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Histological and Biochemical Effect of Galangin Conjugated Gold Nanoparticles on the kidneys Damage Induced by Carbon Tetrachloride of Adult Male Albino Mice

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Abstract

This study aimed to determine the histological and functional effects of Galangin (Gal) conjugated with Gold nanoparticles (AuNPs) on the kidneys of male albino mice treated with CCL_4. Gold nanoparticles were prepared chemically by the Turkevich method. The characterization of the prepared AuNPs and AuNPs+Gal was carried out using UV Spectrophotometry, X-Ray Diffraction (XRD), and particle size. For the *in vivo* study, 42 adult male albino mice were used and randomly distributed into seven groups, and the experiment was extended for 14 days. The first group (G1) was the control group without any treatment, the second group was injected intra-peritoneally (i.p.) with CCl_4 once a week until the end of the experiments, the third group was injected with AuNPs, the fourth and fifth groups were injected with Gal at 10 and 20 mg/kg concentrations, respectively, and the sixth and seventh groups were injected with AuNPs-Gal 10 mg/kg and AuNPs+Gal 20 mg/kg, respectively.

The results showed a spherical shape with an average size of 61.6 nm; the absorption spectrum was 535 nm; and the crystalline nature of the conjugated solution using XRD was confirmed, where the spectrum appears at 45.4, 38, 32, and 24 C°. *In vivo* study results recorded different histopathological alterations in the kidney tissue of mice treated with CCL_4, and treatment with

AuNPs+Gal significantly improved the histology architecture in a dose-dependent manner. Additionally, the levels of urea and creatinine in blood serum were much higher in the CCL_4 group, and they were significantly reduced to normal levels after treatment with Cal and conjugated AuNPs+Gal. These findings revealed that galangin loaded on gold nanoparticles positively improved the damage induced by CCL_4, providing a potentially effective natural treatment following extensive and appropriate developments.

Keywords: Galangin, AuNPs, CCL_4, Albino mice, Kidney histopathology

1. Introduction

Nephritis caused by chemicals, such as industrial detergents, household disinfectants, insecticides, and others, is a serious threat to human health and a global problem [1]. Inflammation of the kidneys caused by CCL_4 in mice is a well-established experimental model of kidney infection, in which inflammatory cells such as T cells, natural killer cells, macrophages, and neutrophils infiltrate, producing a variety of inflammatory cytokines toxic to the kidneys, including interferon (IFN -γ), tumor necrosis factor (TNF-α, β) and interleukin (IL-1 and IL-12), eventually leading to apoptosis, necrosis of kidney cells, and a marked rise in transaminases in the blood [2]. Galangin (3,5,7-trihydroxyflavone)) is a natural active flavonoid active pharmaceutical ingredient extracted from the roots of the *Alpinia officinarum Hance* plant, a plant that has been used in Asia for centuries as a seasoning and as an herbal medicine for various diseases such as vomiting, the removal of colds, and pain [3]. The pharmacological activity of galangin has been studied and includes anti-inflammatory, antioxidant, bactericidal, antioxidant, anti-fibrosis, and antitumor [4]. However, galangin has been reported as a weak soluble in water, which makes it poorly absorbed, which may limit its clinical usability and require a delivery system to reach its therapeutic goal [5]. With the development of nanotechnology in biology and medicine, and due to the features of nanoparticles, the demand for their integration with biomolecules has increased to form conjugated systems because these particles are versatile in applications including catalysts, drug delivery, treatment, imaging sensing, and control of the structure of biomolecules [6]. The unique properties of gold nanoparticles (AuNPs) coupled with their multiple surface functions have enhanced their extensive applications in biotechnology, particularly drug delivery systems, because AuNPs are biocompatible and possess a surface large enough to load the concentration of the delivered drug through their role as carriers of medicines [7].

