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Research article

Amifostine-based nanoemulsion as promising protective agent for nephrotoxicity

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Abstract

Drug-induced kidney injury is the causative of acute kidney failure. Amifostine loaded Silica nanoemulsion was synthesized using water/oil emulsion with the help of ultra-sonication waves. The nanoemulsion was prepared using tetraethyl orthosilicate [TEOS], cetyltrimethyl ammonium bromide [CTAB], castor oil [CAO] and amifostine [AMF] as a source for silica, surfactant, extra stabilizing agent and a model drug respectively. The as synthesized nanoemulsion of silica and silica loaded with amifostine [SiNPs@AMF] was examined via transmission electron microscopy [TEM] and dynamic light scattering [DLS] in terms of particles shape and hydrodynamic average size. The work was extended to achieve the target of the current work target to investigate the protective role of this nanoemulsion model as cytoprotector drug effect against cisplatin-induced nephrotoxicity in male albino rats. It was clearly seen that the successful preparation of the as-synthesized silica nanoemulsion loaded with amifostine [SiNPs@AMF] but the particle size was marginally increased when comparing with silica nanoemulsion. Additionally, blood urea and serum creatinine were increased after cisplatin injection with disorders in oxidative stress and rising of serum KIM-1 and urinary 8-hydroxyguanosine levels. Also, histopathological changes of the kidney tissue were observed. These changes back to normal by treatment with silica nanoparticles loaded amifostine [SiNPs@AMF]. Oil/water nanoemulsion of [SiNPs@AMF] showed a protective and promising preventive strategy against nephrotoxicity due to their cytoprotective and antioxidant effects.

Introduction

Human take drugs for health reasons, it is particularly warranted that these useful drugs make undesirable effects, but some can make positively very dangerous effects. With the complexity of some of those diseases, multi drug treatments are rising, 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 affected due to their roles in glomerular filtrate concentration and drug transport [2]. Some 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 prediction of nephrotoxicity during drug development remains difficult. Many drugs found to cause nephrotoxicity give toxic effects by some 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 considerd 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. The proximal tubules are the main target of cisplatin in kidney, as it accumulates and causes cellular damage [7]. 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 [8]. Oxidative stress injury is actively embroiled in the pathogenesis of acute kidney injury induced by cisplatin. Amifostine is used as organic thiophosphate antioxidant protector for normal tissues in cancer patients from chemotherapeutic or radiotherapy

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drugs [9]. The biological activity is relying on its hydrolysis into the active metabolite with sulfhydryls by alkaline phosphatase. The sulfhydryls could scavenge the oxygen- derived free radicals and so prevent DNA damage [10]. The cytoprotective selectivity of amifostine is mainly caused by the diversity in extracellular acidity and alkaline phosphatase expression between normal tissue and tumor [11]. Since the active metabolite of amifostine showed limited stability in vivo because of rapid formation of disulfide from free sulfhydryls, as high dosage that required to realize eligible the protective effects. In the clinical practices, the amifostine was administered with dose of 740 mg/m2 to reach the drug saturation in blood. Nevertheless, this very high concentration of the drug in blood resulted in reverse effects as nausea, vomiting and hypotension, which had significantly limited the spacious application of amifostine. Strategies are required to at the attenuate safety and efficacy of amifostine to improve the therapeutic effect [11]. 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. Scheme1 represent the steps for the formation of silica nanoparticles loaded amifostine [SiNPs@AMF] 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 macroemulsion. 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 silicananospheres 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 cetyltrimethylammonium 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 amifostine as a model drug for drug delivery domains. The as synthesized nanoemulsion of hollow structured porous silica and silica encapsulated with amifostine 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. Also, this nanoparticle model has the capability for change the unwanted properties of drugs in solubility, immunogenicity, bio distribution, renal filtration, enzymatic degradation and reticulo-endothelial system phagocytosis. So, the circulatory half-life as well as bioavailability of drugs could be increased, while drug toxicity could be decreased [11]. This prompted us to consider if [SiNPs@AMF] could improve the drug stability and efficacy, thereby allowing low injection dosage for safety profile. Therefore, this modification of amifostine by using silica nanoparticles model has never been reported in the literatures. Prevention and/or decreasing of the side effects of drugs are of the main concerns in treating patients who forced to take them for long periods or permanently. So, in this study we elucidate a new way to get these treatments without falling in new drug toxicity. Based on the aforementioned obtained data, nanoemulsion of amifostine silica nanoparticles [SiNPs@AMF] offer a new approach in attenuating of drug induced nephrototoxicity.

