International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2017.292.302

Research Article
Enhancement of Efficacy and Reduced Toxicity of Cisplatin Through Self Nanoemulsifying Drug Delivery System (SNEDDS)

[1,2]Abdel-Moneim M. Osman, [1]Huda M. Al-Kreathy, [1]Amal Al-Zahrani, [3]Osama A. Ahmed, [4]Wafaa S. Ramadan, [5,6]Mohamed F. ElShal, [1]Samir E. Al-Harthi, [1]Ahmed S. Ali and [1]Lateef M. Khan

[1]Department of Pharmacology, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia
[2]Pharmacology Unit, National Cancer Institute, Cairo University, Fom El-Khalig, P.O. Box 11796, Cairo, Egypt
[3]Department of Pharmaceutic, College of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia
[4]Department of Anatomy, College of Medicine, King Abdulziz University, Saudi Arabia
[5]Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
[6]Department of Molecular Biology and Genetic Engeneering and Biotechnology, Minoufia University, Egypt

Abstract

Background: Cisplatin is a potent antineoplastic agent with high therapeutic efficacy against many kinds of tumors. Its clinical use limited by its numerous side-effects, especially nephrotoxicity. **Objective:** The aim of this study was directed to formulate cisplatin self-nanoemulsifying drug delivery system (cisplatin-SNEDDS) as an attempt to improve the therapeutics activity and reduction of cisplatin toxicity. **Materials and Methods:** To evaluate these effects the cytotoxic activity of cisplatin-SNEDDS on the growth of Ehrlich Ascites Carcinoma (EAC) was assessed by determine the survival time of tumor-bearing mice, cisplatin cellular uptake, apoptosis induction, cell cycle distribution and renal function after treatment with cisplatin-SNDDS, compared with free cisplatin. **Results:** Free cisplatin increased the mean survival time of tumor bearing mice to 36 days compared with tumor bearing control mice while treatment of tumor bearing mice with cisplatin-SNEDDS showed a significant increase in their mean survival time to 44.30 days. Also, cisplatin-SNEDDS (7.5 mg kg^{-1}) significantly accumulated the cells in sub-G_1 and dramatically increased the percentage of early apoptotic cells in comparison to free cisplatin. Treatment with cisplatin-SNEDD retained rat's serum urea, creatinine and TAC levels to normal level and significantly increase the reduced glutathione in kidney homogenate compared to animals treated with free cisplatin. **Conclusion:** The SNEDDS enhanced the cytotoxic activity of cisplatin against the growth of EAC *in vivo* and protect against its nephrotoxicity.

Key words: Drug delivery system, cisplatin, cytotoxic activity, Ehrlich ascites carcinoma, apoptosis, cell cycle, renal function

Received: December 03, 2016 Accepted: January 08, 2017 Published: March 15, 2017

Citation: Abdel-Moneim M. Osman, Huda M. Al-Kreathy, Amal Al-Zahrani, Osama A. Ahmed, Wafaa S. Ramadan, Mohamed F. ElShal, Samir E. Al-Harthi, Ahmed S. Ali and Lateef M. Khan, 2017. Enhancement of efficacy and reduced toxicity of cisplatin through self nanoemulsifying drug delivery system (SNEDDS). Int. J. Pharmacol., 13: 292-302.

Corresponding Author: Abdel-Moneim M. Osman, Department of Pharmacology, Faculty of Medicine, King Abdulaziz University, Gamaa Street, P.O. Box 80205, Jeddah, Saudi Arabia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cisplatin is one of the most potent antitumor agents known, displaying clinical activity against a wide variety of solid tumors[1]. It achieved a significant clinical benefit for colon, ovarian and lung cancer despite the chemoresistance development[2]. Individual variations to cisplatin, like cisplatin resistance and toxicity are documented as the crucial obstacles in successful treatment during the course of therapy. The most common toxicities which may occurred during cisplatin therapy include nephrotoxicity, ototoxicty, neurotoxicity, gastrotoxicity, myelosuppression and allergic reactions[3,4]. Consequently, cisplatin effectiveness has been restricted by toxic side effects[5].

A variety of approaches have been tried to enhance the efficacy and reduce the toxicity of cisplatin, one of them is applying the nano-technology as drug delivery system. The entrapment of chemotherapeutic drug in nano-carriers has attracted considerable attention due to their structure, varied composition and surface modifications[6]. The most common architectures for targeted drug delivery applications are nanoparticles, liposomes, micelles and dendrimers.

Now a days, nano-technology in anticancer drugs are rapidly improved and breakthrough and are being applied to resolve some limitations of conventional chemotherapies such as non-specific biodistribution and targeting, poor water solubility, poor oral bioavailability and narrow therapeutic ranges of anticancer drugs. Nano-emulsions are being explored for cancer prevention, detection and treatment. Methods are being proposed and tested that could make diagnosis and treatment of cancer non-invasive which can be targeted directly to tumors. The current drug technologies are modulated that can result in reduced toxicity and in some cases, a 10 fold higher efficacy than when the drug is administered without targeting[7]. To manipulate and improve limitations of conventional chemotherapies, nanoparticles have been formulated and designed with optimal size and surface characteristics to be loaded with active anticancer drugs and carry their loaded drugs to cancer cells selectively by using the unique pathophysiology of tumor tissues, such as their enhanced permeability and retention (EPR) effect and the tumor microenvironment[8]. Steuber *et al*.[9] investigated the efficacy of curcumin in tocotriental nanoemulsion and found remarkable synergism iin antineoplastic efficacy overall in concentration and time dependent manner. However, the local drug delivery systems are not effective in metastatic tumors[10]. Self-nanoemulsifying drug delivery system (SNEDDS) is a strategy that formulates nanoemulsion with particle size less than 200 nm, this strategy drawn wide research interest, basically due to its distinct capacity to solubilise and improve the bioavailability of drugs[11]. Therefore, the present study was directed to investigate whether cisplatin self-nanoemulifying drug delivery system (cisplatin-SNEDDS) may enhance the cytotoxic effects of cisplatin against the growth of Ehrlich Ascites Carcinoma (EAC) inoculated into female Swiss Albino mice. Moreover, we evaluated the nephrotoxic effect of cisplatin-SNEDDS against free cisplatin triggered nephrotoxicity.

MATERIALS AND METHODS

Drugs and chemicals: Cis-diammineplatinum (II) dichloride (cisplatin) and other chemicals were obtained from SIGMA-Aldrich (St. Louis, Missouri, USA). Total Antioxidant Capacity (TAC) and reduced glutathione (GSH) kits were purchased from Biodiagnostic Co. (Dokki, Giza, Egypt). Annexin V-FITC apoptosis detection kit was purchased from Aldrich Chem. Corp., USA. The cell cycle determination kit was purchased from Cayman Chemical Company; USA. Blood Urea Nitrogen (BUN) and creatinine reagent cartridges were purchased from Siemens Healthcare Diagnostics Ltd. (Newark, New Jersey, USA).