Furthermore, AuNPs have been reported as a promising mineral that has been extensively investigated for the delivery of anti-cancer drugs and anti-oxidant [7, 8]. Since AuNPs have a hydrophilic and hydrophobic nature, they easily interfere between cells and change cell function, but their effects generally depend on the size of these nanoparticles [9]. Connor *et al*., (2005) found that the administration of gold nanoparticles in humans showed no toxicity despite being ingested in cells [10]. Furthermore, the experiment revealed that the injection of gold nanoparticles into rats from the tail vein was distributed in different organs such as the brain, lung, liver, heart, kidneys, blood, testicle, and berry but showed no toxicity [11]. Umair *et al*. (2016) considered nanoparticles of gold to be biologically inert [12]. Nanoparticles (NPs) have certain properties that make them suitable for imaging, treatment, and drug delivery [13, 14, 15]. Since AuNPs have shown antioxidant activity and drug delivery, Gal has therapeutic potential, and given the small amount of research done to find out the effects of Gal combined with AuNPs, this pairing is designed to

assess its effect on the function and synthesis of the kidneys of albino mice caused by carbon tetrachloride (CCl_4) through (i.p) injection.

2. Materials and Methods

2.1. Chemicals

Galangin was purcushed from Sigma (St.MO.USA), (AUCl4.3H2O) and Citrate sodium were provided by Fluka (USA), urea kits from LineaR Chemicals, S.L (Spain) and creatinine reagent kits from BEACON (India). All other reagents were obtained from Sigma (St. USA).

2.2. Gold nanoparticles preparation and Galangin conjugation

Gold nanoparticles were chemically prepared following the Turkevich Method, briefly, 0.1 mM of aqueous HAucl4.3H2O solution was let to boil, and 1% trisodium citrate was added and left to develop the red nanoparticle solution [16]. Galangin (10 and 20 mg) was then dropped into AuNPs (1:9 v/v) at 50 °C with stirring for 5 minutes.

2.3. Characterization of AuNPs and AuNPs+Gal

Several techniques have been used to characterize nanoparticles, including the UV- Vis Absorption Spectrophotometry, X-Ray Diffraction (XRD), and particle size.

2.4. Animals

The study was conducted on 42 healthy Swiss male albino albino mice (*Mus musculus*), age 8–10 weeks, with an average weight 25 ± 5 g, obtained from the Razi Center of the Ministry of Industry and Minerals/Industrial Research and Development Authority. All animals were placed under appropriate laboratory conditions (12 hours of darkness and 12 hours of lighting) and a temperature of 25 ± 5 Cº, and offered *ad libitum*. The experiment has the agreement of Committee on Animal use of the University of Baghdad No.7495 in 28/12/2021.

2.5. Experimental Groups

The experimental animals were divided into seven groups (6 in each) and injected intraperitoneal (IP) for 14 days with CCL_4 once/week (apart from the control group) and distributed as follows:

G1: control group, not received any treatment.
G2: injected 0.1 ml of CCl_4 once/week.
G3: injected 0.1 ml of AuNPs, after CCl_4 injection.
G4: injected 0.1 ml of Galangin at concentration 10 mg/kg, after CCl_4 injection.
G5: injected 0.1 ml of Galangin at concentration 20 mg/kg, after CCl_4 injection.
G6: injected 0.1 ml of AuNPs-GAL at concentration 10 mg/kg, after CCl_4 injection.
G7: injected 0.1 ml of AuNPs-GAL at concentration 20 mg/kg, after CCl_4 injection.

2.6. Blood samples collection

Blood samples were collected the next day after the end of the injection period from all study animals through a heart puncture stab after the animals were partially anesthetized with chloroform. Samples were then placed in tubes containing gel tubes and left for half an hour, followed by centrifugation at 4000 rpm for 20 minutes. Serum was then transported in other plastic tubes and kept in 20 C° below zero until used [17]. The urea and creatinine levels in the serum were measured following the manufacturer of the kit protocol.

2.7. Histological study preparation

At the end of experiments, animals were sacrificed by cervical dislocation, and the right and left kidneys were removed, washed, and the weights of the organs were then recorded. Samples were

fixed with formalin (10%). After dehydration in ethanol alcohol ascending grades of ethanol and clearing with xylene, samples were embedded in Paraffin wax. Prepared slide samples were stained with Hematoxylin-eosin (H&E) according to standard histological protocol [18]. Slides were observed and photographed under 40X Olympus microscopy (Germany).

2.8. Statistical analysis:

Statistical analysis was performed using Social Package for Social Sciences (SPSS) version 24. Analysis of variance (ANOVA) was applied to calculate statistically significant differences between the groups, followed by the least statistically significant differences (LSD), and the significance was accepted at $P \leq 0.05$.

