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RESEARCH ARTICLE

Nephrotoxicity: Protection of Silica Nanoparticles Loaded Sodium Salicylate and Ginger in Experimental Rats

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Abstract

Background: Ultrasound-assisted sol–gel method was used to prepare hollow structured porous silica Nano emulsion loaded with sodium salicylate as a model drug. Objective: This work was extended to achieve the target of the current work via investigating the protective role this Nano emulsion model as anti-inflammatory drug or ginger for its antioxidant effect against cisplatin-induced nephrotoxicity in male albino rats. Results: The results clarify that the Nano emulsion model was synthesized using ultrasonic assisted with small size and well stabilization as proved by TEM and DLS analysis. Additionally, blood urea and serum creatinine were increased after cisplatin injection with disorders in oxidative stress and rising of homocysteine levels. Also, histopathological changes of the kidney tissue were observed. These changes back to normal via treatment with silica nanoparticles loaded sodium salicylate (Si-Sc-NPs), ginger or both. Oil /water Nano emulsion of (Si-Sc NPs) and ginger showed a protective and promising preventive strategy against nephrotoxicity due to their antioxidant and anti-inflammatory effects.

Keywords: *Drug nephrotoxicity, Silica nanoparticles, Sodium salicylate, Ginger, Cisplatin.*

Introduction

Humans take the drugs for health reasons, it is particularly warranted that all useful drugs will make undesirable effects, but some can make positively very dangerous effects. With the complexity of some of these diseases, multidrug treatments are elevating, with a high probability of adverse effects of different organs or systems [1]. The kidney is a major target for drug-induced toxicity. The renal proximal tubular cells (PTC) are frequently influenced due to their roles in glomerular filtrate concentration and drug transport [2]. Many drugs used in markets including anti-cancer drugs, antibiotics, immune suppressants and radiocontrast agents are nephrotoxic and injure PTC[3]. Drug which induced nephrotoxicity can lead to acute kidney injury (AKI) or chronic kidney disease (CKD) and is a main problem for clinicians**.** Development of less nephrotoxic drugs is defying due to the fact that the foretelling of nephrotoxicity during drug development remains difficult. Most drugs found to cause nephrotoxicity give toxic effects by many pathogenic mechanisms. Those in close altered intra-glomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic micro-angiopathy [4]. Knowledge about offending drugs and their specific pathogenic mechanisms of renal injury are critical to recognize and prevent drug-induced renal impairment. Cisplatin (cis-diamminedichloroplatinum II) is example of a drug which displays multiorgan toxicity with redox imbalance of a possible mechanism and is considered one of the platinum-containing chemotherapeutic agents that are highly effective antineoplastic drug [5]. Commonly, it is used in treatment of cancers [6]. Nephrotoxicity is reckoned the main and specific dose-limiting side effect of cisplatin. This drug is cleared in the kidney by both tubular secretion and glomerular filtration [7].

The proximal tubules are the main target of cisplatin in kidney, as it accumulates and causes cellular damage [8]. The in vivo mechanisms as nephron toxicity induced by cisplatin are complex and involve oxidative stress, inflammation, fibro-genesis and apoptosis. High doses of cisplatin result in necrosis in the cells of the proximal tubules, while low doses result in apoptosis [9].Oxidative stress injury is actively entangled in the pathogenesis of acute kidney injury induced by cisplatin. Reactive oxygen species (ROS) act directly on cellular components such as proteins, lipids and DNA to destroy their structure [10]. The lipid components of the cell membrane are destroyed by the free radicals through peroxidation and denature proteins leading to enzymatic inactivation and result in mitochondrial dysfunction [11].

Cisplatin may also inhibit antioxidant enzymes as glutathione peroxidase, superoxide dismutase and catalase. Therefore, the evaluation of nephrotoxicity is important for determination of the safety of drug nominees. Furthermore, cisplatin activates some of inflammatory changes which mediate renal injury and coordinates the activation of a large numbers of chemokines and cytokines in the kidney. Thereafter, the use of anti-inflammatory or antioxidants agents may play a very important role in preventing the cisplatin induced nephrotoxicity [12]. Several urinary tests as blood urea and serum creatinine are considered the common clinical pathology markers for the detection of kidney injury. However, neither marker is very specific or very sensitive, as there is usually a significant loss of renal function and renal mass before increases of urea and creatinine [13].

Homocysteine (hcy) is a sulfur-containing amino acid formed during the metabolism of the essential amino acid methionine. Hcy may directly cause renal vascular damage throughout the production of ROS, endothelial injury, effects on vascular smooth muscle cells, reduction in plasma, levels, tissue interstitium adenosine, mesangial cell proliferation and apoptosis [14]. The models of experimental animal of homocysteine-induced glomerular damage have also been developed [15]. Shankar et al [16].Found the subjects with CKD have higher levels of serum hcy than subjects with normal renal function. Serum hcy levels are negatively associated with glomerular filtration rate (GFR). Some evidence proposes that the apoptosis and inflammatory mechanisms play a serious role in the pathogenesis of drug nephrotoxicity [17, 18]. Therefore, there is a great need for the presence of the treatment ameliorate the toxic effect of drugs that the patient is forced to take them for long periods or permanently. Salicylates are widely applied in the treatment of inflammatory conditions such as rheumatic fever and rheumatoid arthritis. The anti-inflammatory actions of salicylates and other non-steroidal anti-inflammatory agents have generally been attributed to their inhibition of cyclooxygenase activity and prostaglandin synthesis. Many extractions from natural products were used as antioxidants against nephrotoxicity [19].

It has been shown that dietary antioxidants may detoxify ROS and decrease their side effects. Some of natural products have been used to for protection against the toxicities induced by drugs. Herbs are considered safe and proved to be efficacious against various human diseases and their medicinal uses have been gradually elevating in developed countries. *Zingiberofficinale* roscoe (ginger) belonged to Zingiberaceae family, is a free radical scavenger and has been shown to be effective on injury in the rat's kidney [20]**.** Ginger extracts are rich in shagaols and gingerols that show anti-inflammatory, anti-carcinogenic and anti-oxidant proprieties under '*in vitro* 'and "*in vivo* 'systems [21]. Recently, hollow structured porous nanospheres integrating hollow interior with porous shell into one nanostructure have attracted much research attentions due to their unique properties, such as low density, good permeability, high surface area and excellent loading capacity.