Animals and tumor: Female Swiss Albino mice (8 weeks of age, 20-22 g b.wt.) and male Wistar Albino rats (8-10 weeks of age, 180-200 g b.wt.) were obtained from King Fahd Medical Research Center (KFRC), King Abdulaziz University, Jeddah, Saudi Arabia. The animals were acclimatized for 1 week before each experiment. A commercial balanced diet and water, *ad libitum* were provided throughout the experiments.

The Ehrlich Ascites Carcinoma (EAC) cells acquired through the courtesy of National Cancer Institution, Cairo University and maintained in our laboratory byweekly i.p., transplantation of 2.5×10^6 cell mouse^{-1}. Ascites fluid was withdrawn under aseptic condition (ultraviolet laminar airflow system) from the peritoneal cavity of the inoculated mice 10 days after EAC cells implantation. This study was approved by the institutional ethical committee of King Abdulaziz hospital.

Development of drug containing SNEDD formulation: The drug containing formulations were prepared by dissolving 5 mg of cisplatin in oil mixture and respective surfactant and co-surfactant on the vortex mixer and required quantity of aqueous phase added with gentle agitation[12].

The selected loaded formulations were subjected to dipersibility test and thermodynamic stability test to confirm the stability of SNEDDS formulation.

Evaluation of antitumor activity: The antitumor activity effect of cisplatin or cisplatin-SNEDDS was evaluated using the modified regimen of Donenko *et al.*[13]. The EAC cells were inoculated i.p., into 40 Swiss Albino mice (20-22 g) 2.5×10^6 cells mouse^{-1}. About 24 h later, mice were equally divided into four groups. Group I injected with normal saline i.p. (0.2 mL/20 g) and served as control group. Group II was administered plain-SNEDDS i.p. (0.2 mL/20 g). Group III was received a single i.p., injection of cisplatin (5 mg kg^{-1}) while group IV received a single i.p., injection of selected cisplatin-SNEDDS (5 mg kg^{-1}).

Mean survival times of mice and long term survivors were defined as the mice survived to the end of the experiment (45 days) with no apparent tumor.

Assessment of cisplatin cellular uptake: The EAC were inoculated i.p., into 36 Swiss Albino mice (20-22 g) 10×10^6 cells mouse^{-1}. About 24 h later mice were divided into six groups (six mice each). Groups 1-3 injected with cisplatin (7.5 mg kg^{-1} i.p.). Groups 4-6 injected with cisplatin-SNEDDS. Animals were sacrificed by cervical dislocation at 3, 24 and 48 h after treatment. Cell were withdrawn from peritoneal cavity and washed twice with Phosphate Buffer Saline (PBS) and then suspended in 1 mL PBS and counted. For drug uptake analysis, cells (1×10^6) should be suspended in 2 mL 1% HNO_3 for 24 h at 70°C to be digested. The volume of samples was completed to 5 mL with PBS then cisplatin concentration measured by CP-Optical Emission Spectrometer (Optima 7000DV, ICP-OES, PerkinElmer, INC, Waltham. USA) at wavelength 203, 210 and 214 nm, respectively. Cellular uptake of platinum was expressed as nanogram platinum per 1×10^6 cells.

Cell cycle analysis: The EAC were inoculated i.p., into 40 Swiss Albino mice (20-22 g) 10×10^6 cells mouse^{-1}. About 24 h later mice were divided into four groups (10 mice each). Group I injected with normal saline i.p. (0.2 mL/20 g) and served as control group. Group II was administered plain-SNEDDS i.p. (0.2 mL/20 g). Group III was received a single i.p., injection of cisplatin (7.5 mg kg^{-1}) while group IV received a single i.p., injection of selected cisplatin-SNEDDS (7.5 mg kg^{-1}). Animals were sacrificed by cervical dislocation at 48 h after treatment. Cells were withdrawn from peritoneal cavity and washed twice with assay buffer. The cells pellet was resuspended to a density of 10^6 cells mL^{-1} in assay buffer. After that 1 mL of fixative agent was added to each sample to fix and permeabilize the cells for atleast 2 h prior to PI staining. Fixed cells were centrifuged at 500×g for 5 min, where the fixative decanted thoroughly. The cells pellet was suspended in staining solution which prepared by mixing (10 mL assay buffer with 200 µL RNase A solution and 200 µL PI solution) for every 20 samples to be stained, then the cells were incubated for 30 min at RT in the dark. Cell cycle analysis was performed by using flow cytometry (Becton DICNSON (BD) FACS Calibur)[14].

Assay of apoptosis: Apoptosis cells were quantified by annexin V-FITC-propodium iodide double staining, using an annexin V-FITC apoptosis detection kit. The EAC were inoculated i.p., into 40 Swiss Albino mice (20-22 g) 10×10^6 cells mouse^{-1}. About 24 h later mice were divided into 4 groups (10 mice each) and injected as mentioned in cell cycle analysis paragraph. Animals were sacrificed by cervical dislocation 48 h after treatment. Cells were withdrawn from peritoneal cavity and washed twice with PBS and then resuspended in 100 µL annexin V incubation reagent prepared by mixing (binding buffer 10x, PI, annexin V-FITC and deionized water) for each sample. The solution was incubated in the dark for 15 min at room temperature. Then 400 µL 1x of binding buffer were added to each sample and process by flow cytometry (NAVIOS Beckman Coulter, U.S.A.) within 1 h for maximal signal.

Nephrotoxic effect of cisplatin-SNEDDS compare to free cisplatin: Twenty male Wister rat were divided into four equal groups, each composed of 6 animals. Group I received normal saline i.p. (0.5 mL/200 g) and reserved as control group. Group II was administered plain-SNEDDS i.p. (0.5 mL/200 g). Group III was received i.p., injection of cisplatin (2.5 mg kg^{-1}, every other day for 3 doses) while group IV received i.p., injection of selected cisplatin-SNEDDS (2.5 mg kg^{-1}, every other day for 3 doses). At the end of the experiment (day 7), rats were anesthetized and blood samples were collected from the ophthalmic artery in the orbital rim and rapidly centrifuged for serum separation that was stored at −80°C to evaluate BUN, serum creatinine levels by commercial kits[15,16] and TAC according to the method of Koracevic *et al.*[17]. The dissected rat kidney was cut into small pieces and immersed immediately in 10% neutral buffered formalin, for light microscope study. The residual kidney pieces were weighted and homogenized in 7 mL cold buffer (50 mM potassium phosphate, pH 7.5, 1 mM EDTA), (1 g tissue/7 mL cold buffer) by a homogenizer (Potters, German) then it was centrifuged at 4000 rpm for 15 min at 4°C. The supernatant was removed and stored in -80°C and used for the evaluation of GSH according to the method of Beutler and Gelbart[18].