3. Results

3.1. Preparation and characterization of AuNPs and AuNPs+Gal

Tetra-chloroauric acid (HAuCL4.3H2O) was mixed with tri-sodium citrate as a reducing mediator; color changes from yellowish to dark red indicate the creation of AuNPs, as shown in **Figure1.** Then Galangin was progressively added to the AuNPs solution under heating condition and the color changed from red to viole indicating the conjunction of Galangin in **Figure1**.

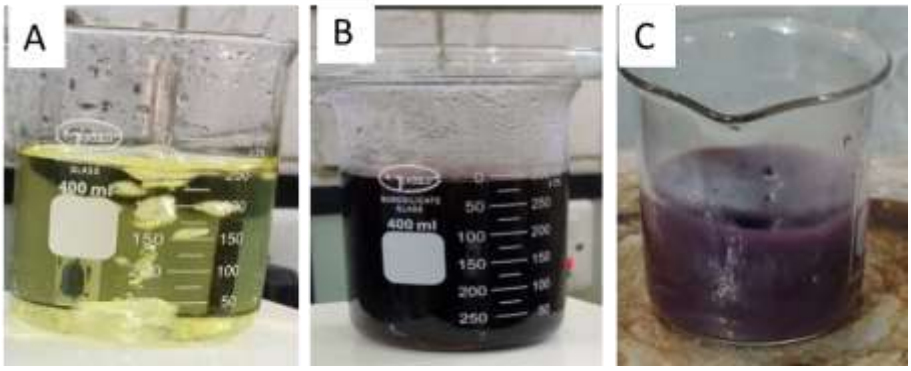

Figure 1. Color changes throw-out gold nanoparticles preparation and galangin loaded. (A) Tetrachloride acid (HAuCL4.3H2O), (B) AuNPs with tri-sodium citrate as a reducing medium, and (C) AuNPs+Gal.

Following AuNPs preparation and galangin loading, the characterizations were carried out, and the results of UV-Vis spectrophotometry revealed a peak at 522 nm for the AuNPs, while the maximum absorbance for the AuNPs+Gal was at 535, as shown in **Figure 2.** This minor shift is due to the polymer layer, which changes the refractive index of the AuNPs. The results are in agreement with Yang *et al*., (2018) who reported that the citrate reduction process can synthesize AuNPs at a range of 520-535 nm depending on the gold-to-citrate ratio [19] .

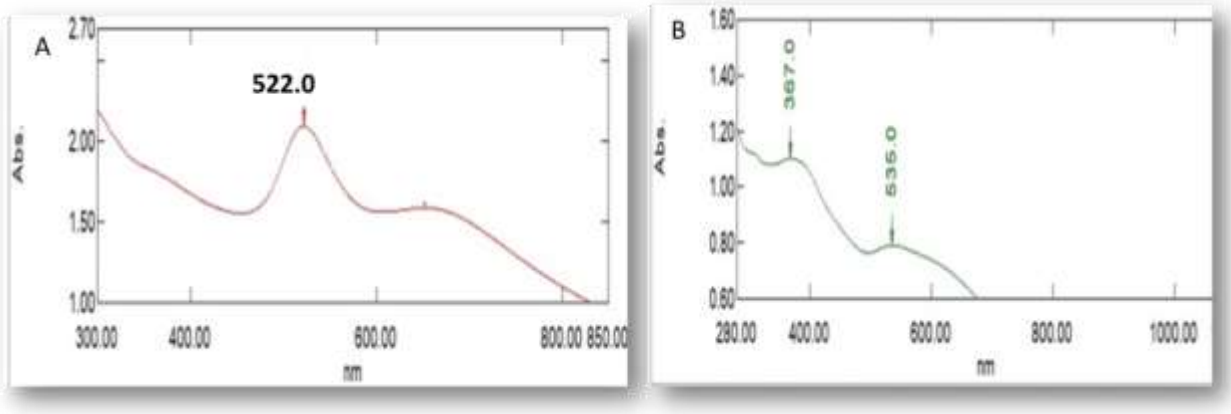

Figure 2. UV spectra of prepared (A) AuNPs and (B) AuNPs+Gal .