The void in the hollow structures can provide space for loading of guest molecules or particles, making them attractive in drug delivery. Scheme 1 represents the steps for the formation of silica nanoparticles loaded sodium salicylate. Ultra-sonication has been used for the preparation of hollow structured porous via the creation of silanol species through free radical process providing the strong shearing of the initial oil-in-water macro emulsion. The strong shearing action on oil-in-water droplet would produce long and thin structure which finally breaks up

into nanodroplets. Hereby, the current research was designed to prepare oil/water (O/W) nanoemulsion of compartmentalized hollow silica nanospheres by ultrasonication of an oil–water–surfactant system in the presence of silica source (tetraethyl orthosilicate; TEOS) in the presence of common cationic surfactant and oil nominated cetyltrimethyl ammonium bromide (CTAB) and castor oil respectively. The vegetable oil (castor oil) was chosen for its biocompatibility and low cost. The silica source is tetraethyl orthosilicate (TEOS).

The research work was extended to prepare compartmentalized hollow silica nanospheres (HSN) encapsulated with sodium salicylate as a model drug for drug delivery domains. The as synthesized nanoemulsion of hollow structured porous silica and silica encapsulated with sodium salicylate will be extensively characterized to determine the hydrodynamic size, particle shape via TEM and DLS techniques. Moreover, the research study was extended to ameliorate the toxic effect of drugs without falling in new drug toxicity. This study aims to elucidate agents ameliorating of drug nephrotoxicity induced by cisplatin in experimental animals using medicinal plant (ginger) for its antioxidant effect.

Materials and Methods

Chemicals and Drugs

All reagents were used as received without further purification. Cetyltrimethylammonium bromide (CTAB, 99 + %), tetraethyl orthosilicate (TEOS, 98), castor oil was purchased from Across Co (Germany). Ultrapure deionized (D.I.) water was generated using a Millipore Milli-Q plus system. Homocysteine standards (HPLC grade), Cisplatin, and sodium salicylat were purchased from Sigma-Aldrich Chemical Company, St. Louis, MO, USA. Ginger extracts were obtained from Arab Company for Pharmaceuticals and Medicinal Plants (MEPACO, Cairo, Egypt) in tablet form.

Animals

Male albino rats from the National Research Centre animal house (Cairo, Egypt), weighing 180-200 g, were used. Rats were group-housed under temperature- and light-controlled conditions and allowed standard laboratory rodent chow and water ad libitum. Animal procedures followed the recommendations of the Ethics Committee of the National Research Centre (Cairo, Egypt) and the United States National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Methods

Chemical Synthesis of Silica Nano Particles Loaded with Sodium Salicylate

For preparing silica nanospheres (Si NPs), 1 g of CTAB was dissolved in 183 ml of deionized water at room temperature. Afterward, an oil phase solution containing 15 ml of tetraethyl orthosilicate (TEOS) and 2 ml of castor oil was added to the aforesaid aqueous solution with stirring at 1000 rpm for 5 min to generate a simple oil-in-water (O/W) emulsion system. The reaction mixture was then sonicated using an ultrasonic bath (FALC Instruments s.r.i., Italy), supplemented with mechanical stirring at 1000 rpm (Heidolph, Germany) for 15 min at room temperature. After that, a cloudy mixture was obtained and then left to stand for another 24 h.

The SiNPs were isolated by centrifugation at 15000 rpm for 30 min (K241R, Centurion Scientific, UK). The product obtained was further washed in sequence with ethanol and deionized water to remove unreacted chemicals. To encapsulate sodium salicylate (Sc) into the formed silica nanoemulsion 2g of sodium salicylate was added to the oil part of TEOS and castor oil and then added to aqueous phase of CTAB solution. The as produced nanoparticles of Si loaded Sc was coded as (Si-Sc-NPs).

Study Design

Rats were randomly divided into eight equal groups, with ten rats in each group. Rats were given saline I.P (group 1) or cisplatin I.P. (12 mg/kg BW) (group 2) or carrier I.P 100mg/kg BW /day (group 3). This dose of cisplatin produces nephrotoxicity in rats. Some groups also received I.P injection of Si-Sc-NPs (100 mg/kg BW/day) either alone (group 4) or with cisplatin (group 5). Ginger was administered with stomach tube 310mg/kg BW /day alone (group 6) or with cisplatin (group 7) or both ginger and Si-Sc-NPs (100 mg/kg BW/day) with cisplatin (group 8).The Si-Sc-NPs, ginger or

combination of both were administered for three weeks alone before the injection of cisplatin and then were continued for one week.

Sample Collection

Animals were then euthanized by decapitation one week after cisplatin injection. Blood and kidney tissues were collected. Tissues were fixed in 10% neutral buffered formalin for histopathological examination or homogenized for estimation of kidney parameters.

Physical and Biochemical Characterization

Physical Characterization for the Formed Nanoparticles of Si and Si Loaded SC as a Model Drug

The particles shape of the formed nanoemulsion of Si NPs loaded with and without (Sc) was investigated using transmission electron microscopy (TEM) technique. The images were taken by a JEM-2011F microscope (JEOL, Japan) operated at 200 kV. In addition, the hydrodynamic size of Si nanoemulsion and the formed nanoemulsion of Si-Sc was determined by diluting 1 ml of the as prepared nanoemulsion in 10 ml of deionized water. Followed by the samples sonication for 10 min at room temperature. Size distributions of the nanoparticles were determined with a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., GB) by the DLS technique.

Biochemical Analysis

Preparation of Kidney Homogenate

Kidneys were removed quickly and placed in iced normal saline and homogenized cold buffer pH= 7.4 for parameters estimation [22].Blood urea was performed according to the method of [23] and serum creatinine according to the kinetic method of [24]. Kits were supplied from Spectrum Company.

Lipid peroxidation was assayed by measuring the level of malondialdehyde (MDA) in kidney tissue homogenate using the method of [25]. Nitric oxide (NO) was determined in kidney tissue homogenate using Griess reagent, according to the method reported by Moshage et al. [26]. The arylesterase activity of paraoxonase was measured spectrophotometrically in kidney tissue homogenate supernatants using phenyl acetate as a substrate [27].