Statistical analysis: Data are presented as Mean±SD. Multiple comparisons were performed using one-way ANOVA followed by Tukey-Kramer as a *post hoc* test. The 0.05 level of probability was used as the criterion for significance. All statistical analyses were performed using GraphPad Instat software version 3. Graphs were sketched using GraphPad Prism software version 4 (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Survival of tumor bearing mice: Table 1 and Fig. 1 show the effect of intraperitoneal administration of free cisplatin (5 mg kg^{-1}) or cisplatin-SNEDD (5 mg kg^{-1}) on the survival of female Swiss Albino mice bearing EAC cells. Control group showed a mean survival time of 17.2 days, whereas, the administration of free cisplatin showed significant increase in the mean survival time to 35.1 days with two mice survived to the end of experiment in 45 days. The administration of cisplatin-SNEDDS (5 mg kg^{-1}) showed significant increased in the mean survival time to 44 days with 80% long-term survivor (45 days). No significant difference between control group and plain-SNEDD (0.2 mL/ 20 g) treated group has been observed.

Effect of SNEDDS on cellular uptake of cisplatin in EAC cells: Table 2 shows cellular level of cisplatin concentration in EAC cells after treatment with a single dose of free cisplatin (7.5 mg kg^{-1}) or a single dose of cisplatin-SNEDDS (7.5 mg kg^{-1}). Treatment with cisplatin-SNEDDS significantly increased the cellular level of cisplatin by 1.5 fold after 24 and 48 h of treatment.

Table 1: Effects of free cisplatin or cisplatin-SNEDDS on the survival time of mice bearing EAC cells

Treatment groups	Mean survival time (days)	45-day survivors
Control (normal saline, 0.2 mL/20 g)	17.20±2.04	0/10
Plain-SNEDDS (0.2 mL/20 g)	15.90±1.66	0/10
Cisplatin (5 mg kg^{-1})	35.10±6.35[a]	2/10
Cisplatin-SNEDDS (5 mg kg^{-1})	44.30±1.64[a,b]	8/10

Data represent as Mean±SD (n = 10), [a]Significantly different from control at $p<0.05$, [b]Significantly different from free cisplatin at $p<0.05$, one way ANOVA followed by tukey post test

Table 2: Effect of SNEDDS on cellular uptake of cisplatin in EAC cells

	Cisplatin concentration (ng 10^{-8} cells)		
Treatment groups	3 h after treatment	24 h after treatment	48 h after treatment
Cisplatin (7.5 mg kg^{-1})	2.78±0.32	5.88±0.47	6.81±0.21
Cisplatin-SNEDDS (7.5 mg kg^{-1})	3.94±0.41[b]	8.78±0.66[a,b]	10.67±0.78[a,b]

Data represent as Mean±SD (n = 6), [a]Significantly different from corresponding free cisplatin at $p<0.05$, [b]Significantly different from 3 h one way ANOVA followed by tukey post test

Effect of cisplatin or cisplatin-SNEDDS on cell cycle analysis: Figure 2 shows the percent of distribution of G_0, G_1, S and G_2/M of tumor cells after 48 h of treatment with free cisplatin or cisplatin-SNEDDS. Free cisplatin treatment (7.5 mg kg^{-1}) accumulated the cells in G_0 phase by 42.20%. However, cisplatin-SNEDDS treatment (7.5 mg kg^{-1}) significantly increased the accumulation in G_0 phase by 78.80%, 48 h after treatment.

Effect of cisplatin or cisplatin-SNEDDS on the induction of apoptosis: The percentage of early apoptotic cells (Annexin V-positive cells) were dramatically increased after treatment with free cisplatin or cisplatin-SNEDDS in comparison to the control cells (Early apoptosis cells (%). Also, treatment with cisplatin-SNEDDS (7.5 mg kg^{-1}) increased the percentage of early apoptotic cells (Annexin V-positive cells) significantly by 78.10% after 48 h of treatment compared with EAC cells withdrawn from animals treated with free cisplatin (55.15%) (Fig. 3).

Protective effect of SNEEDS against cisplatin-induced nephrotoxicity: Cisplatin treatment (2.5 mg kg^{-1} every other days for 3 doses) caused a significant 4 and 6 fold increase in serum creatinine and blood urea nitrogen, respectively (Table 3). However, cisplatin in SNEEDS formulation showed decrease in the cisplatin-induced increase in creatinine and blood urea by 41.5 and 57%, respectively. Moreover, there was decrease in reduced glutathione in kidney homogenate and serum total antioxidant capacity by 55 and 39% after

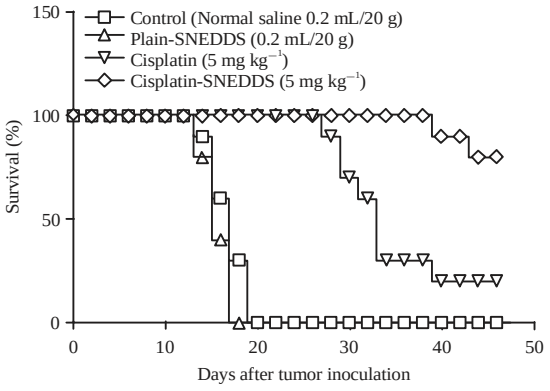

Fig. 1: Effects of free cisplatin or cisplatin-SNEDDS on the survival time of mice bearing EAC cells