The resulting Dynamic light spectra (DLS) of the AuNPs, showed an average size of about 33.8 nm. While the results of the coupling examination showed that the average pairing particle size was 61.6 nm (**Figure 3).**

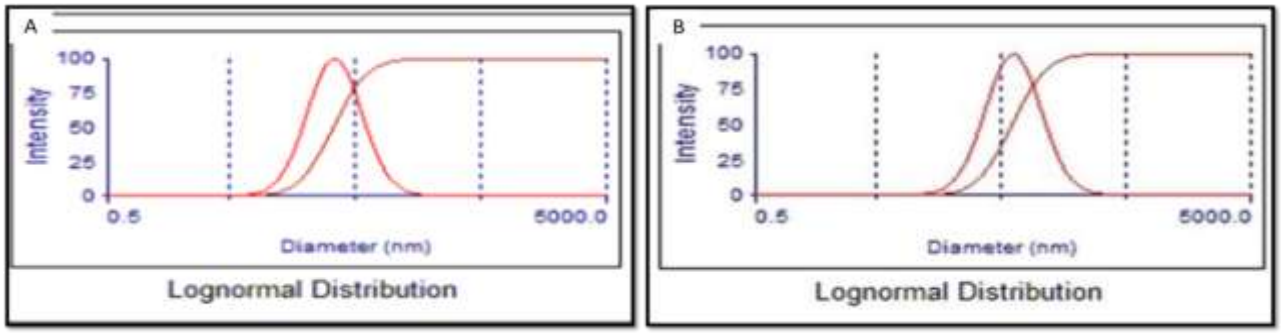

Figure3. (A) The particle size distribution AuNPs revealed that the average size was 33.8 nm. (B) AuNPs+Gal have a size range of 61.6nm.

The crystalline nature of the AuNPs solution was emphasized using XRD, where the spectrum shows two peaks at 45.4°C and 38°C. the crystalline nature of the coupling solution using XRD was confirmed, where the spectrum appears at 45.4, 38, 32, and 24 ° C. which represents the crystalline nature of Galangin and gold nanoparticles (**Figure 4).**

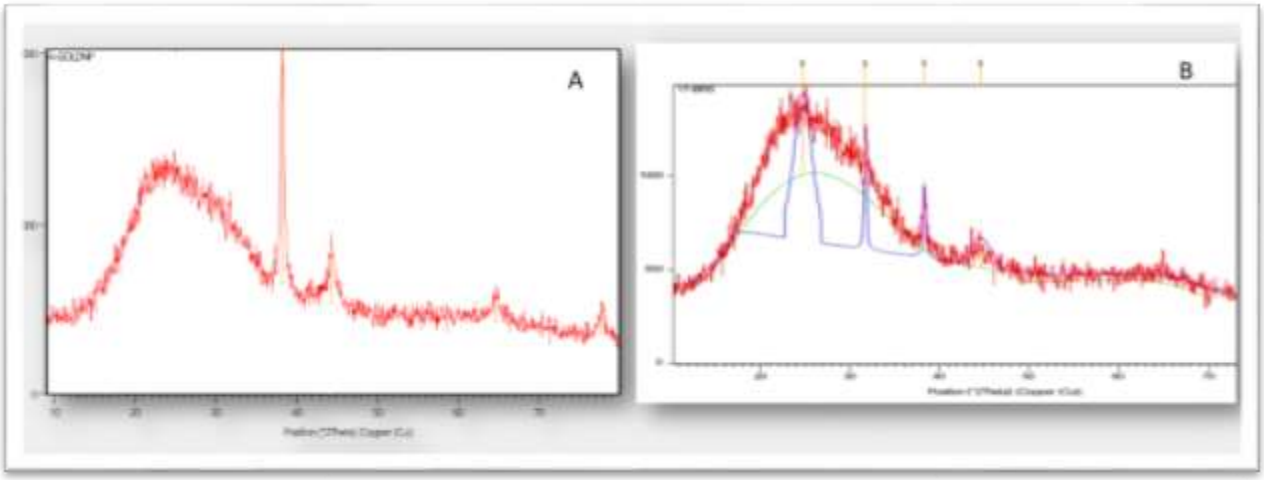

Figure 4. X-ray diffraction (XRD) of prepared (A) AuNPs where the spectrum shows two peaks at 45.4 C ° and 38 C°. (B) AuNPs-Gal where the spectrum appears at 45.4, 38, 32, and 24 C °.