Serum hcy was estimated by high performance liquid chromatography (HPLC) system, Agilent technologies 1100 series, equipped with a quaternary pump (G131A model) [28].

Sample Extraction

Briefly, 400 µl from serum samples were treated with 30 µl of1.2 mol/ L trichloroacetic acid (TCA) mixed well and incubated in ice for 30 min to precipitate protein. After centrifugation for20 min at G-value (3200g) and 4 ºC, supernatants were filtered through hydrophilic 0.45 ml polyvinylidene floride (PVDF) membrane filter.

HPLC Condition

50 µl from the filtered supernatant were injected into HPLC; separation was achieved on reversed phase column (C18, 25, 0.46 cm i.d. 5 lm). The mobile phase consisted of 40 mmol/L sodium phosphate monobasic monohydrate; 8 mmol/L heptanes sulfonic acid and 18% (v/v) methanol pH was adjusted to 3.1 by addition of phosphoric acid; filtered two times through a 0.45- lm membrane filter.

The mobile phase was then delivered at a flow rate of 1 ml/min at 40 ºC. UV detection was performed at 260 nm. Serial dilutions of standards were injected into HPLC, and their peak areas were determined. A linear standard curve was constructed by plotting peak areas versus the corresponding concentrations. Concentrations in samples were obtained from the standard curve.

Histopathological Analysis

For microscopic evaluation kidneys were fixed in 10% neutral buffered formalin. The fixed samples were dehydrated in ascending series of ethanol, cleared in zylene, and embedded in paraffin wax. Sections 5 µm thickness was prepared using a microtome stained with hematoxylin and eosin (H & E), and examination under a light microscope [29].

Statistical Analysis

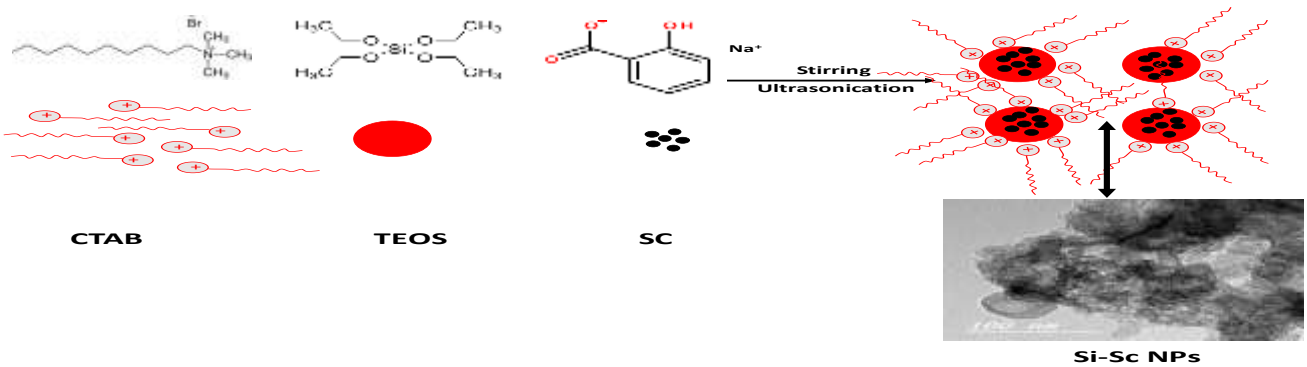
All data will be expressed as mean ± SE. Distribution of the data will be verified to be normal using Tests of Normality (SPSS version 12).

Statistical significance will be tested by one-way analysis of variance (ANOVA) followed by Bonferroni post hoc analysis.

Results and Discussion

Utilization of silica materials as drug carriers and controlled release of drug has been extensively used since about 15 years ago due to their biocompatibility, biodegradability and easy preparing with drugs. By methods for utilizing SiNPs to transport bioactive molecules can shield them from degradation underneath physiological conditions, permit for controlled release, extend their blood movement, enhance disease targeting, and minimize side effects to healthful tissues. In a sol–gel preparation without sonoexcitation, there is necessities to use acid or base catalysts during the hydrolysis of tetraethyl orthosilicate (TEOS).On the other hand, the hydrolysis reaction of TEOS is very slow at neutral pH.

To increase the efficiency of TEOS hydrolysis, it can be hydrolyzed under sonoexcitation using ultrasonic irradiation with there is no need to use basic or acidic catalyst. These irradiations produced from ultrasonication have the ability to harvest acoustical cavitation within reactants.



Scheme 1: steps for the formation of silica nanoparticles loaded sodium salicylate (Si-Sc-NPs)

The fabricated hydrolyzed silica species is prepared at neutral pH in absence of acid or base catalysts due to the effect of ultrasonic cavitation. Schematic diagram clarifies the steps for the formation of hollow structured porous silica encapsulated with (Sc) as a model drug. The nanoemulsion was successfully prepared using TEOS, CTAB and (Sc) as precursors for silica, surfactant and model drug respectively.

Characterization of Silica and Silica Loaded Sodium Salicylate

Figure (1) represents the TEM images of silica nanoemulsion (SiNPs) and nanoemulsion of silica encapsulated with sodium salicylate (Si-Sc-NPs). It can be clearly seen (Figure 1 A) that the silica nanoemulsions are hollow. On the other hand, Figure 1 B demonstrates the particle shape of (Si-Sc-NPs). It is clearly seen that the particle shape significantly changed when compared with the hollow structure of SiNPs. it is observed in Figure 1 B that there is dark middle region due to the encapsulation of Sc. In addition, the internal space of the inner hollow spheres is filled with (Sc) as clearly identified in TEM figure with high magnification (Figure 1C).