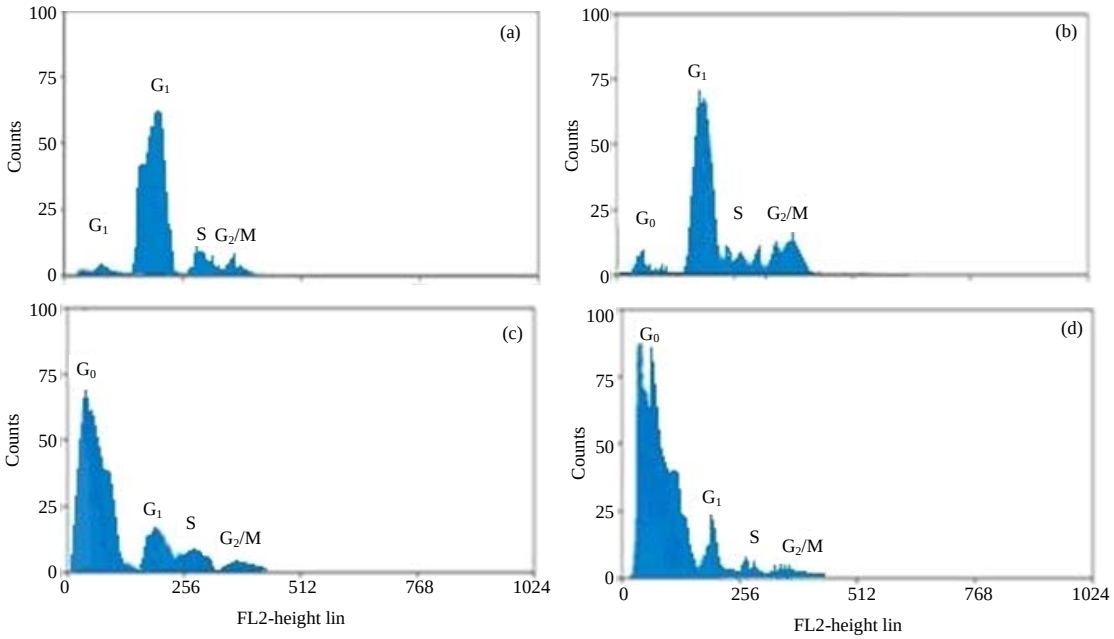

Fig. 2(a-d): Effect of free cisplatin or cisplatin-SNEDDS on cell cycle phase distribution of EAC cells after 48 h of treatment. Cell cycle distribution was analyzed by staining with PI, cells that withdrawn from animals treated with (a) Normal saline (0.2 mL/20 g), (b) Plain-SNEDDS (0.2 mL/20 g), (c) Free cisplatin (7.5 mg kg^{-1}) and (d) Cisplatin-SNEDDS (7.5 mg kg^{-1})

Table 3: Effect of free cisplatin or cisplatin-SNEDDS on blood urea and serum creatinine level of normal rats

Treatment groups	BUN (mg dL^{-1})	Creatinine (mg dL^{-1})
Control (normal saline, 0.2 mL/20 g)	31.00±3.58	0.47±0.05
Plain-SNEDDS (0.2 mL/20 g)	38.50±2.17	0.60±0.09
Cisplatin (7.5 mg kg^{-1})	182.33±11.15[a]	1.70±0.30[a]
Cisplatin-SNEDDS (7.5 mg kg^{-1})	77.67±12.75[a,b]	1.00±0.08[a,b]

Data represent as Mean±SD (n = 6), [a]Significantly different from control at p<0.05, [b]Significantly different from free cisplatin at p<0.05, one way ANOVA followed by tukey post test

Table 4: Effect of free cisplatin or cisplatin-SNEDDS on GSH activity in rat's kidney homogenate

Treatment groups	GSH (mg g^{-1})
Control (normal saline, 0.2 mL/20 g)	45.71±2.76
Plain-SNEDDS (0.2 mL/20 g)	36.78±3.26
Cisplatin (7.5 mg kg^{-1})	20.67±1.71[a]
Cisplatin-SNEDDS (7.5 mg kg^{-1})	38.49±8.38[a,b]

Data represent as Mean±SD (n = 6), [a]Significantly different from control at p<0.05, [b]Significantly different from free cisplatin at p<0.05, one way ANOVA followed by tukey post test

Table 5: Effect of free cisplatin or cisplatin-SNEDDS on the serum level of TAC in rats

Treatments	TAC (mmol L^{-1})
Control (normal saline, 0.2 mL/20 g)	1.13±0.26
Plain-SNEDDS (0.2 mL/20 g)	1.11±0.28
Cisplatin (7.5 mg kg^{-1})	0.69±0.08[a]
Cisplatin-SNEDDS (7.5 mg kg^{-1})	1.08±0.21[b]

Data represent as Mean±SD (n = 6), [a]Significantly different from control at p<0.05, [b]Significantly different from free cisplatin at p<0.05, one way ANOVA followed by tukey post test

treatment with cisplatin. However, cisplatin in SNEEDS showed nearly normal level in both reduced glutathione and total antioxidant capacity (Table 4, 5). Histopathological examination of kidney tissue of normal rats showed normal morphology of a glomerulus (G) and the subcapsular space (Fig. 4), plain SNEEDS treatment did not show any alterations of kidney tissue slight congestion of the intertubular capillaries (arrows) as well as the glomerular tuft of capillaries (Fig. 5). Treatment with cisplatin showed vaculoation of the cuboidal cells lining the tubules (Fig. 6) and areas of desquamated tubular epithelium are noted. However, treatment with cisplatin-SNEDDS showed widened of subcapsular space (Fig. 7). The DCT appear within normal structure.

DISCUSSION

Nano-technology is one of the most popular areas of scientific research particularly used in developing a new generation of drug delivery system with greater targeting selectivity and better delivery efficiency, capable to overcome the limitations and improve the overall pharmacological properties of anticancer drugs by taking advantages of tumor microenvironment.

Cisplatin is the most widely used cytotoxic drug in the treatment of many kinds of tumors either alone or in