3.2. Animal Body weights

The results of the current study showed a significant decrease (P≤0.01) in the weight gain of all treated groups of animals at the end of extermination compared to the control group. Supplementation with AuNPs or AuNPs+Gal significantly reduced the body weight gain compared to the groups of animals treated with CCL4 over 14 days of treatment, as shown in **Table 1.**

Table 1. Body weight gain after 14 days of treated animals for 14 days.

Groups	Weight gain (gm)
Control	4.517 ± 0.265
CCL_4	2.668 ± 0.15 *b
Galangin 10 mg	2.118 ± 0.354 *
Galangin 20 mg	2.645 ± 0.89
AuNPs	-1.515 ± 1.093 *a
Galangin 10 mg + GNPs	-0.910 ± 1.206 *c
Galangin 20 mg + GNPs	-0.182 ± 0.39 * d

*** Significant differences at *P≤0.05* vs control group, different letters means significant difference between groups. Data represent mean ± SD.**

3.3. Kidney weight index

The study recorded a significant increase (*P≤0.05*) in kidney weight index in the group of animals injected with 10 mg/kg Galangin alone and Galangin conjugated with gold nanoparticles.

Table 2. Shows the average weight of the kidneys relative to the body weights of the experimental animals 14 days after the treatment**.**

Groups	Kidney/Wb (gm) (Mean ± S.E.)
Control	0.0083 ± 0.0002
CCL4	0.0086 ± 0.0004
Gal 10 mg	0.0095 ± 0.0004 * a
Gal 20 mg	0.0077 ± 0.0003 b
Au NPs	0.0085 ± 0.0003
AuNPs + Gal 10 mg	0.009 ± 0.0003 a
AuNPs + Gal 20 mg	0.007 ± 0.0002 b

*** Significant differences at *P≤0.05* vs control, different letters means significant difference between groups. Data represent mean ± SD.**

3.4. Urea concentration test

The statistical results of this study showed a significant increase (P≤0.05) in the concentration of urea in the serum of mice in the group of animals injected with CCL4 solution, where 62.027 (mg/dL) was recorded compared to all other experimental groups, where the control group animals recorded 25.130 (mg/dL), however, there was no significant difference within groups.

Table 3. Shows changes in urea concentration ratio (mg/dL) among all groups of experimental animals 14 days after treatment

Groups	Blood Urea (mg/dL) (Mean ± S.E.)
Control	25.130 ± 1.021
CCL4	62.027 ± 3.788 *
Gal 10 mg	25.981 ± 1.731
Gal 20 mg	24.048 ± 1.759
AuNPs	24.500 ± 1.099
AuNPs + Gal 10 mg	23.994 ± 0.548
AuNPs+ Gal 20 mg	21.327 ± 0.197

*** Significant differences at *P≤0.05* vs control**

3.5. Creatinine concentration test

The study recorded a significant increase (P≤0.05) in the concentration of creatinine in the serum of the group of animals injected with CCL4 solution compared to all other treated groups. The statistical difference was not significant within the treated groups.

Table 4. Shows changes in the concentration of creatinine (mg/dL) among all experimental animals after treatment for 14 days.

Groups	Serum Creatinine (mg/dL) (Mean ± S.E.)
Control	0.427 ± 0.046
CCL4	2.035 ± 0.172 *
Gal 10 mg	0.523 ± 0.088
Gal 20 mg	0.358 ± 0.069
AuNPs	0.346 ± 0.086
AuNPs +Gal 10 mg	0.289 ± 0.033
AuNPs + Gal 20 mg	0.238 ± 0.053

*** Significant differences at *P≤0.05* between CCL4 group vs all other groups**

3.6. Histological changes

The control group shows a normal histological structure of the kidney with cortex and medulla and the presence of renal corpuscles, which appear normally in the cortex area and are formed by glomeruli surrounded by Bowman's capsule. (**Figure 5,A)**. The G2 group injected with CCL_4 once a week for 14 days sections showed various alterations, including blood congestion, atrophy, and necrosis in some cells lining the tubules. As well as Sloughing and separation of epithelial endothelial cells of the tubules from the basement membrane and shrinkage of some glomeruli (Figure 5, B). Furthermore, a group of animals injected with gold nanoparticles (G3) showed an expansion of Bowman's capsule in the renal particle and deformation in the distal and proximal tubules, with necrosis of some cells lining the renal tubules **Figure 5**C.