Materials and methods

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. Cis, and amifostine were purchased from Sigma-Aldrich Chemical Company, St. Louis, MO, USA.

Animals

Male albino rats from the animal house of the National Research Centre [Cairo, Egypt], weighing 180–200 g, were used. 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].

Preparation of silica hollow nanoemulsion loaded with amifostine as a model drug

Typically, 0.66 g of CTAB was dissolved in 12.2 mL of deionized water at room temperature. Afterward, an oil phase solution containing 1 ml of TEOS and 0.13 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 [Branson 2510R-DTH Ultrasonic Cleaner, 100 W, 42 kHz], supplemented with mechanical stirring at 1000 rpm [IKA, EuroST D S1] for 15 min at room temperature. After that, a cloudy mixture was obtained and then left to stand for another 24 h. The nanoemulsion particles of silica [SiNPs] were isolated by centrifugation at 15000

rpm for 30 min. The product obtained was further washed in sequence with ethanol and deionized water to remove unreacted chemicals. To encapsulate the drug into the formed silica nanoemulsion 150 mg of Amifostine was added to the oil part of TEOS and castor oil and then added to aqueous phase of CTAB solution.

In vitro experimental design

Rats were randomly divided into five equal groups, with ten rats in each group. Rats were given saline I.P. [group 1] or Cis I.P. [12 mg/kg BW] [group 2] or carrier I.P. 100mg/kg BW /day [group 3]. This dose of Cis produces nephrotoxicity in rats [12]. Some groups also received I.P injection of [SiNPs@AMF] [150 mg/kg BW three times a week] either alone [group 4] or with Cis [group 5]. The [SiNPs@AMF] was administered for three weeks alone before the injection of Cis and then were continued for one week. Animals were then euthanized by decapitation. Blood, urine and liver tissues were collected. Tissues were fixed in 10% neutral buffered formalin for histopathological examination or homogenized for estimation of liver parameters.

Physical characterization for the formed nanoparticles of Si and Si loaded [Am] as a model drug

The particles shape of the formed nanoemulsion of Si NPs loaded with and without amifostine [Amf] 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-amifostine 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

Kidneys were homogenized in cold buffer pH= 7.4 then centrifuged and the supernatant was removed for parameters estimation [13]. Blood urea was performed according to the method of Young [14] and serum creatinine according to the kinetic method of Young [15]. Kits were supplied from Spectrum Company. Serum KIM-1 was estimated by enzyme-linked immunosorbant assay [ELISA], the kit was purchased from Biovision, Inc. Beijing, China. Lipid peroxidation was assayed by measuring the level of malondialdehyde [MDA] in kidney tissue homogenate using the method of Ruiz-Larrea *et al.* [16]. Nitric oxide [NO] was determined in kidney tissue homogenate using Griess reagent, according to the method reported by Moshage *et al.* [17]. The arylesterase activity of paraoxonase was measured spectrophotometrically in kidney tissue using the method of Patil *et al.* [18].

Estimation of urinary 8-hydroxyguanosine by HPLC

Protocol for urinary 8-hydroxyguanosine [8-OHdG] analysis was modified from the method described by Kim *et al.* [19]. 8-OHdG was extracted from 1 ml urine. The eluents were dried under ultrapure N2 stream and were reconstituted in 5 ml deionized water 20 µl from each sample and also from the different concentrations of the standard were injected in HPLC, and the concentration of urinary 8-OHdG was calculated from the standard curve and then was divided on the urinary creatinine. Urinary creatinine was estimated by kinetic method as described by Larsen [20].

HPLC condition

HPLC column C18 [260 × 4.6, particle size 5 µl] using mobile phase acetonitrile/methanol/phosphate buffer [25/10/965] v/v. Phosphate buffer was prepared by dissolving 8.8 g of potassium dihydrogen phosphate in 1000 ml deionized water and pH was adjusted at 3.5. The buffer was then filtered 2 times before being used at a flow rate of 1 ml/min using electrochemical detector with cell potential of 600 mV [21].