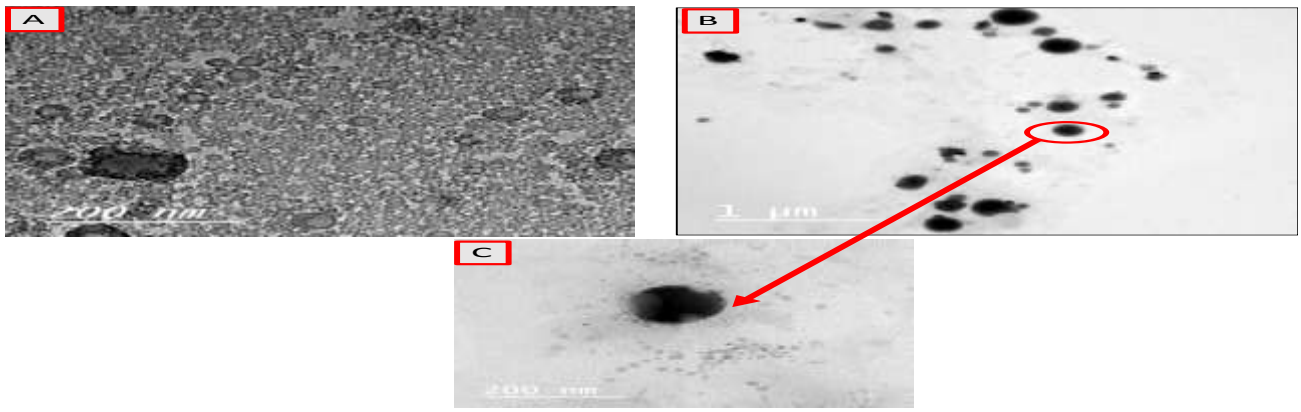


Figure 1: TEM images of (a) hollow structured porous silica nanoemulsion (SiNPs) and (b) silica nanospheres encapsulated with sodium salicylate (Si-Sc-NPs)

By and large, all particles of silica and silica loaded with the model drug (Sc) are still in the form of nanometre size. However, the size is marginally increased which may be assigned to the encapsulation of (Sc). The addition of CTAB assumes an essential part to stabilize the as synthesized silica nanoemulsion with and without (Sc). In presence of CTAB, many well-separated hollow silica nanospheres (SiNPs) are seen due to the presence of positive charges. It is also observed that the solubility of CTAB in the encapsulated oil phase is high with the addition of castor oil. The ultrasound-induced silica helps in the rapid formation of homogeneity and well-stabilized nanoemulsion.

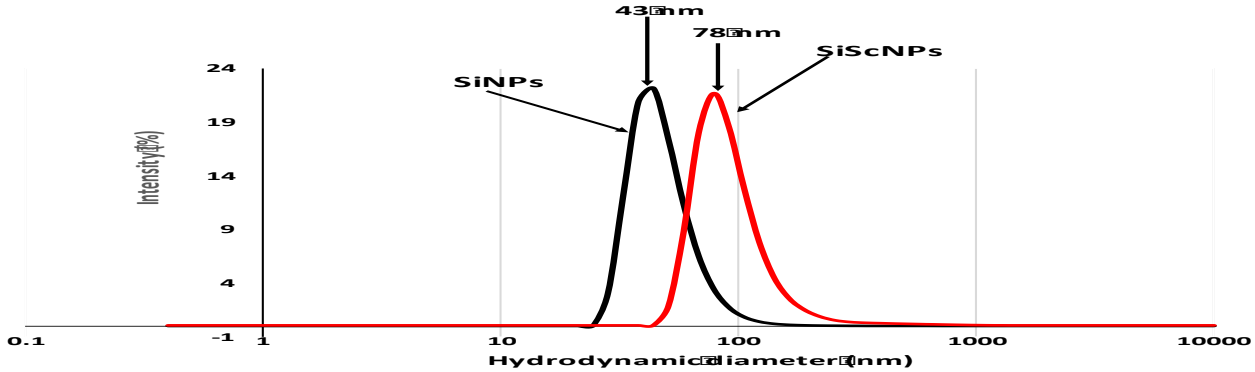


Figure 2: hydrodynamic size of the produced nanoemulsion of silica nanospheres(SiNPs) and silica nanospheres encapsulated with sodium salicylate (Si-Sc-NPs)

The average hydrodynamic size of the synthesized nanoemulsion of SiNPs is 43 nm in water as proved from dynamic light scattering (DLS) and clarified from the graph in Figure 2, indicating that SiNPs is non-aggregating. After encapsulation with (Sc), the average hydrodynamic size increases to 78 nm. This means that the negative charge of silicate is strong enough for compensation of the positive charge of CTAB, and that has the capability to repulsion the silica particles from each other. The obtained data of DLS analysis (Figure 2) is in a good accordance with TEM images (Figure 1).

In Vivo Study

Many physicians prescribe drugs to treat or prevent diseases. Those drugs possibly be toxic to certain patients, however, on account of nonselective action, genetic predisposition or in appropriate use or administration of the drug. Whole drug molecules are metabolized by the liver and/or other tissues. Occasionally, metabolism produces a pharmacologically active metabolite which can have an adverse effect. Although however, the biological mechanisms are still not well understood, some of these complexes between the drug metabolite and cellular proteins are highly toxic to many organs as kidney, liver, testes ….etc [30].

A drug and/or its metabolites interact with specific receptors to mediate on-target or off-target adverse effects. In addition, these metabolites can be detoxified and excreted, or can react with a variety of macromolecules including DNA, small antioxidants such as glutathione (GSH) and cellular or plasma proteins. The formation of unrepaired or misrepaired DNA adducts is predominatingly mutagenic and may lead to cancer. The impairment of oxidative defenses can lead to inflammation and cell death (apoptosis or necrosis).

The formation of drug-protein adducts can trigger immune responses that can damage cells and tissues. Regardless of the mechanism of damage, a gradation of acute responses from protection of apoptosis and necrosis can result, relying on the extent of damage and the temporal and dose relationships. Chronic inflammation and repair may be also lead to tissue fibrosis [31]. The following study was performed to investigate the nephro-protective potentials by using technique of nanotechnology drug delivery using (Si-Sc-NPs) nanoemulsion as anti-inflammatory agent, medicinal plant (ginger) for its antioxidant effect or in combination together.

The renal damage caused by cisplatin depends on the dose or duration of treatment. The study was issued with high dose of cisplatin to induce nephrotoxicity which corresponds to the dosage of cisplatin being

used in clinical study[32] and that showed in the present histopathological results of cisplatin group (Figure 3C).These histological examinations showed changes: degenerative affecting glomeruli, epithelial cells of tubules, and in the interstitium. Marked atrophy of some glomerular tufts, showing large Bowman's space was also evident.