Kidney tissue in the group treated with galangin at two concentrations of 10 and 20 mg/kg (G4 and G5) showed almost normal architecture with mild alterations, including necrosis and atrophy in some renal tubule cells, as in **Figures 5D** and **E**.

Kidney tissue in the group of mice treated with CCl_4 followed by treatment with AuNPs+Gal conjugation solution at a concentration of 10 and 20 mg/kg showed some similarity in structure to that in the control group, in which the epithelial cells in the glomerulus did not show changes, however, some other alterations were indicated, such as blood hemorrhage, damage to the tubules, renal space enlargement and atrophy, as shown in **Figure 5 F and G**.

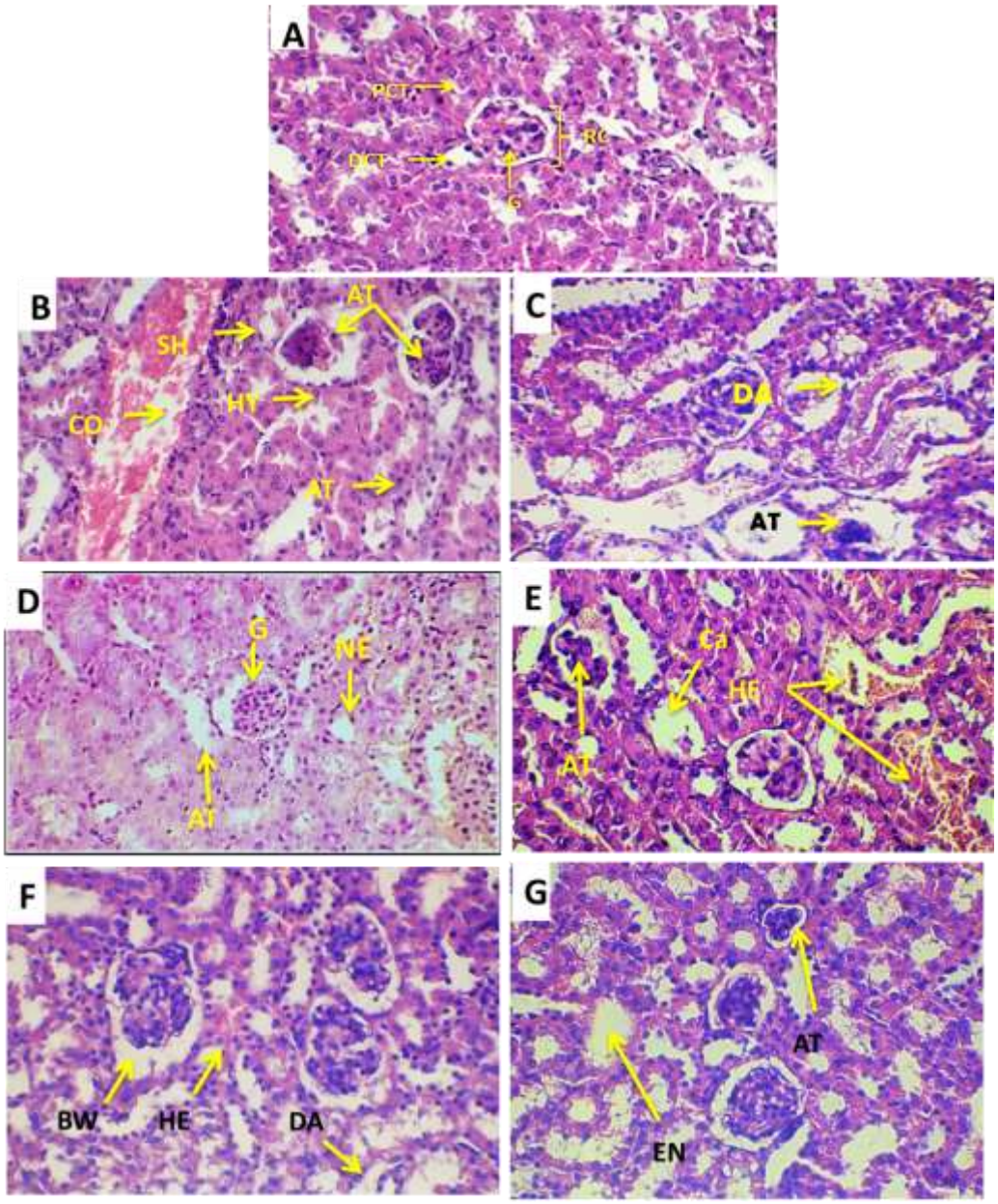

Figure 5. Transverse sections of kidneys in male Swiss albino mice in different groups. (A) The cortex of the control group displayed renal corpuscle (RC) with glomerulus (G), distal convoluted tubules (DCT), and proximal convoluted tubules (PCT); (B) The abnormal kidney structure in mice that were injected with CCL4 for one week for 14 days showed blood congestion of blood vessels (CO) and atrophy in cells lining the tubules (AT). shrinkage of some glomeruli (SH), (AT) atrophy in glomeruli, nucleus hypertrophy (Hy), (C) showed an expansion of Bowman's capsule in the renal particle and deformation (DA) in the distal and proximal tubules with atrophy (AT) of some cells lining the renal tubules in the group of animals injected with gold nanoparticles, (D and E) showed almost normal architecture with mild alterations including necrosis (NE) and atrophy (AT) in some renal tubule cells and blood hemorhage (HE), and deposition of calcium in the lumen of the renal tubule (Ca). Galangin with two concentrations of 10 and 20 mg/kg (F and G) with AuNPs + Gal 10–20 mg/kg caused hemorrhage (HE), renal space enlargement (EN), damage to the tubules (DA), widening of the Bowman's space (BW), and atrophy (AT).

4. Discussion

In the present study, the effects of gold nanoparticles with and without galangin and galangin alone were investigated in kidney mice damaged by CCL4. The results demonstrated that loading galangin on gold nanoparticles exhibited an improvement in kidney histology and function alterations induced by CCL4.