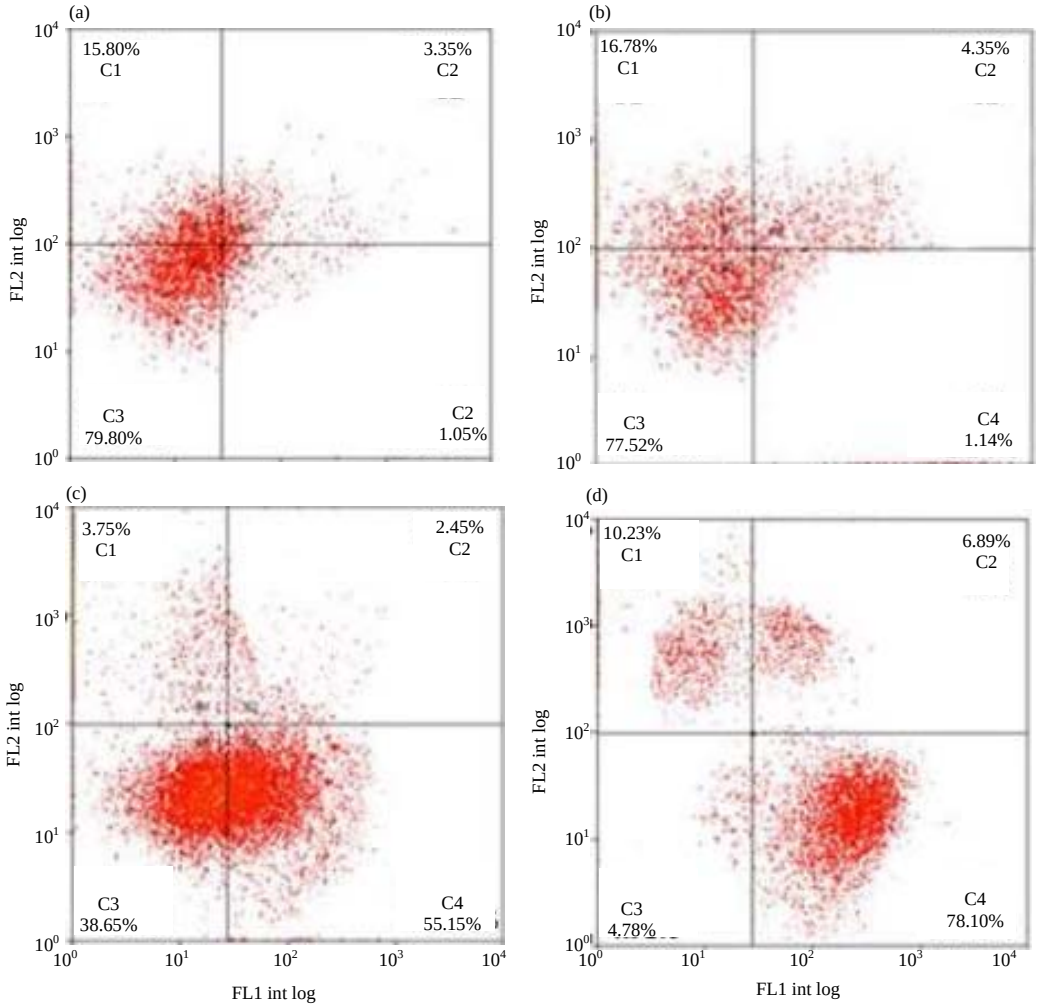

Fig. 3(a-d): Effect of free cisplatin or cisplatin-SNEDDS on apoptosis induction in EAC cells withdrawn 48 h after treatment. Apoptosis was analyzed by staining with PI (y-axis) and annexin-FITC (x-axis), EAC cells withdrawn from animal treated with (a) Normal saline (0.2 mL/20 g i.p.), (b) Plain-SNEDDS (0.2 mL/20 g i.p.), (c) Free cisplatin (7.5 mg kg^{-1}) and (d) Cisplatin-SNEDDS (7.5 mg kg^{-1}). The percentage of cells in each quadrant is indicated (C1: Necrosis, C2: Late apoptosis, C3: Live cells, C4: Early apoptosis)

combination with other cytocidal drugs. However its clinical uses are limited by its detrimental adverse effects including nephrotoxicity[19]. Recent studies have discovered new protocols, compounds, enzymes and molecular alteration that reduced the side-effects of anticancer drugs and enhanced their cytotoxic effects[20,21]. In an attempt to increase the cytotoxic activity of cisplatin and minimizing its nephrotoxicity, cisplatin-SNEDDS a promising drug delivery vehicles that form nanosized emulsions loaded with cisplatin was formulated as new strategy to increase its efficacy therefore decrease the antitumor dose and so decrease the toxicity.

The present study showed that the treatment of tumor bearing mice with cisplatin-SNEDDS (5 mg kg^{-1}), significantly enhances the cytotoxic activity of cisplatin against the growth of EAC cells by 1.25 fold increase in the long term survivor compared with animals treated with free cisplatin. It is well known that cisplatin induced formation of intra and inter-DNA strand linkage lead to severe local distortion in the DNA double helical structure lead to cell death[22,23]. The mentioned results have been confirmed by the observed increase in cisplatin cellular uptake after cisplatin-SNEDDS treatment. Treatment with cisplatin-SNEDDS lead to almost 2 folds increase in the cisplatin accumulation ratios in EAC cells especially after 24 and 48 h compared with corresponding cells treated with free cisplatin (Table 2). In a good agreement with the results, Paraskar *et al.*[24] reported that the treatment with the cisplatin nanoparticle resulted in

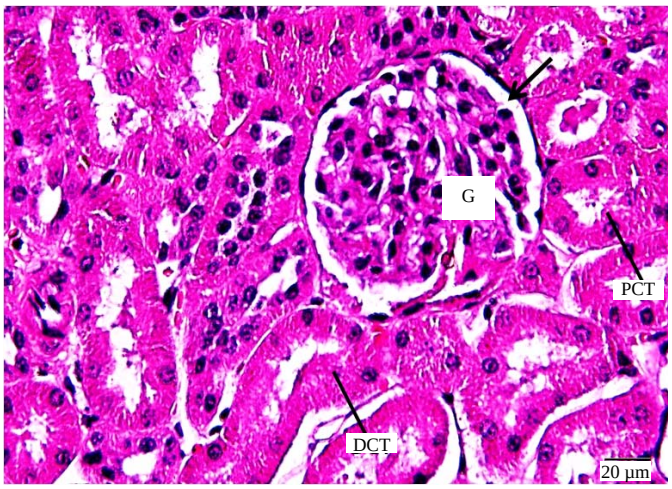

Fig. 4: Photomicrograph of a section of the kidney cortex of a rat in control group. It is showing normal morphology of a glomerulus (G) and the subcapsular space (arrow). The proximal (PCT) and distal (DCT) convoluted tubules are lined with cuboidal cells with eosinophilic cytoplasm and round basal nuclei

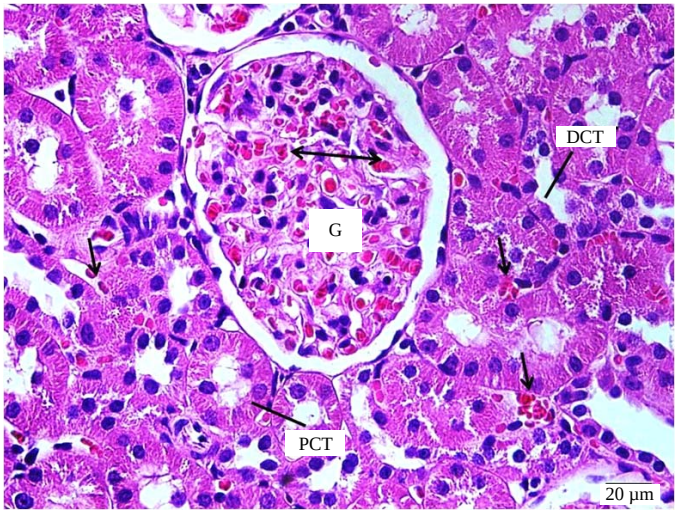

Fig. 5: Photomicrograph of a section of the kidney cortex of a rat receiving plain-SNEDDS. It is showing slight congestion of the intertubular capillaries (arrows) as well as the glomerular tuft of capillaries (double head arrow)

significant tumor ablation in breast cancer bearing mice and significantly increased platinum concentration in the tumor as compared with the free drug.