The renal sections displayed marked tubular degeneration, necrosis, dilatation, hyaline cast, with pyknoticneucli, apoptotic bodies were apparently. Loss of inner brush border lining proximal convoluted tubules and hyaline cast was observed. In the interstitial of the renal cortical regions, there was focal infiltration of mononuclear inflammatory cells which was accompanied by hemorrhage in agreement with Meral et al [33].Who reported that the histopathological examinations exhibited rife tubular necrosis and dilatation in cisplatin-treated rats which states cisplatin induced nephrotoxicity.

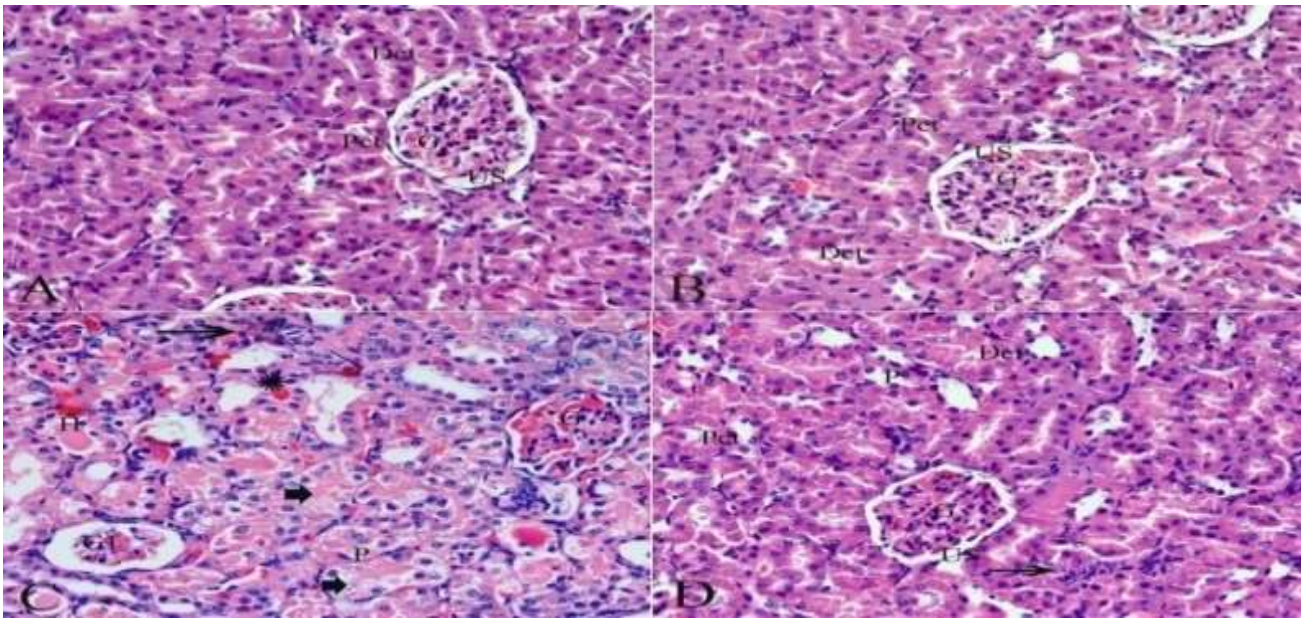


Figure 3: Light photomicrographs of kidney sections from different experimental groups stained with H&E: A. Control group showing normal glomeruli (G) with an intact urinary space (Us), proximal convoluted (Pct) and distal convoluted (Dct) tubules. B. Carrier group showing normal glomeruli (G) urinary space (Us), proximal convoluted (Pct) and distal convoluted (Dct) tubules. C. Cisplatin group showing atrophy of glomerulus (G), congestion of glomerulus (G1),widening of urinary space(Us), necrosis of epithelium renal tubules (arrow head), pyknotic nuclei (P), interstitial inflammatory cells (arrow) intertubular hemorrhage (star) and hyaline cast (H) D. Sodium salicylate group showing nearly similar to the control group with few inflammatory cells (arrow). (H & E X 400)

Table 1: Blood urea levels (mg/dl) in different studied groups

Group	Range	Mean+SE
Control	25.8-33.0	29.55±0.7
carrier	27-38.1	31.7±20.1
Cisplatin	67.4-90.5	77. 8 ± 3.7 [a,b]
Ginger	24.9-31.4	27.65 ± 0.72 [C]
Sodium salicylate	26.8-34.5	30.5±1.0 [C]
Sodium salicylate+cisplatin	48.5-70.1	59.1±2.29 [a,b,c,e]
Ginger+Cisplatin	55.3-80.4	67.1 ±3.5 [a,b,c,d]
Combination+ cisplatin	48.1-67.5	55.3±3.0 [a,b,c,f,g]

Table 2: Blood creatinine levels (mg/dl) in different studied groups

Group	Range	Mean+SE
Control	0.59-0.59	0.59±0
carrier	0.4-0.6	0.78±0.06
Cisplatin	2.3-3.4	2.81±0.16 [a,b]
Ginger	0.58-0.81	0.683±0.03 [c]
Sodium salicylate	0.6-1.00	0.83±0.07 [c]

Sodium salicylate + cisplatin	**1.8-2.3**	**2.03±0.051** [a,b,c,e]
Ginger + Cisplatin	**2.0-2.8**	**2.3± 0.12** [a,b,c,d]
Combination + cisplatin	**1.7-2.1**	**1.9±0.05** [a,b,c,g,f]

Significant P value <0.001.[a]Significant difference compared to control group; [b]Significant difference compared to carrier group, [c] Significant difference compared to cisplatin group, [d]Significant difference compared to treated group with ginger.[e] Significant difference compared to treated group with sodium salicylate, [f]Significant difference compared to Sodium salicylate+ group, [g]Significant difference compared to ginger+ group Number of cases = 10.

In the current study, cisplatin elevated blood urea and serum creatinine (Table 1, 2) and Figure4 (A,B), this in accord with Qi et al [34].Who mentioned that chronic renal injuries by cisplatin intoxication were associated with blood urea and serum creatinine increases which are indicators of kidney injury. Administration of nano model alleviated renal tissue damage and improved renal function. This alleviation was improved in combination group.