The presence of the chemical synthesis of AuNPs has been demonstrated via various descriptions in **Figure 1**, where AuNPs were present from the reaction of tetra-chloroauric acid with trisodium

citrate. The results were in agreement with previously published research, which reported that the wavelength peaks of AuNPs typically occur between 520 and 530 nm depending on the diameter of gold nanoparticles, occur at a longer wavelength for larger gold nanoparticles, and that changing the concentration of gold nanoparticles only changes the absorption volume (peak height) but not the peak wavelength. This summit confirmed the preparation of spherical nanoparticles [20, 21]. According to the current study using the dynamic light scattering (DLS) technique, particle size analysis revealed that the sizes of AuNPs and AuNPs-Gal (33.8 and 61.6 nm, respectively) appeared, and they correspond to the analysis data of absorption spectroscopy, which have a characteristic range at 522 and 535 nm and are within the reported nanoparticle range for photoluminescence [22]. The bandwidth rises with the increase in unit size [23], and nanoparticles exhibit different optical bonds depending on the particle size [24]. The crystalline nature of the AuNPs solution was confirmed using XRD, where the spectroscope shows two peaks at 45.4 C° and 38 C°. AuNPs+Gal. XRD showed where the spectrum appears at 45.4, 38, 32, and 24 C°. This represents the crystalline nature of galangin and gold nanoparticles. XRD is a suitable tool for defining the physical nature of particles [25]. These values are constant with a study to evaluate the crystal state of galangin, where characteristic peaks such as 23.4, 25.7, and 27.8 C° were formed, indicating a good crystal phase [26]. It is also consistent with a study on gold, and the results were 38.22, 64.71, and 44.42 C° [27]. Thus, chemically manufactured AuNPs indicate acceptable stability with a reading not less than the desired stable expression. The current results are consistent with previous studies. No additional peak was observed at the diffraction tops, which indicates that the manufactured AuNPs were highly pure without any pollution.