Moreover, Lee *et al.*[25] showed that cisplatin-incorporated nanoparticles poly (acrylic acid-co-methyl methacrylate) (PAA-MMA) showed a significant inhibitory effect on the growth of tumor mass of carcinoma cells (CT26) bearing mice in comparison with cisplatin alone. Recently Andey *et al.*[26] reported that mannosylated noscapine self-emulsifying solid dispersions (Mann-Nos_SESDs) are bioavailable and potentiate the antineoplastic effect of cisplatin-based chemotherapy in cisplatin-resistant NSCLC. The increase in the cisplatin cytotoxicity and accumulation in cells treated with dispersions (Mann-Nos_SESDs) are bioavailable and potentiate the antineoplastic effect of cisplatin-based chemotherapy in cisplatin-resistant NSCLC. The increase in the cisplatin cytotoxicity and accumulation in cells treated with cisplatin-SNEDDS could be explained by the unique pathophysiologic characteristics of tumor vessels enable macromolecules, including nanoparticles, to selectively accumulate in tumor tissues[27]. Fast-growing cancer cells demand the recruitment of new vessels

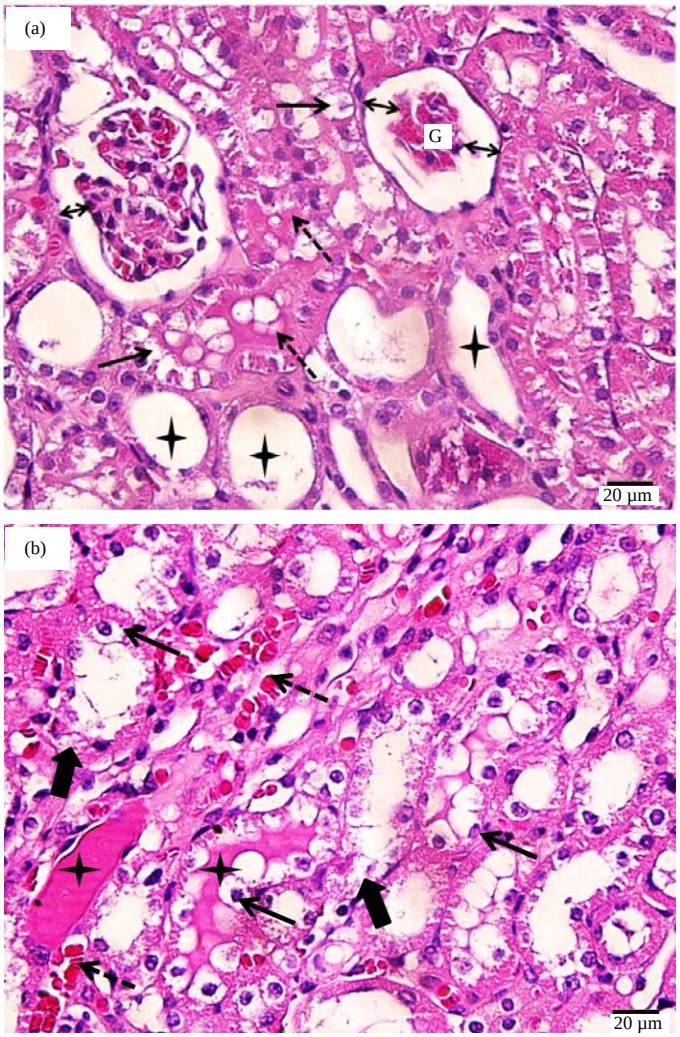

Fig. 6(a-b): Photomicrograph of a section of the kidney (a) Cortex and (b) Medulla of a rat treated with free cisplatin (2.5 mg kg×3 every other day), (a) Vaculoation of the cuboidal cells lining the tubules (arrows). Many tubules are dilated with apparent thinning of the epithelium (stars) while others are filled with homogenous cast material (dashed arrows). Some glomerular tufts of capillaries (G) are shrunken with widening of the subcapsular space (double head arrow) and (b) Vacuolation of the cytoplasm and ballooning of most epithelial cells lining the tubules (arrows). Areas of desquamated tubular epithelium are noted (thick arrow). Some tubules are filled with cast material (✦). Congestion of the intertubular capillaries (dashed arrows) are also noticed

(neovascularization) to supply them with oxygen and nutrients. The resulting imbalance of angiogenic regulators such as growth factors and matrix metalloproteinases makes tumor vessels highly disorganized and dilated with numerous pores showing enlarged gap junctions between endothelial cells and compromised lymphatic drainage by which nanoparticles, can selectively accumulate in the tumor tissues[28]. As a result cisplatin-SNEDDS accumulated more inside the tumor tissues which may contribute to more cisplatin uptake and consequently more cell death.

Furthermore, the SNEDDS retained all the advantages that associated with nano-scale emulsions such as improve bioavailability, enhance drug permeation[29] entrap a larger amount of drug; high drug loading capacity, reduce the toxicity of the incorporated drug[30,31] and possibility of dose reduction[32].

It is known that DNA damage caused by different cytotoxic agents, induced cell cycle arrest at G_1, S and G_2, thereby preventing replication of damaged DNA or aberrant mitosis which if not repaired, may result in either tumorigenesis or apoptosis[33,34].

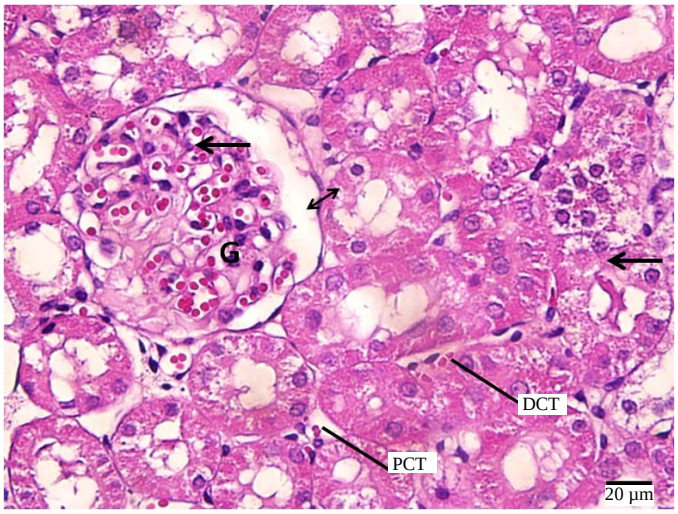

Fig. 7: Photomicrograph of a section of the kidney cortex of a rat treated with cisplatin-SNEDDS (2.5 mg kg×3 every other day). It is showing vacuolation of epithelium of some of proximal convoluted tubules (arrows). The subcapsular space appear widened (double head arrow) on one side and obliterated on the opposite side. The DCT appear within normal structure

These results showed that the tumor cells withdrawn from animals treated with cisplatin-SNEDD showed a significant increase in the arrested cells in G_0 compared with cells treated with free cisplatin specifically after 48 h (Fig. 3). These results could be attributed to the nanosized emulsion which preferentially accumulated inside the tumor cells as a result of enhanced permeability and retention (EPR) effect and also related to the underlying mechanism for nanosize emulsion entering cells being endocytotic and/or pinocytotic rather than merely a simple passive permeation, thus reducing excretion of cisplatin from the cells. This suggests that the long presence of cisplatin inside the tumors due to the nanoemulsion formulation, thus can effectively activates apoptosis by inhibiting DNA replication and transcription, through the formation of covalent adducts between its chloride atoms and DNA bases[35].

