 ± 0.55^{b}	37.33 ± 0.91^{c}	27.00 ± 1.09^{d}
	T-test	—	−25.10***	07.95***	07.82***	11.77***
Creatinine	Mean ± SE LSD 0.05 = 0.091	0.53 ± 0.02^{e}	2.16 ± 0.04^{a}	1.70 ± 0.03^{b}	1.32 ± 0.02^{c}	1.01 ± 0.01^{d}
	T-test	—	−28.61***	10.87***	11.750***	18.46***
Uric acid	Mean ± SE LSD 0.05 = 0.229	3.83 ± 0.05^{e}	7.66 ± 0.09^{a}	6.36 ± 0.11^{b}	5.30 ± 0.07^{c}	4.60 ± 0.03^{d}
	T-test	—	−45.45***	11.86***	25.75***	23.91***

T-test values ***: significant at $P < 0.001$. ANOVA analysis: means with different superscripts (a, b, c, d, or e) are significantly different at $P < 0.05$. LSD: least significant difference.

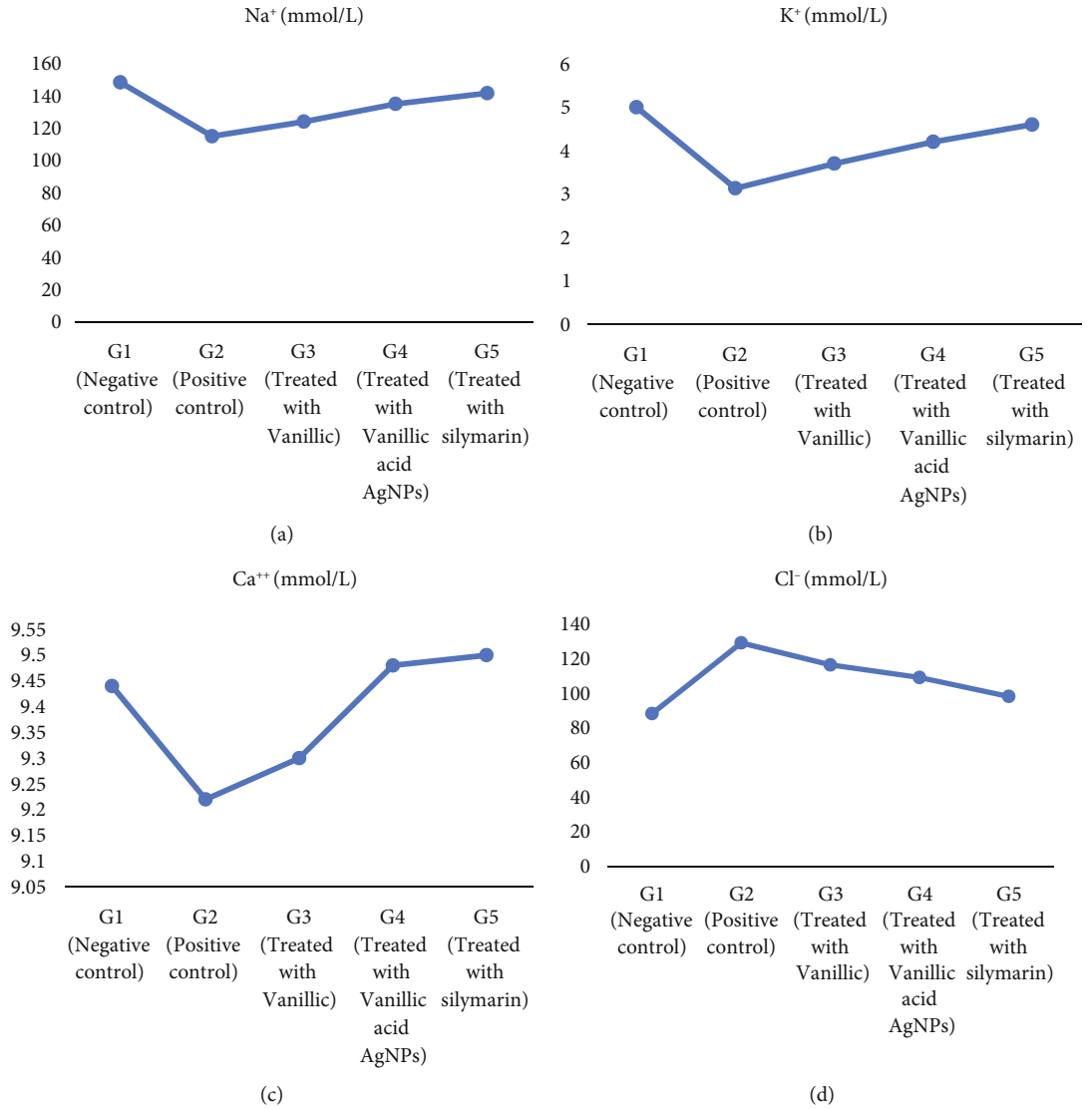


Figure 4: Effect of treating CCl_4-induced nephrotoxicity in male rats vanillic acid, vanillic acid AgNPs, and silymarin on serum electrolytes levels. (a) Na^+. (b) K^+. (c) Ca^{++}. (d) Cl^-.

Table 2: Effect of treating CCl_4-induced nephrotoxicity in male rats with vanillic acid, vanillic acid AgNPs, and silymarin on antioxidant enzymes (GST, GSH, SOD, and CAT) and TAC.

Antioxidant kidney tissue (U/g)	Statistics	G1 (negative control)	G2 (positive control)	G3 (treated with vanillic)	G4 (treated with vanillic acid AgNPs)	G5 (treated with silymarin)