In this study, CCl_4 did not affect kidney weight and was close to the weight of the control group, due to the lack of a significant difference in body weight for this group compared to the control group, which is due to the low concentration of CCL4 (V-V) in olive oil and to the short duration and low dose given to animals once a week for fourteen days. Comparison with [28] study that found a significant decrease in body weight in the CCL_4 group compared to the control group due to high concentration 1-1 (V-V) with olive oil at a daily dose for 21 days. Reach [29] reported that the normal weight of the kidneys and the degree of pathological damage are good indicators of kidney function in nephrotoxicity caused by toxic chemicals. As for the group of animals injected with AuNPs solution, the study recorded a decrease in kidney weight, which may be due to a significant loss of fat and inhibition of inflammatory effects caused by carbon tetrachloride. These results are consistent with a study by [30], where they indicated a reduction in kidney weight after three days after injection of AuNPs. In animals injected with Gal at 20 mg/kg, despite the low concentration and short duration, kidney weights showed a reduction compared to the control group due to the antioxidant effect of Gal. This is confirmed by researcher [31]. Kidney weights were reduced with Gal at concentrations of 50, 100, and 200 mg/kg for 40 days. The effect of AuNPs + Gal conjugation was pronounced for the two concentrations, as the average kidney weights and body weights were low for the rest of the groups, indicating the effect of delivering galangin to the kidneys by gold nanoparticles and the effect on lower body weight and kidney weight.

As for the measurement of urea and creatinine in this study, the results gave similar levels to most previous studies. CCL_4 was found to show high renal toxicity due to histological and renal cellular changes that affected tubular absorption capacity. As indicated by the study [34], which used the same concentration as our study, the injection of mice for 1 ml/kg body weight with intraperitoneal

CCL_4 (IP) resulted in high levels of creatinine, urea, and uric acid in plasma, and histological examination of the kidneys showed glomerular hypertrophy and tubular dilatation. Also, when the dose was increased, higher levels were given in the study of [32], where it was reported that injecting mice with a single dose of CCL4 at a concentration of 10 ml/kg resulted in higher levels of urea, creatinine, and uric acid, as well as a higher level of sodium, potassium, chloride, and calcium. It also led to a significant decrease in the activity of antioxidant enzymes [33].

Another study reported that treating white rats with 2 ml/kg of CCL_4 resulted in a significant rise in the level of liver enzymes (glucose, uric acid, urea, creatinine, and malonaldehyde) compared to the control group and a significant decrease in the level of antioxidant enzymes (glutathione, catalysis, ascorbic acid, and pyrooxydone glutathione) in liver and kidney tissue [34]. The current study showed a protective effect of AuNPs by eliminating the toxic effects of CCL4, evident from the results of urea and creatinine levels, where a previous study indicated that there were no significant differences in urea and creatinine levels between groups of animals injected with AuNPs compared to animals injected with CCL_4 [35]. As for galangin in two concentrations, it showed a protective effect of toxicity as shown by CCL4, which is consistent with the result reached by [36], where it was found that galangin acts as an anti-inflammatory and antioxidant and is not toxic to cells. As for the conjugation of AuNPs and Gal, which is the purpose of this study, it was found that there is a significant prevention of the toxicity effect of CCl_4 through the ratios of low urea and creatinine levels compared to CCL4 and close to the proportions of the control group and relatively lower levels. The reason may be due to the ability of gold nanoparticles to combine with different molecules, high stability, biocompatibility, and the formation of bonds with amine and thiol groups, in addition to the large surface area relative to the size that allows loading drug to the inside of the cell [37]. Histological evaluation showed abnormalities in cells and renal tubule structure in the CCl_4 group, but gold nanoparticles did not show a significant protective effect, while animals treated with galangin at two concentrations showed slight improvements in histological structure, while animals treated with conjugation solution had a better protective effect in the tissue as well as improved kidney functional indicators. [35] pointed to the complete destruction of the proximal and distant tubules, an increase in capsule size in the renal corpuscle, hyperplasia of the cells lining the renal tubules, semi-normal renal particles, the presence of hyperemia in the renal tubules, and glomerular contraction when animals were treated with 100 PPM for 14 days due to nontoxicity that causes functional and structural disorders, but when using a concentration of 10 PPm, no severe toxicity was shown. [38] showed that exposure to AuNPs caused pathological changes in the form of contractile and deformed glomeruli, dilated tubes, edema secretions, mild necrosis, and infiltration of inflammatory cells, but pathological histological changes in the kidneys did not reach statistical significance due to high differences within the group.

5. Conclusions:

The current study found that Galangin can be conjugated to gold nanoparticles and have therapeutic action and high efficiency by retrieving normal ratios of renal enzymes and histological structure at a high rate after pathological exposure, and the effectiveness of AuNPs+GAL was very high compared to AuNPs and Galangin-free.

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