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 [22].

Statistical analysis

All data expressed as mean ± SE. Distribution of the data verified to be normal using Tests of Normality [SPSS version 12]. Statistical significance tested by one-way analysis of variance [ANOVA] followed by Bonferroni post hoc analysis.

Results

Nanoemulsion based on silica nanoparticles are chosen for encapsulation the model drug; amifostine [Amf] for many reasons. Of these reasons are the silica nanoemulsion can be used for carrying bioactive molecules and protect them from degradation can shield them from degradation under the effect of physiological conditions. On the other side, allow the drug to releases in controlled state. Extend their blood movement, enhance disease targeting, and minimize side effects to healthful

tissues. The ultra-sonication technique is selected due to its capability to disperse the formed nanoemulsion and kept these particles in dispersing state without noticeable aggregation. In addition to there is no need to use an extra chemical such as acids or base to accelerate and catalyze the hydrolysis of TEOS. Therefore, the prepared nanoemulsion occurred at neutral pH using such technique. Given below the full characterization of silica nanoemulsion with and without Amf.

Characterization of the as-synthesized silica nanoemulsion loaded with and without amifostine

The particle shape and morphology of the as-prepared nanoemulsion of silica nanoparticles loaded with Amf was evaluated by making use of TEM and compared with that of silica nanoemulsion (Figure 1).