GST (U/g)	Mean ± SE LSD 0.05 = 8.808	265.66 ± 3.72^{a}	86.66 ± 3.04^{e}	128.66 ± 2.83^{d}	169.66 ± 2.56^{c}	188.00 ± 1.59^{b}
	T-test	—	39.89***	−07.37***	−42.95***	−41.67***
GSH (mmol/g)	Mean ± SE LSD 0.05 = 7.407	273.66 ± 3.31^{a}	153.33 ± 3.65^{e}	235.00 ± 2.28^{b}	213.66 ± 3.80^{c}	180.00 ± 2.19^{b}
	T-test	—	051.25***	−024.79***	−286.18***	−005.76***
TAC (IU/g)	Mean ± SE LSD 0.05 = 0.329	4.10 ± 0.16^{a}	0.17 ± 0.02^{e}	0.98 ± 0.03^{d}	1.80 ± 0.13^{c}	2.56 ± 0.09^{b}
	T-test	—	20.41***	−41.92***	−15.25***	−23.19***
SOD (IU/g)	Mean ± SE LSD 0.05 = 12.552	221.00 ± 5.87^{a}	94.66 ± 4.26^{d}	149.66 ± 2.28^{c}	142.33 ± 3.10^{c}	194.66 ± 4.10^{b}
	T-test	—	13.95***	−08.31***	−14.04***	−11.95***
CAT (mmol/g)	Mean ± SE LSD 0.05 = 0.210	5.30 ± 0.10^{a}	1.50 ± 0.07^{e}	2.31 ± 0.01^{d}	3.14 ± 0.04^{c}	4.29 ± 0.04^{b}
	T-test	—	23.87***	−13.86***	−45.02***	−28.41***

T-test values ***: significant at $P < 0.001$. ANOVA analysis: means with different superscripts (a, b, c, d, or e) are significantly different at $P < 0.05$. LSD: least significant difference.

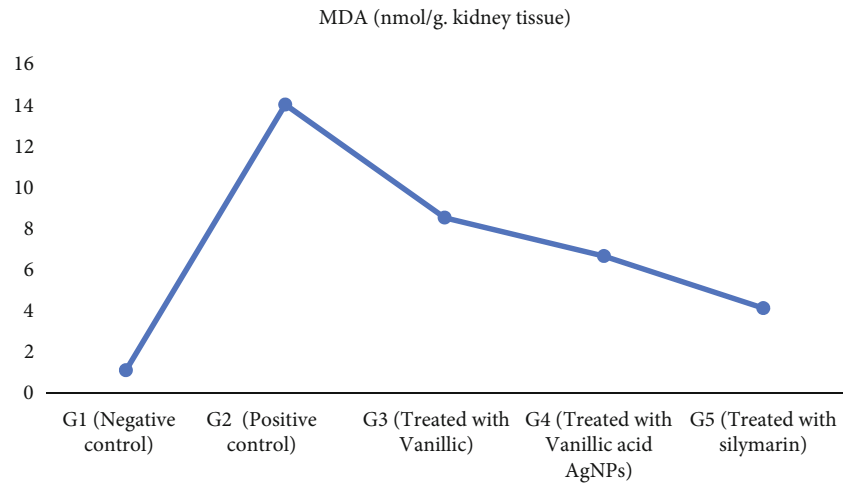


Figure 5: Effect of treating CCl_4-induced nephrotoxicity in male rats with vanillic acid, vanillic acid AgNPs, and silymarin on MDA.