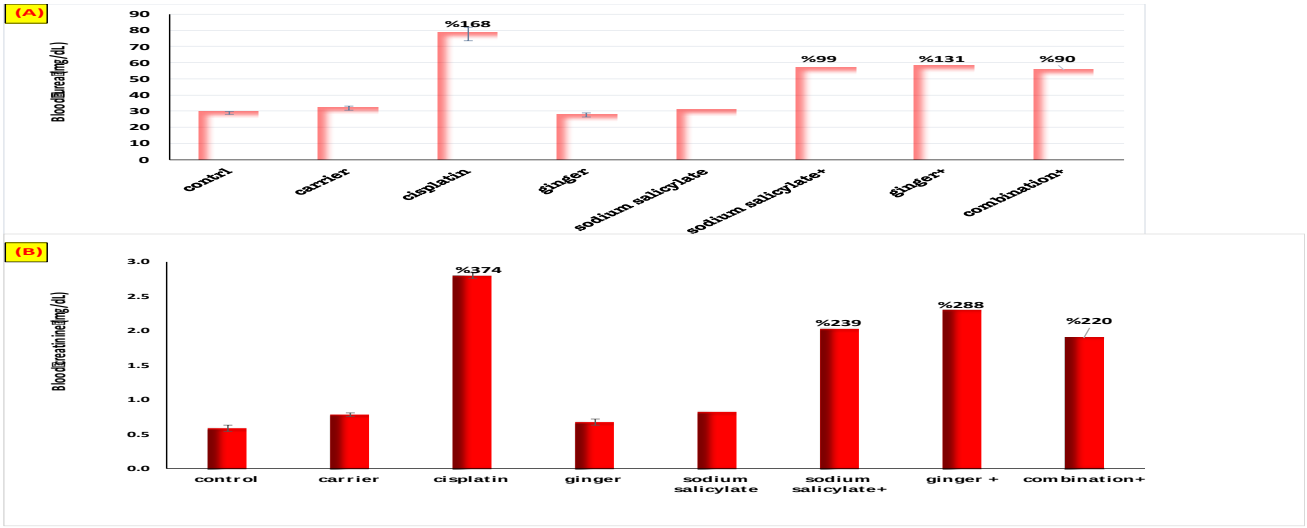


Figure 4: (A) Blood urea levels (mg/dl) and (B) Blood creatinine levels (mg/dl) in different studied groups with % change

Cisplatin-induced nephrotoxicity provides an example of chronic oxidative stress. Thus, in the following study we evaluated the kidney oxidative stress in experimental animals and examined the potential protective role of (Si-Sc-NPs) and ginger against the changes of cisplatin induced as set out in Tables (3, 4, 5) and Figure 5(A, B, C). Our data showed an elevation of MDA and NO levels in cisplatin group along with a lowering of antioxidant enzyme; 1(PON-1) activity in comparison with the control groups. Cisplatin enhances the production of lipid peroxidation and expulsion of ROS, in addition to the decreasing of antioxidant enzymes activity; resulting in the imbalance between oxidation and anti-oxidation activates [35].It has been reported that cisplatin leads to alterations in the mitochondrial electron transport chain; thus, mitochondrial energy dysfunction, ROS formation, and oxidative stress were recognized and associated with cisplatin related complications [36]. Both ROS and NO can alter mitochondrial membrane potential by opening the mitochondrial permeability transition pores releasing cytochrome C and following by activation of caspases 3 resulting in cell death [37].

Table 3: Kidney MDA levels (nmol/g tissue) in different studied groups

Group	Range	Mean+SE
Control	**59.4-90.6**	**78.9±5.2**
carrier	**78.6-99.5**	**87.5±2.8**
Cisplatin	**153.5-188.5**	**171.1±5.2** [a,b]
Ginger	**69.62-96.72**	**80,5±4.2** [c]
Sodium salicylate	**67.7-96**	**82.5±4.9** [c]
Sodium salicylate + cisplatin	**90.6-116.1**	**104±2.7** [a,b,c,d]
Ginger+ Cisplatin	**133.6-144.7**	**137.4±1.5** [a,b,c,e]
Combination + cisplatin	**97.7-116.4**	**104±2.7** [a,b,c,g,f]

Table 4: Kidney NO levels (µmol/g tissue) in different studied groups

Group	Range	Mean+SE
Control	6.67-9.6	8.3±0.4
carrier	7.38-9.10	8.1±0.3
Cisplatin	16.6 - 21.1	19.02±0.7 [a, b]
Ginger	6.6-10.4	8.5±0.53 [c]
Sodium salicylate	7.65-11.1	9.4±0.54 [c]
Sodium salicylate + cisplatin	14.3-17.19	11.6±0.5 [a,b,c,e]
Ginger + Cisplatin	19.5-27.1	15.6±0.5 [a,b,c,d]
Combination + cisplatin	8.72-13.6	10.8±0.7 [a,b,c,g,f]

Significant P value <0.001.[a]Significant difference compared to control group; [b]Significant difference compared to carrier group, [c] Significant difference compared to cisplatin group, [d]Significant difference compared to treated group with ginger.[e] Significant difference compared to treated group with sodium salicylate, [f]Significant difference compared to Sodium salicylate+ group, [g]Significant difference compared to ginger+ group Number of cases = 10.