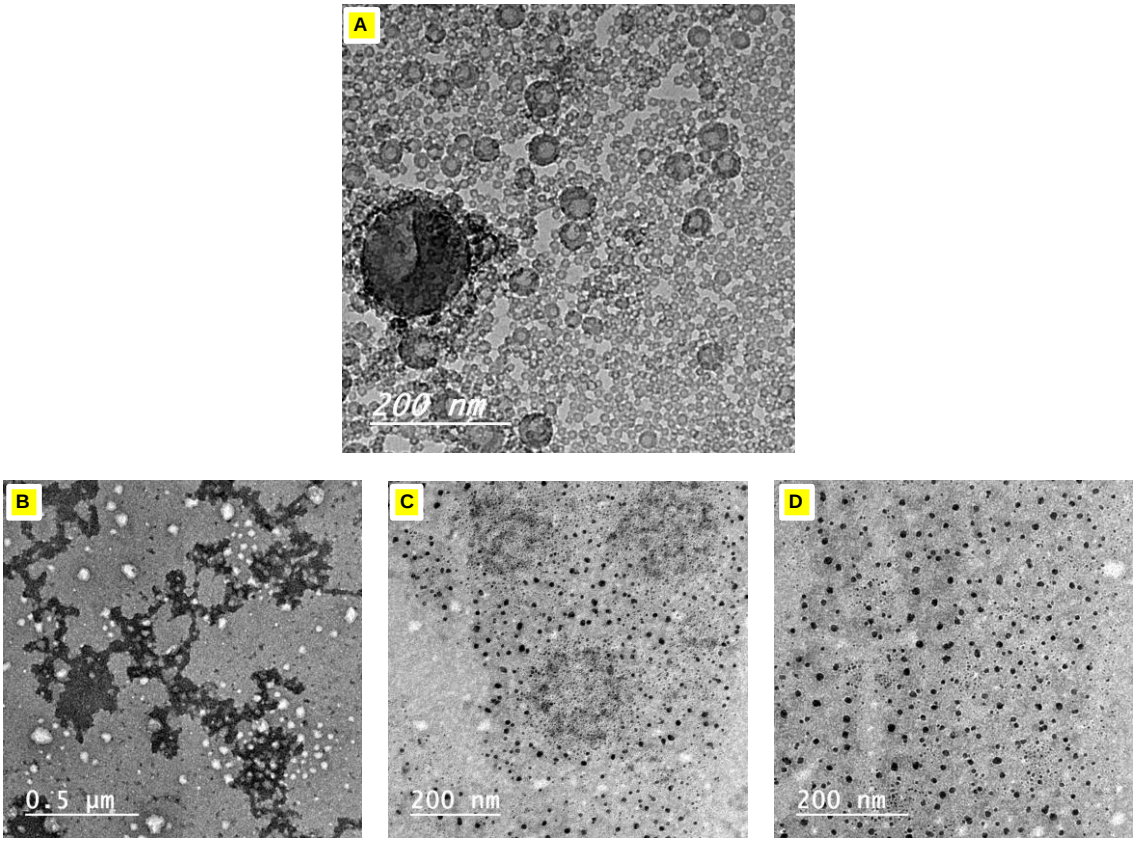


Figure 1. TEM images of [a] hollow structured porous silica nanoemulsion [SiNPs] and [b] silica nanospheres encapsulated with amifostine [SiNPs@AMF].

It is observed form Figure 1 [A] that the silica nanoemulsions are hollow with small size and homogeneity. The obtained small size and homogeneity may be attributed to the stabilizing effect of CTAB and castor oil. Loading the silica nanoemulsion with AMF as shown in Figure 1 [B] leads to make the particles very dense with also small size. It is clearly seen that the dark region may be attributed to the encapsulation of AMF the interior cavity of the inner hollow silica particles. This observation is clearly identified in TEM figure with high magnification as shown in Figure 1 [C and D]. Therefore, TEM images of silica nanoemulsion and AMF loaded silica nanoemulsion provide noticeable change in the shape of the as formed particles. However, the size is marginally increased which may be assigned to the encapsulation of [Amf].

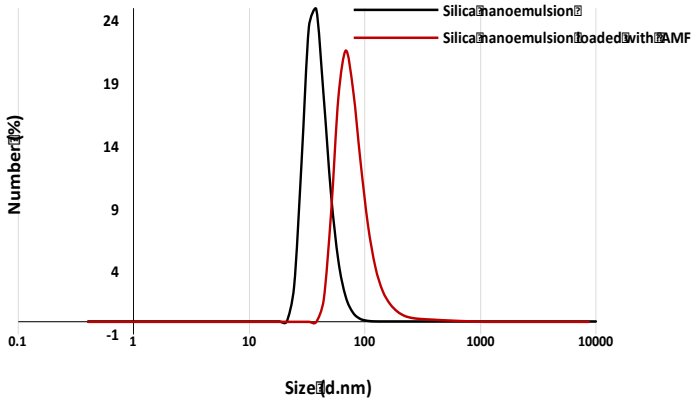


Figure 2. Hydrodynamic size of the produced nanoemulsion of silica nanoemulsion loaded with and without amifostine [Amf].

The majority size of the synthesized silica nanoemulsion with and without Amf is determined using dynamic light scattering [DLS] and the obtained data is plotted in figure [2]. The average particle size of silica nanoemulsion after dilution with deionized water is 37 nm with homogeneity as illustrated from polydispersity index [0.03] confirming the successful preparation of well stabilized silica nanoemulsion in non-aggregated form. Loading the silica nanoemulsion with AMF leads to enlarge the size to be 68 nm. The obtained data of DLS analysis of Figure [2] is in a good agreement with TEM images [Figure 1].

In vivo results

Kidney MDA and NO were significantly elevated, while PON 1 was significantly reduced in cis group in comparison with control, denoting the increase of oxidative stress. Otherwise, kidney MDA and NO were significantly reduced, but PON 1 was significantly elevated in SiNPs@AMF protected groups in comparison with cis group [Table 1] denoting the antioxidant potential of these supplemented agents.

Also, the detected significant increase in the blood urea and serum creatinine levels in cis group were found to be significantly decreased in groups protected with SiNPs@AMF and then injected with cis [Table 2]. While blood urea and serum creatinine levels in SiNPs@AMF alone were insignificantly changed in comparison with control group.

The other findings in current study were the significant increase of serum KIM-1and urinary 8-OHdG levels in cis group compared with control group [Table 3]. Also, the values of these parameters were significantly reduced in SiNPs@AMF protected group compared to cis and control groups.

Table 1. Kidney oxidant and antioxidants in different studied groups.