In addition, Alamri et al. [12] reported that the maximum absorption was 450 nm.

On the other hand, Figure 2 shows the TEM image of the vanillic acid AgNPs showing a spherical shape with a diameter ranging from 12.1 to 15.3 nm [12, 39]. In addition, the synthesized vanillic acid AgNPs were seen based on the electron plasmon oscillations of their free surface as seen by the UV–Vis spectra. Moreover, the shape and size of the synthesized vanillic acid AgNPs peaks in the spectra are due to the (SPR peaks. In addition, locating the peaks at 450 nm confirmed that there was no significant difference in the size of the synthesized vanillic acid AgNPs [12, 39]. In addition, the result of the DLS analysis (Figure 3) shows that the synthesized vanillic acid AgNPs has an average particle size of 21.6 nm [12, 40].

3.2. Effect of CCl_4-Induced Nephrotoxicity on Renal Function Indices. Table 1 shows the effect of treating CCl_4-induced

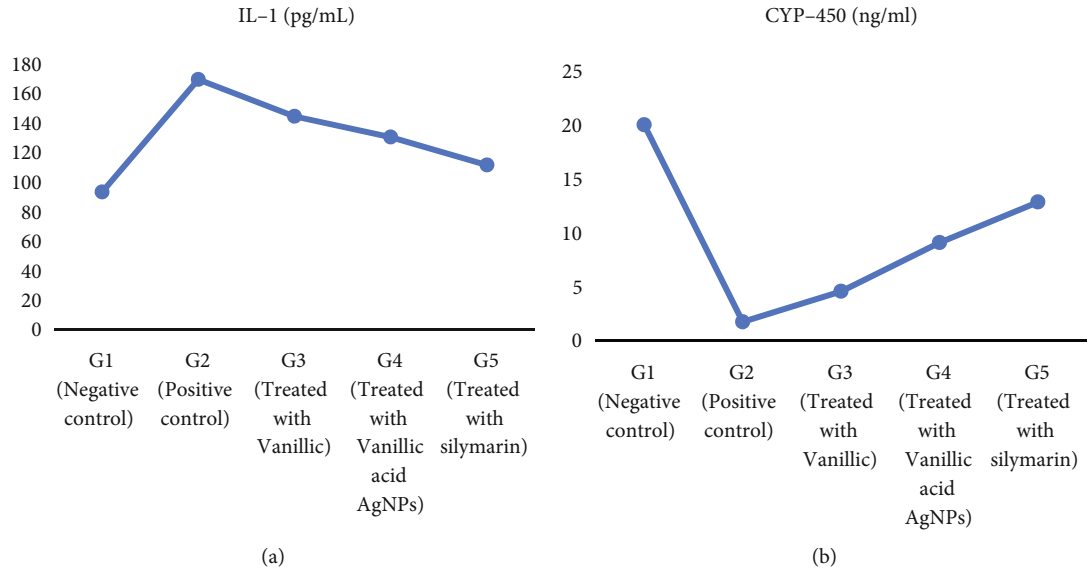


FIGURE 6: Effect of treating CCl_4-induced nephrotoxicity in male rats with vanillic acid, vanillic acid AgNPs, and silymarin on (a) serum IL-1 and (b) CYP-450 levels.