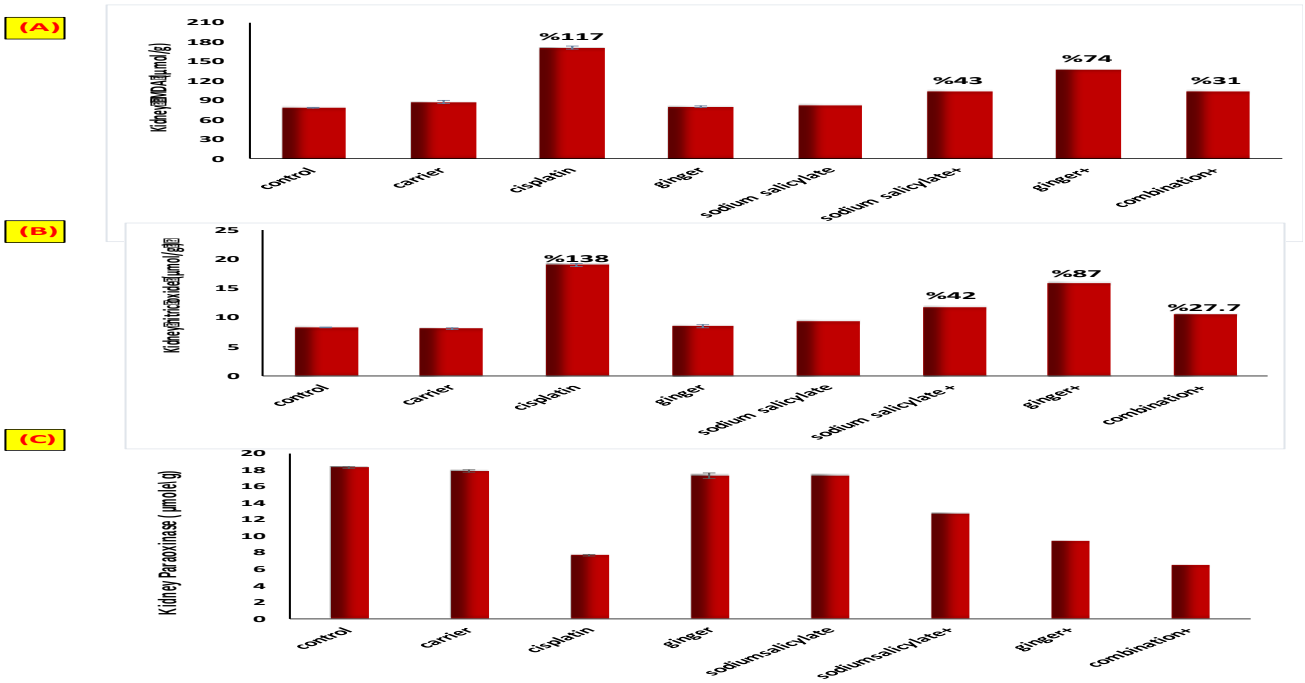


Figure 5: (A) KidneyMDA levels (nmol/g tissue), (B) Kidney NO levels (µmol/g tissue) and (C) Kidney PON1 levels (µmol/g tissue) in different studied groups with %change

Table 5: Kidney PON1 levels (umol/g tissue) in different studied groups

Group	Range	Mean+SE
Control	12-24	18.3±1.7
carrier	12-23	17.9±1.85
Cisplatin	6.16-4.58	7.7±0.85 [a, b]
Ginger	12.99-22.2	17.3±1.2 [c]
Sodium salicylate	13.6-21.33	17.4±1.1 [c]
Sodium salicylate + cisplatin	8.4-17.56	12.7±1.3 [a,b,c,e]
Ginger + Cisplatin	7.04-12.21	9.45±0.7 [a,b,c,d]
Combination + cisplatin	11.4-18.4	15.6±1.07 [a,b,c,g,f]

Table 6: Serum homocysteine (µmole/L) in different studied groups

Group	Range	Mean+SE
Control	4.5-4.9	4.7±0.08
carrier	5.09-5.3	5.1±0.04
Cisplatin	9.3-10. 8	10.1±0.3 [a, b]
Ginger	5.2-6.4	5.8±0.27 [c]
Sodium salicylate	5.2-6.4	5.8±0.27 [c]
Sodium salicylate + cisplatin	7.3-7.8	7.6±0.1 [a,b,c,e]
Ginger + Cisplatin	8.04-8.6	8.3±0.13 [a,b,c,d]
Combination + cisplatin	6.2-6.9	6.5±0.15 [a,b,c,g,f]

Significant P value <0.001.[a]Significant difference compared to control group; [b]Significant difference compared to carrier group, [c] Significant difference compared to cisplatin group ,[d]Significant difference compared to treated group with ginger.[e] Significant difference compared to treated group with sodium salicylate, [f] Significant difference compared to Sodium salicylate+ group, [g]Significant difference compared to ginger+ group Number of cases = 10

Protection with (Si-Sc-NPs) recorded improvement of the PON1, NO and MDA. Also these protections were more improved in the combination group. Preventive effects of ginger against cisplatin-induced oxidative stress could be referred to its high level of polyphenol compounds (6-gingerol and its derivatives), which have a high antioxidant activity. Those compounds could scavenge the free radicals of cisplatin generated throughout P450 enzyme system and so diminish the oxidative injuries. Ginger may also impair cisplatin-mediated lipid peroxidation through decreased production of free radical derivatives. Administration of ginger alone to the animals showed significant changes in all the antioxidant parameters confirming the potency of the ginger extracts as anti-free radicals-producer. Overall, these results demonstrate that (Si-Sc-NPs) has greater potential in the elimination of free radicals and prevention of drug-induced toxicity in cells. One of our important results in the following study is the increasing of hcy level in cisplatin group in comparison with control group as shown in Table (6) and Figure (6). Hcy is a by-product of transmethylation reactions and it is detoxified by methionine syntheses, which depends on vitamin B12 and folate as co-enzymes for its proper function [38].

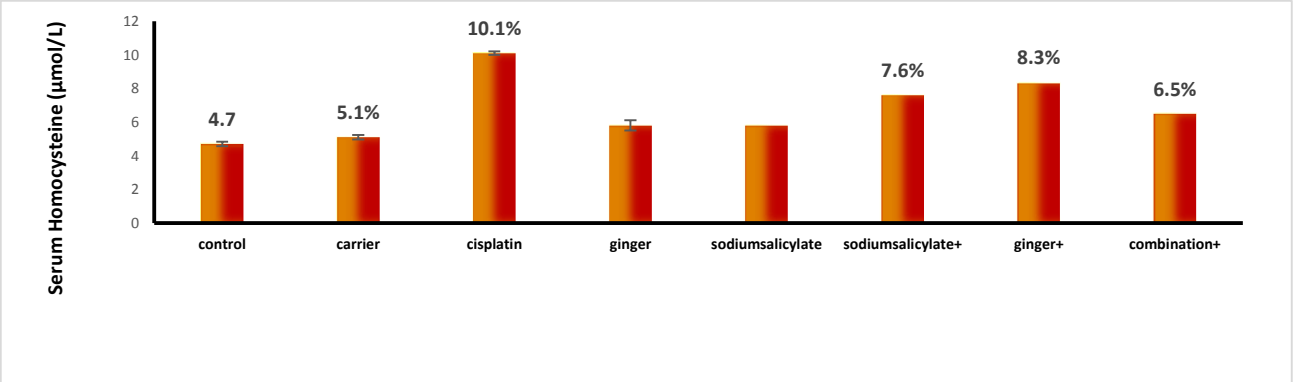


Figure 6: Serum homocysteine levels (umol/g tissue) in different studied groups

Hyperhomocysteinemia and its determinants such as low concentrations of folate and/or vitamin B coenzymes and alteration of enzymes that involved in the breakdown of hcy, are also associated with the risk of cisplatin complications [39].In agreement, Ho et al. [40] and James et al. [41] reported that hcy induces enhancing of ROS production and oxidative activation of NO and lipid peroxidation as was stated in our study. The improvement of drug delivery of salicylate such as using nanoformulation method will make it easier and more potent for treatment of some diseases.