Groups	Parameter		
	MDA [nmol/g]	NO [nmol/g]	PON1 [nmol/g]
Control	78.9±5.2	8.3±0.45	18.3±1.7
Carrier	87.57±2.8	8.1±0.3	16.9±2.02
Cis	171.1±12.5a,b	19.0±0.69a,b	7.7±0.85a,b
[SiNPs@AMF]	81.6±5.04 c	8.6±0.42b,c	16.9±1.7c,d
[SiNPs@AMF]+Cis	94.2±4.1a,c	9.9±0.79a,b,c	11.5±1.2a,b,c,d

Data presented as mean±SE. Significant P value <0.001. a Significant difference compared to control group; b Significant difference compared to carrier group, c Significant difference compared to Cis group, d Significant difference compared to treated group with loaded amifostine, e Significant difference compared to Cis treated group with loaded amifostine.

Table 2. Serum Creatinine and urea in different studied groups.

Groups	Parameters	
	Creatinine [mg/dl]	Urea [mg/dl]
Control	0.68±0.04	29.5±1.0
Carrier	0.78±0.06	31.7±2.0
Cis	2.8±0.16a,b	77. 9 ± 3.7a,b
[SiNPs@AMF]	0.86±0.04c,e	31.1 ± 1.c,e
[SiNPs@AMF]+Cis	1.8±0.07a,b,c,d	59.4±3.6a,bc,d

Data presented as mean±SE. Significant P value <0.001. a Significant difference compared to control group; b Significant difference compared to carrier group, c Significant difference compared to Cis group, d Significant difference compared to treated group with loaded amifostine, e Significant difference compared to Cis treated group with loaded amifostine.

Table 3. Serum 8-OHdG and KIM-1 levels in different studied groups.

Groups	Parameters	
	8-OHdG [ng/mg creatinine]	KIM-1[pg/ml]
Control	3.6±0.15	62.3±1.6
Carrier	4.13±0.26	57.7±0.56
Cis	15.6±1.3a,b	195.1±3.2^{a,b}
[SiNPs@AMF]	4.5±0.36a,c	60.9±0.5^{c}
[SiNPs@AMF]+Cis	6.8±0.11a,b,c,d	105.7±3.5^{a,b,c}

Data presented as mean±SE Significant P value <0.001. a Significant difference compared to control group; b Significant difference compared to carrier group, c Significant difference compared to Cis group, d Significant difference compared to treated group with amifostine, e Significant difference compared to treated group with amifostine nanoemulsion.

Histopathological results

The sections of control group showed normal renal glomeruli surrounded by urinary space and normal proximal, distal and convoluted tubules [Figure 3]. Renal sections from the carrier groups showed no histological changes [Figure 4]. Histological examination of cis group showed types of changes: degenerative affecting glomeruli, epithelial cells of tubules, and in the interstitium. Marked atrophy of many glomerular tufts, showing large Bowman's space was also evident. The renal sections showed 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 [Figure 5]. In Figure 6 Ami treated group showed nearly normal structure with minimal inflammatory cells. Kidney specimens from rats treated with Ami and cis showed moderated reduction in cis-induced pathological alterations. Focal inflammatory cells, interstitial haemorrhage with few pyknotic nuclei were observed [Figure 7].

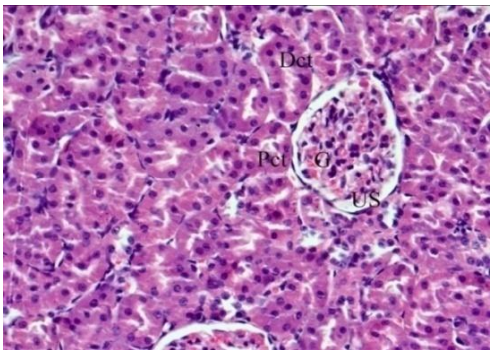


Figure 3. Photomicrograph of section kidney of control group showing normal glomeruli [G] with an intact urinary space [Us], proximal convoluted [Pct] and distal convoluted [Dct] tubules. [H and E X 400].