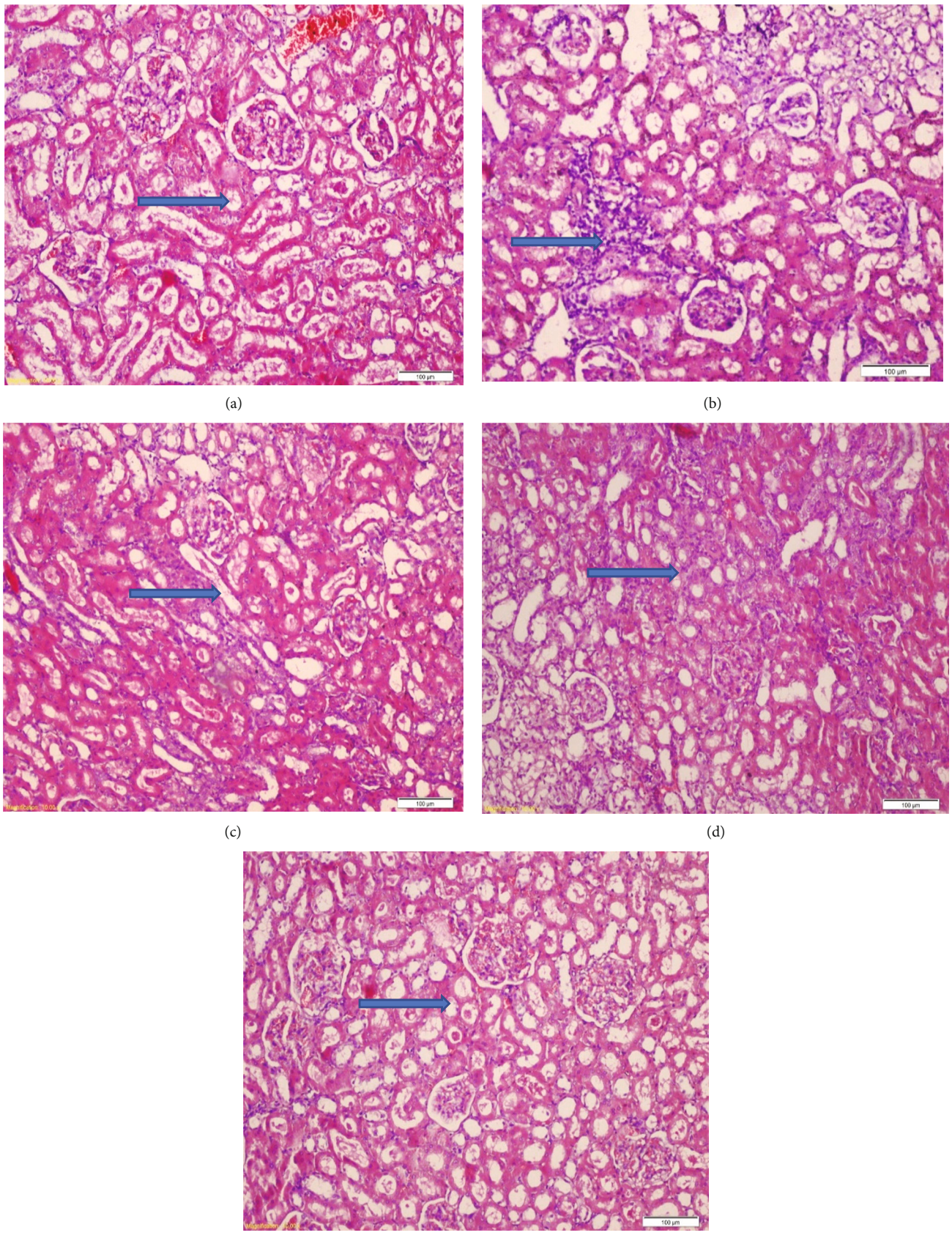
nephrotoxicity in male rats with vanillic acid, vanillic acid AgNPs, and silymarin on kidney function indices: serum creatinine, serum urea, and serum uric acid. The CCl_4-induced nephrotoxicity in the positive control (G2) significantly increases all kidney function indices compared with that of the negative control [30, 31]. While treating the nephrotoxic rats with vanillic acid, vanillic acid AgNPs, and silymarin in G3, G4, and G5, respectively, greatly ameliorated the altered renal parameters and nearly restored them to normal levels. Vanillic acid treatment in G3 and the AgNPs vanillic acid in G4 greatly ameliorated the altered kidney indices due to the antioxidant activity of vanillic acid conferred by its methoxy group [12, 17, 19, 21]. The treatment with AgNPs of vanillic acid in G4 was more effective than vanillic acid in G3 [12, 21, 22]. In addition, the treatment with silymarin in G5 was more effective than the other groups. Silymarin is known for its protective activity as an antioxidant and anti-inflammatory activity [35]. The current result is consistent with that of Rafieian-Kopaie and Nasri [30], they stated that silymarin prevents diabetic nephropathy. In addition, the result of the current study showed that the biosynthetic polymeric vanillic acid AgNPs increased the protecting activity of vanillic acid [27–29].