In consistence with our results, Li *et al.*[36] reported that, the apoptotic rate in Non Small Cell Lung Cancer (NSCLC) was significantly higher in the group treated with magnetic iron oxide nanoparticles loaded with cisplatin (Fe_3O_4-MNP-DDP) than in the groups treated with free cisplatin. They interpreted the high rate of apoptosis as a result of intracellular accumulation of cisplatin nanoparticles in tumor tissues and down regulation of transmembrane transporters, such as P-glycoprotein and Multidrug Resistance Proteins 1 (MRP1), both are members of the ATP-binding cassette protein transporter which acting as drugs efflux pumps. Moreover, an *in vitro* cell assay done by Li *et al.*[37] demonstrated the high biocompatibility of poly (acrylic acid) modified mesoporous silica nanoparticles (PAA-MSNs) increased cytotoxicity of the combination of doxorubicin (DOX) and cisplatin ($Pt@PAA\text{-}MSN_{DOX}$) nanocomposites in both HeLa and A357 (human melanoma cells) tumor cells with respect to free single drug or single drug loaded nanoparticles at the same dosage. This unique drug co-delivery system using an anticancer drug as a cross-linking linkage suggests a promising application in multi-drug delivery for combination cancer therapy.

In the current study, rats treated with free cisplatin showed a significant increase in the levels of serum creatinine and BUN levels, while in the cisplatin-SNEDDS treated animals the levels nearly return to normal (Table 3). Histopatholgical evaluation in this study showed that cisplatin treatment causes marked necrosis in proximal tubules and degeneration of the tubular epithelial cells (Fig. 6). Whereas the treatment with cisplatin-SNEDD display more intact kidney structure than that happened with cisplatin (Fig. 7). The neglected nephrotoxicity of cisplatin-SNEDDS could be illustrated by reduced clearance of nanoparticles through reticuloendothelial system. It is well documented that nanoparticles larger than 10 nm avoid renal clearance[24] and they potentially decrease cisplatin nephrotoxic effect. Furthermore, it is well established that nanoparticles in the size range of 10-100 nm preferentially accumulate in the tumor due to the EPR effect and prevent their extravasations in normal tissues such as kidney and liver, therefore reduce the toxicity of incorporated drug[25].

Cisplatin is highly reactive oxygen species can cause extensive tissue damage through reactions with all biological macromolecule, e.g., lipids, proteins and nucleic acids, leading

to the formation of oxidized substances[38]. It also induces a fall in plasma antioxidant levels, which reflect a failure of the antioxidant defense mechanism[39]. Reduced glutathione is the most abundant intracellular thiol and antioxidant which play an important role in protecting cells from apoptosis[40,41]. In our result study cisplatin-SNEDDS significantly increase GSH level in kidney tissues compared to free cisplatin (Table 4) while the level of TAC was return to normal level (Table 5). The most convincing explanation to GSH and TAC reduction after cisplatin administration is their over consumption in non-enzymatic removal of Reactive Oxygen Species (ROS) which produce as a result of cisplatin administration, the production of ROS by cisplatin in kidney is crucial to the progression of nephrotoxicity. Cisplatin toxicity is mainly due to the systemic distribution of free cisplatin which involve specific renal transport systems while the particles size of cisplatin-SNEDDS prevent their systemic distribution and hinder them from passage through kidney tissues therefore reduce the spending of GSH and TAC to counteract cisplatin free radicals[42].

CONCLUSION

It seems that SNEDDS improved the cytotoxic activity of cisplatin and reduced their systemic toxicity as a result of physical and chemical characteristic of nano-emulsion itself and the unique pathophysiologic characteristics of tumor cells which passively accumulate cispatin-SNEDDS inside them.

ACKNOWLEDGMENTS

The present study has been funded by grant from King Abdulaziz City for Science and Technology Research, Saudi Arabia (No. 317-36 ط.أ).

REFERENCES

1. Siddik, Z.H., 2003. Cisplatin: Mode of cytotoxic action and molecular basis of resistance. Oncogene, 22: 7265-7279.
2. Germani, A., A. Matrone, V. Grossi, A. Peserico and P. Sanese *et al*., 2014. Targeted therapy against chemoresistant colorectal cancers: Inhibition of p38α modulates the effect of cisplatin *in* vitro and *in vivo* through the tumor suppressor FoxO3A. Cancer Lett., 344: 110-118.
3. Hartmann, J.T., L.M. Fels, S. Knop, H. Stolte, L. Kanz and C. Bokemeyer, 2000. A randomized trial comparing the nephrotoxicity of cisplatin/ifosfamide-based combination chemotherapy with or without amifostine in patients with solid tumors. Invest. New Drugs, 18: 281-289.
4. Hartmann, J.T. and H.P. Lipp, 2003. Toxicity of platinum compounds. Expert Opin. Pharmacother., 4: 889-901.
5. Chen, D., V. Milacic, M. Frezza and Q.P. Dou, 2009. Metal complexes, their cellular targets and potential for cancer therapy. Curr. Pharmaceut. Des., 15: 777-791.
6. Kijanka, M., B. Dorresteijn, S. Oliveira and P.M.P. van Bergen en Henegouwen, 2015. Nanobody-based cancer therapy of solid tumors. Nanomedicine, 10: 161-174.
7. Kumar, G.P. and A. Divya, 2015. Nanoemulsion based targeting in cancer therapeutics. Med. Chem., 5: 272-284.
8. Cho, K., X. Wang, S. Nie, Z.G. Chen and D.M. Shin, 2008. Therapeutic nanoparticles for drug delivery in cancer. Clin. Cancer Res., 14: 1310-1316.
9. Steuber, N., K. Vo, R. Wadhwa, J. Birch, P. Iacoban, P. Chavez and T.A. Elbayoumi, 2016. Tocotrienol nanoemulsion platform of curcumin elicit elevated apoptosis and augmentation of anticancer efficacy against breast and ovarian carcinomas. Int. J. Mol. Sci., Vol. 17, No. 11. 10.3390/ijms17111792.
10. Ordikhani, F., M.E. Arslan, R. Marcelo, I. Sahin, P. Grigsby, J.K. Schwarz and A.K. Azab, 2016. Drug delivery approaches for the treatment of cervical cancer. Pharmaceutics, Vol. 8, No. 3. 10.3390/pharmaceutics8030023.
11. Balakumar, K., C.V. Raghavan, N.T. Selvan, R.H. Prasad and S. Abdu, 2013. Self Nanoemulsifying Drug Delivery System (SNEDDS) of rosuvastatin calcium: Design, formulation, bioavailability and pharmacokinetic evaluation. Colloids Surf. B: Biointerfaces, 112: 337-343.
12. Elnaggar, Y.S., M.A. El-Massik and O.Y. Abdallah, 2009. Self-nanoemulsifying drug delivery systems of tamoxifen citrate: Design and optimization. Int. J. Pharm., 380: 133-141.
13. Donenko, F.V., T. Efferth, J. Mattern, L.V. Moroz and M. Volm, 1991. Resistance to doxorubicin in tumor cells *in vitro* and *in vivo* after pretreatment with verapamil. Chemotherapy, 37: 57-61.
14. Sulic, S., L. Panic, I. Dikic and S. Volarevic, 2005. Deregulation of cell growth and malignant transformation. Croat Med. J., 46: 622-638.
15. Bonsnes, R.W. and H.H. Taussky, 1945. On the colorimetric determination of creatinine by the Jaffe reaction. J. Biol. Chem., 158: 581-591.
16. Fawcett, J.K. and J.E. Scott, 1960. A rapid and precise method for the determination of urea. J. Clin. Pathol., 13: 156-159.
17. Koracevic, D., G. Koracevic, V. Djordjevic, S. Andrejevic and V. Cosic, 2001. Method for the measurement of antioxidant activity in human fluids. J. Clin. Pathol., 54: 356-361.
18. Beutler, E. and T. Gelbart, 1986. Improved assay of the enzymes of glutathione synthesis: γ-glutamylcysteine synthetase and glutathione synthetase. Clinica Chimica Acta, 158: 115-123.
19. Yao, X., K. Ichpisal, N, Kurtzman and K. Nugent, 2007. Cisplatin nephrotoxicity: A review. Am. J. Med. Sci., 334: 115-124.