In our study hcy was significantly reduced in treated group in comparison to cisplatin group. These results confirmed anti-inflammatory potential of (Si-Sc-NPs). Hcy could directly cause renal vascular damage throughout the production of ROS, effects on vascular smooth muscle cells, endothelial injury, reduction in plasma and tissue interstitium adenosine levels, and mesangial cell proliferation and apoptosis. The experimental animal models of hcy-induced glomerular damage have also been developed [42].

Two mechanisms may explain the elevated plasma hcy level. Firstly, the primary defect in the sulphur amino acid metabolism may be an impairment of hcy transsulphuration, which is offset by higher plasma hcy after which the daily methionine load can again be metabolized through the transsulphuration pathway. This higher Hcy level would slow down the methylation cycle by inhibiting transmethylation. Secondly, the primary defect may be a block in hcy remethylation, which can only be partially indemnification for by elevation in plasma hcy, resulting in a further reduction of the methylation cycle without compromising transsulphuration [43].

Figure 3(B) and Figure 3 (D) of the carrier group and (Si-Sc-NPs) group respectively showed that no histological changes indicating the safety of their use. While Figure 7 (C) showed the histopathological results of group (Si-Sc-NPs) group with cisplatin; as it showed that the kidney tissues were protected against the cisplatin-induced toxicity and revealed moderate improvement, however the histology of renal tissue showed

congestion glomeruli accompanied by moderate necrosis of tubules, interstitial haemorrhage and pyknotic nuclei when compared with the control group (Figure 3A) or cisplatin group as shown in Figure 3(C).

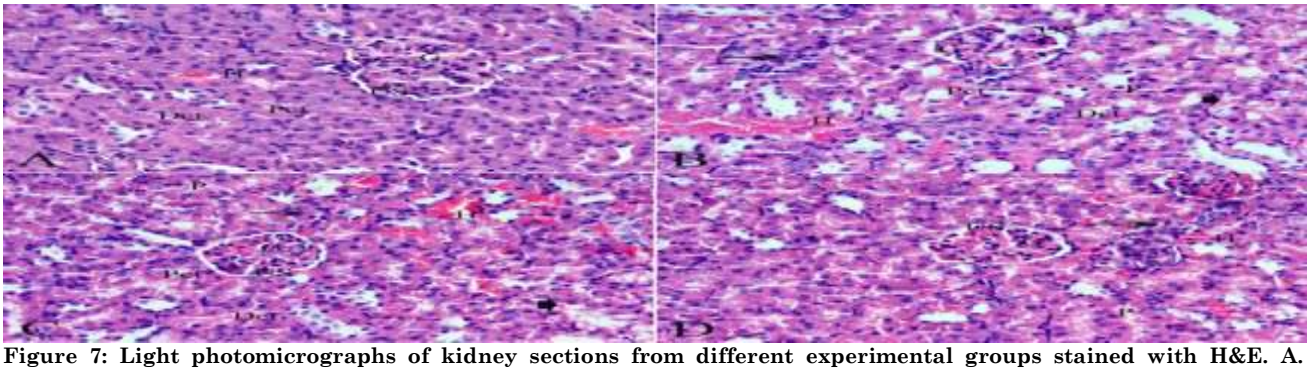


Figure 7: Light photomicrographs of kidney sections from different experimental groups stained with H&E. A. Ginger group showing nearly normal structure except intertubular hemorrhage (H). B.Ginger + Cisplatin group showingcongestion glomeruli (G), and dilated urinary space (US), tubular necrosis (arrowhead), pyknotic nuclei(P), inflammatory cells (arrow) and interstitial haemorrhage (H). C. Sodium salicylate + Cisplatin group showing moderate improvement, congestion glomeruli (G) accompanied by moderate necrosis of tubules (arrowhead), interstitial haemorrhage (H) and pyknotic nuclei (P). D.Ginger + Cisplatin + Sodium salicylate group showing congestion some glomeruli (G), mild necrosis of tubules (arrowhead), interstitial haemorrhage (H) and pyknotic nuclei (P). (H & E X 400)

However, Fig 7 (B) showed the histological examination in ginger group; as it revealed slightly improvement against induction with cisplatin as it revealed congestion glomeruli and dilated urinary space. Also, tubular necrosis, pyknotic nuclei, inflammatory cells and interstitial haemorrhage that in comparison with control and cisplatin groups.

The corrective histopathological findings were observed in Fig 7 (D) related to combination group (ginger and Si-Sc-NPs) group against induction with cisplatin; which showed congestion some glomeruli, mild necrosis of tubules, interstitial haemorrhage and pyknotic nuclei. These findings gave an additional support that this combination mops up free radicals generation by cisplatin, reduces inflammation, improves kidney function, and induces healthy state of renal cells, suggesting their role as renal protective agent as antioxidant and anti-inflammatory agents

Conclusions

Nanoemulsion of hollow structure silica nanoparticles and silica nanoemulsion encapsulated with (Sc) as a model drug was synthesized using ultrasound-assisted sol-gel method. CTAB and TEOS were used as precursor and surfactant respectively. Addition of CTAB had a vital rule in controlling the particle shape of silica nanoparticle in well stabilized size without noticeable agglomeration. The obtained spherical particles of silica and silica encapsulated (Sc) had 43 and 78 nm respectively. In vivo studies have been examined against drug nephrotoxicity showing increase in blood urea and serum creatinine in cisplatin -induced nephrotoxicity rats associated with disturbances in oxidative stress parameters and upgrading of hcy level. Contrarily, these disturbances were augmented by (Si-Sc-NPs) supplementation alone or in combination with ginger. The obtained results are considered as potential implications that offer a new approach in attenuating of drug induced nephrotoxicity.

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