3.3. Effect of CCl_4-Induced Nephrotoxicity on Serum Electrolytes. The concentration of serum positive ions of sodium, potassium, and calcium was decreased, whereas the negative chlorine ions concentration was increased as a result of CCl_4-induced nephrotoxicity in the positive control group as shown in Figures 4a, 4b, 4c, and 4d and Supplemental Table S1 [30, 31]. However, this altered ions concentration was nearly restored after treatment with vanillic acid, vanillic acid AgNPs, and silymarin in G3, G4, and G5, respectively. Vanillic acid and vanillic acid AgNPs succeeded to restore the altered Na, K, Ca, and Cl ions due to the antioxidant effect of vanillic acid that reflected in restoring the kidney function parameters [19, 30]. The altered ions concentration was efficiently restored by silymarin treatment in G5 compared with vanillic acid in G3 and vanillic acid AgNPs in G4 thanks to the highly protecting and anti-inflammatory effects of silymarin to tissues that restored their normal functions [12, 30, 31].

3.4. Effect of CCl_4-Induced Nephrotoxicity on Antioxidants and Total Antioxidant Capacity. Table 2 shows the effect of treating CCl_4-induced nephrotoxicity in male rats with vanillic acid, vanillic acid AgNPs, and silymarin on antioxidants (GST, GSH, SOD, and CAT) and TAC [30, 31]. All antioxidant enzymes and TAC were decreased in the kidney tissue homogenate as a result of CCl_4-induced nephrotoxicity in the positive control group (G2) compared with the negative control [12]. Rats of the treated groups G3, G4, and G5 showed significant protection and the antioxidant enzyme activity and the TAC were increased, especially in the fourth and fifth groups that were treated with vanillic acid AgNPs and silymarin [12, 21, 31, 35].

3.5. Effect of CCl4-Induced Nephrotoxicity on Lipid Peroxidation as Revealed by MDA Level. In the positive control group (G2), lipid peroxidation as revealed by MDA level in the kidney tissue homogenate was increased as a result of CCl_4-induced nephrotoxicity in comparison with that of the negative control as shown in Figure 5 and Supplemental Table S2 [30, 31]. On the other hand, lipid peroxidation level was decreased with vanillic acid, vanillic acid AgNPs, and silymarin treatment in G3, G4, and G5, respectively, compared with that of the positive control. Treating with vanillic acid AgNPs and silymarin in G4 and G5 were more efficient than vanillic acid in G3 [12, 35].

3.6. Effect of CCl4-Induced Nephrotoxicity on IL-1 and CYP-450 Levels. Serum IL-1 level was increased, whereas CYP-



(a) (b) (c) (d) (e)

Figure 7: (a) The renal tissues of G1 with normal renal architecture and glomeruli (arrow). (b) The renal tissues of G2 with drastically injured renal tissues (arrow). (c) The renal tissues of G3 with mildly injured tissues (arrow). (d) The renal tissues of G4 with nearly normal renal tissues (arrow). (e) The renal tissues of G5 with nearly normal renal tissues (H&E, $X = 200$).

450 level was decreased as a result of CCl4-induced nephrotoxicity in the positive control group (G2) compared with that of the negative control (G1) as shown in Figures 6(a) and 6(b) and Supplemental Table S3. This result is consistent with that of El Rabey et al. [31]. The levels of IL-1 and CYP-450 were nearly restored to the normal values in G1 with treatment with vanillic acid in G3, vanillic acid AgNPs in G4, and silymarin in G5 [12]. In addition, silymarin was more effective than vanillic acid and vanillic acid AgNPs [12, 31, 35].