20. Yildirim, Z., S. Sogut, E. Odaci, M. Iraz, H. Ozyurt, M. Kotuk and O. Akyol, 2003. Oral erdosteine administration attenuates cisplatin-induced renal tubular damage in rats. Pharmacol. Res., 47: 149-156.
21. Osman, A.M.M., A.A. Ali, Z.A. Damanhouri, S.E. Al-Harthy and M.F. ElShal *et al*., 2015. Dimethylsulfoxide excerbates cisplatin-induced cytotoxicity in Ehrlich ascites carcinoma cells. Cancer Cell Int., Vol. 15. 10.1186/s12935-015-0258-1.
22. Eastman, A., 1987. The formation, isolation and characterization of DNA adducts produced by anticancer platinum complexes. Pharmacol. Therapeut., 34: 155-166.
23. Danford, A.J., D. Wang, Q. Wang, T.D. Tullius and S.J. Lippard, 2005. Platinum anticancer drug damage enforces a particular rotational setting of DNA in nucleosomes. Proc. Natl. Acad. Sci. USA., 102: 12311-12316.
24. Paraskar, A.S., S. Soni, K.T. Chin, P. Chaudhuri and K.W. Muto *et al*., 2010. Harnessing structure-activity relationship to engineer a cisplatin nanoparticle for enhanced antitumor efficacy. Proc. Natl. Acad. Sci. USA., 107: 12435-12440.
25. Lee, K.D., Y.I. Jeong, D.H. Kim, G.T. Lim and K.C. Choi, 2013. Cisplatin-incorporated nanoparticles of poly (acrylic acid-co-methyl methacrylate) copolymer. Int. J. Nanomed., 8: 2835-2845.
26. Andey, T., A. Patel, S. Marepally, M. Chougule, S.D. Spencer, A.K. Rishi and M. Singh, 2016. Formulation, pharmacokinetic and efficacy studies of mannosylated self-emulsifying solid dispersions of noscapine. PLoS ONE, Vol. 11. 10.1371/journal.pone.0146804.
27. Maeda, H., 2001. The Enhanced Permeability and Retention (EPR) effect in tumor vasculature: The key role of tumor-selective macromolecular drug targeting. Adv. Enzyme Regul., 41: 189-207.
28. Carmeliet, P. and R.K. Jain, 2000. Angiogenesis in cancer and other diseases. Nature, 407: 249-257.
29. Lei, B., W. Zha, Y. Wang, C. Wen and E.J. Studer *et al*., 2010. Development of a novel self-microemulsifying drug delivery system for reducing HIV protease inhibitor-induced intestinal epithelial barrier dysfunction. Mol. Pharm., 7: 844-853.
30. Mistry, R.B. and N.S. Sheth, 2011. A review: Self emulsifying drug delivery system. Int. J. Pharm. Pharmaceut. Sci., 3: 23-28.
31. Kumar, S., S.K. Gupta and P.K. Sharma, 2012. Self-Emulsifying Drug Delivery Systems (SEDDS) for oral delivery of lipid based formulations-a review. Afr. J. Basic Applied Sci., 4: 7-11.
32. Shen, H. and M. Zhong, 2006. Preparation and evaluation of Self-Microemulsifying Drug Delivery Systems (SMEDDS) containing atorvastatin. J. Pharm. Pharmacol., 58: 1183-1191.
33. Walworth, N.C., 2000. Cell-cycle checkpoint kinases: Checking in on the cell cycle. Curr. Opin. Cell Biol., 12: 697-704.
34. Zhou, B.B.S. and S.J. Elledge, 2000. The DNA damage response: Putting checkpoints in perspective. Nature, 408: 433-439.
35. Choi, H.S., W. Liu, P. Misra, E. Tanaka and J.P. Zimmer *et al*., 2007. Renal clearance of quantum dots. Nat. Biotechnol., 25: 1165-1170.
36. Li, K., B. Chen, L. Xu, J. Feng and G. Xia *et al*., 2013. Reversal of multidrug resistance by cisplatin-loaded magnetic Fe_3O_4 nanoparticles in A549/DDP lung cancer cells *in vitro* and *in vivo*. Int. J. Nanomed., 8: 1867-1877.
37. Li, H., H. Yu, C. Zhu, J. Hu, M. Du, F. Zhang and D. Yang, 2016. Cisplatin and doxorubicin dual-loaded mesoporous silica nanoparticles for controlled drug delivery. RSC Adv., 6: 94160-94169.
38. Gutteridge, J.M. and B. Halliwell, 1992. Comments on review of free radicals in biology and medicine, second edition, by Barry Halliwell and John M. C. Gutteridge. Free Radical Biol. Med., 12: 93-95.
39. Weijl, N.I., A. Wipkink-Bakker, E.G.W.M. Lentjes, H.M. Berger, F.J. Cleton and S. Osanto, 1998. Cisplatin combination chemotherapy induces a fall in plasma antioxidants of cancer patients. Ann. Oncol., 9: 1331-1337.
40. Husain, K., C. Morris, C. Whitworth, G.L. Trammell, L.P. Rybak and S.M. Somani, 1998. Protection by ebselen against cisplatin-induced nephrotoxicity: Antioxidant system. Mol. Cell. Biochem., 178: 127-133.
41. Huang, Q., R.T. Dunn II, S. Jayadev, O. DiSorbo and F.D. Pack *et al*., 2001. Assessment of cisplatin-induced nephrotoxicity by microarray technology. Toxicol. Sci., 63: 196-207.
42. Almaghrabi, O.A., 2015. Molecular and biochemical investigations on the effect of quercetin on oxidative stress induced by cisplatin in rat kidney. Saudi J. Biol. Sci., 22: 227-231.