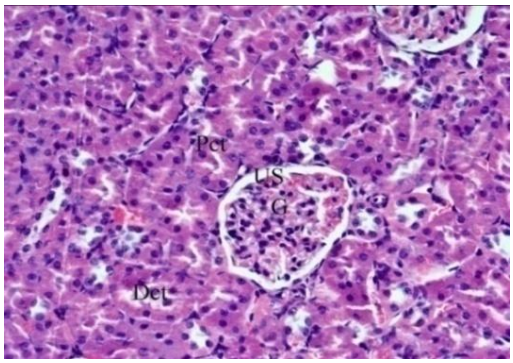


Figure 4. Photomicrograph of section kidney of carrier showing normal glomeruli [G] urinary space [Us], proximal convoluted [Pct] and distal convoluted [Dct] tubules. [H and E X 400].

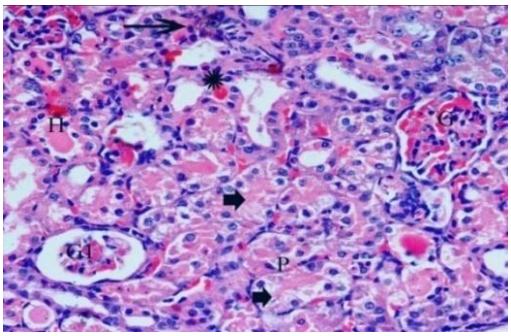


Figure 5. Photomicrographs of section kidney Cis group showing atrophy of glomerulus [G], congestion of glomerulus [G1], widening of urinary space [Us], necrosis of epithelium renal tubules [arrow head], pyknotic nuclei [P], interstitial inflammatory cells [arrow] intertubular hemorrhage [star] and hyaline cast [H].

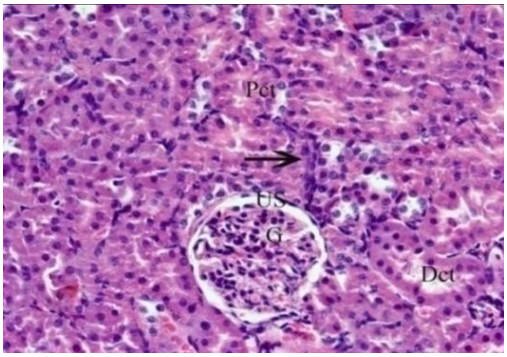


Figure 6. Photomicrographs of section kidney of SiNPs@AMFshowing nearly normal structure with minimal inflammatory cells [arrow].

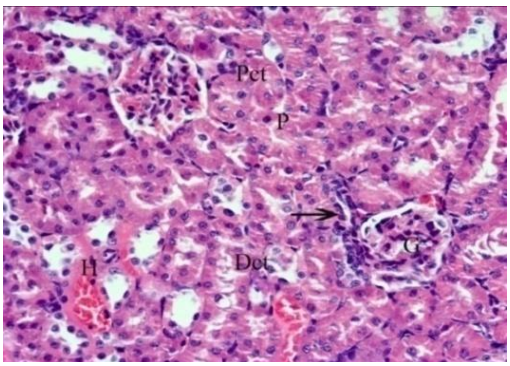


Figure 7. Photomicrographs of section kidney of SiNPs@AMF+ Cis group showing moderated reduction in Cis-induced pathological alterations. Focal inflammatory cells [arrow], interstitial haemorrhage [H] with few pyknotic nuclei [P].