3.7. Histopathology. Figure 7(a) shows the normal renal tissue of the negative control revealing the normal histological structure of renal parenchyma, normal renal tissue, normal blood vessels, and interstitial with no histopathological changes and normal composition and normal blood vessels. The glomeruli were normal in structure and pattern, and the renal tubules were normal lining epithelium. In the second group G2 (the positive control group) the renal cortical tissue showed glomerular ischemia with shrinkage of vascular tuft, marked tubular atrophy, and moderate interstitial mononuclear inflammatory infiltrate as shown in Figure 7(b). This result is consistent with Elbakry et al. [30] and El Rabey et al. [31], they reported CCl_4-induced nephrotoxicity and with other studies confirming histological damage as a result of CCI_4-induced nephrotoxicity. In G3, which was treated with vanillic acid, the renal cortical tissue showed moderate glomerular ischemia, moderate tubular atrophy, and mild interstitial inflammation (Figure 7(c)). Figure 7(d) shows nearly normal renal tissues of G4 with nearly normal renal cortical tissue with mild glomerular ischemia, mild tubular atrophy, and minimal interstitial infiltrate. In G5, the renal tissues showed nearly normal cortical tissue with only very mild tubular atrophy (Figure 7(e)). Herbal active constituents, such as vanillic acid protected against nephrotoxic effects [15, 21]. In addition, the biosynthesis of vanillic acid in G4 showed more effective protection than vanillic acid in G3 [28, 29]. Due to its highly protective and anti-inflammatory effects, silymarin was used in protecting against a variety of hepatotoxic and nephrotoxic agents [12, 30, 31, 35].

4. Conclusion

In the current study, the nephrotoxic effect of CCl_4 was confirmed as revealed by the altered kidney function indices, electrolyte concentrations, antioxidant, lipid peroxidation, IL-1, CYP-450, and the injured kidney tissues. The biosynthetic vanillic acid, vanillic acid AgNPs, and silymarin were used in treating the induced CCl_4-nephrotoxicity in male rats. Vanillic acid showed a moderate protective effect against the CCl_4-induced nephrotoxicity compared with vanillic acid AgNPs and silymarin, which showed higher protection by nearly restoring both the biochemical and histological changes nearly to the normal state. In addition, silymarin showed the highest protection compared with vanillic acid and vanillic acid AgNPs. More studies are needed for an in-depth understanding of the exact nephrotoxic effects of CCl_4 and the exact protecting action of vanillic acid AgNPs and silymarin in protecting against CCl4-induced nephrotoxicity.

Abbreviations

AAN: Aristolochic acid nephropathy
AgNPs: Silver nanoparticles
CAT: Catalase
CYP-450: Cytochrome P-450
DLS: Dynamic light scattering
G1: A negative control group was intraperitoneally injected with 1 : 1 water to olive oil on the first and fourth day of every week
G2: A positive control group was intraperitoneally injected with 1 : 1 CCl_4 to olive oil on the first and fourth day of every week and left without treatment
G3: Intraperitoneally injected with 1 : 1 CCl_4 to olive oil on the first and fourth day of every week and was treated with vanillic acid
G4: Intraperitoneally injected with 1 : 1 CCl_4 to olive oil on the first and fourth day of every week and was treated with vanillic acid silver nanoparticles
G5: Intraperitoneally injected with 1 : 1 CCl_4 to olive oil on the first and fourth day of every week and was treated with silymarin
GSH: Glutathione reduced
GST: Glutathione-S-transferase
IL-1: Interleukin-1
MDA: Malondialdehyde
ROS: Reactive oxygen species
SOD: Superoxide dismutase
TAC: Total antioxidant capacity.

Data Availability

Data supporting this research article are available from the corresponding author on reasonable request.

Conflicts of Interest

The author(s) declare(s) that they have no conflicts of interest.

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Supplementary Materials

Table S1. Effect of treating CCl_4-induced nephrotoxicity in male rats vanillic acid, vanillic acid AgNPs, and silymarin on serum electrolytes levels. Table S2. Effect of treating CCl_4-induced nephrotoxicity in male rats with vanillic acid, vanillic acid AgNPs, and silymarin on malonaldehyde (MDA). Table S3. Effect of treating CCl_4-induced nephrotoxicity in male rats with vanillic acid, vanillic acid AgNPs, and silymarin on serum IL-1 and CYP-450 levels. *(Supplementary Materials)*

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