Discussion

Drugs were prescribed by many physicians to treat or prevent diseases. These drugs possibly are toxic to certain patients, 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 [23]. Many systemic approaches with selective protection of normal cells from the toxic effects of chemotherapy also have been investigated [24]. The most promising results have been obtained with the organic thiophosphate compound – amifostine. Ami [WR-2721] is a pro-drug dephosphorylated to its active metabolite WR-1065. WR-1065 has been rapidly cleared from the plasma compartment. The short initial half-life can be explained by the fast uptake in tissues and approximately 90% of the drug is cleared from plasma within 6 minutes [25]. Several factors favor the uptake and the activity of Ami in normal tissue rather than in tumor tissues. Among these are the localization of Alkaline phosphatase and its activity in normal tissues; the neutral pH of normal cells versus the acidic pH in many tumors, and differences between normal and tumor cells in membrane transport rate. In normal cells Ami protects against cytotoxic agents by several mechanisms, including direct binding and detoxifying of platinum agents, by reduction of platinum-DNA adduct formation and by scavenging oxygen-free radicals [26]. In the current study, cisplatin elevated blood urea and serum creatinine this in accord with Qi *et al.*, [27] 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 [SiNPs@AMF] model alleviated renal tissue damage and improved renal function. Cisplatin-induced nephrotoxicity provides an example of chronic oxidative stress, where cisplatin enhances the lipid peroxidation production and ROS expulsion, in addition to the decreasing of the activity of antioxidant enzymes; which lead to the imbalance between oxidation and anti-oxidation activates [28]. It was reported that cisplatin results in disturbances in the mitochondrial electron transport chain; thus, mitochondrial energy dysfunction, the formation of ROS, and oxidative stress were recognized and linked with cisplatin complications [29]. Both of ROS and NO could alter mitochondrial membrane potential through opening the mitochondrial permeability transition pores, releasing cytochrome C and followed by caspases 3 activation resulted in cell death [30]. While, the capability of [SiNPs@AMF] in attenuating the oxidative stress was observed indicating the protective effect that the form of nanoparticles could potentially do. The biomarker, 8-OHdG, released secondary to DNA damage, is regarded as the most significant indicator of DNA damage [31]. Hydroxyl radicals eliminate hydrogen from nucleic acids or react with double bonds, leading to 8-OHdG elevation [32]. Altuner suggested that DNA damage is increased by following single dose Cis [5 mg/kg/d] in rat ovaries. Our results also suggesting that increasing 8-OHdG levels is also a good indicator of tissue damage in Cis-induced toxicity group [33]. In the current study, the Cis group treated with [SiNPs@AMF] has a significant decreasing in 8-OHdG levels when compared with the Cis group and in agree with Stankiewicz and Skrzydlewska [34], indicating the prevention of DNA damage and a significant protective antioxidant ability of ami by increasing PON1 levels. Moreover, Kidney specimens from rats treated with Ami and Cis showed moderated reduction in Cis-induced pathological alterations. Takaharu., *et al.*, [35] reported detection of rat Kim-1 protein in the tissue and urine samples, suggesting that Kim-1 may be a sensitive general renal injury or early repair biomarker in animals exposed to nephrotoxicants. Measurement of Kim-1 protein expression may also be beneficial for detection of subtle abnormalities of tubular epithelial cells during injury/repair in the kidney at a time distant from an insult when gross morphology and physiological parameters have already returned to baseline. Kim-1 may be useful in preclinical and clinical studies vital to drug development and evaluation. It may also serve in the monitoring of disease states that manifest as injury to the proximal tubule and be useful in guiding interventional strategies. Our study deduce increase in KIM1 levels in cis group and this elevation returned to almost normal level indicating the protective effect of our model [SiNPs@AMF]. All these results infer that Silica nanospheres encapsulated with ami [SiNPs@AMF] improve its ability to diffuse by endocytosis as a behavior of small particles, and furthermore transformed to its metabolites gradually in acidic lysosomes organelles. Furthermore, the free sulphhydryls have been prevented from forming disulfide in acidic pH. Controlled production of sulphhydryl groups from conjugated amifostine was potentially to offer a protracted antioxidative environment because of the reduction in hydrolysis rate. Moreover, ROS generation will be repressed by this prevention from contact with other biomolecules by the polymeric structure.

Conclusions

Oil/water emulsion was used for the preparation of silica nanoparticles loaded with ami as a model drug. The preparation was carried out using ultra-sonication waves to enhance in the dispersing of nanoemulsion with the help of surfactant as well stabilizing and controlling agent for the formed nanosize. The data obtained proves that the produced nanoemulsion based on silica loaded with ami had small size with well distribution particles without any agglomeration or aggregation due to the steric effect of cetyltrimethyl ammonium bromide [CTAB] and castor oil [CAO]. In vivo studies were tested against drug nephrotoxicity resulting in increase of blood urea and serum creatinine in Cis–induced nephrotoxicity rats

concomitant with the disturbances of oxidative stress parameters, serum KIM 1 and upgrading of urinary 8-OHdG levels. Contrarily, these disturbances were augmented by protection of [SiNPs@AMF] supplementation. The obtained results are considered as potential implications that offer a new approach in attenuating of drug induced nephrotoxicity.

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Conflict Interests

The authors declare no conflict of interests.

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