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Hepatoprotective Efficacy Of Amifostine Nanoemulsion In Cisplatin Hepatotoxic Rat.

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ABSTRACT

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 these diseases, multi drug treatments are rising, with a high probability of adverse effects of different organs or systems. The liver is a major organ for drug-induced toxicity. This study aimed to ameliorate the toxic effects of drugs that the patient is forced to take them for long periods or permanently and to evaluate the changes of liver functions, oxidative stress, anti-oxidants and histopathological investigations in cisplatin-induced hepatotoxicity and the SiNPs@AMF ameliorative effects. This article describes the preparation and characterization of nanoemulsion of amifostine silica nanoparticles [SiNPs@AMF]. Cisplatin had given to mice induced marked hepatic injuries, characterized with a significant increase in serum ALT, AST, MDA, LN and TIMP1. In the groups that were administered SiNPs@AMF in association with cisplatin, improvement was observed in oxidative stress parameters (MDA), other hepatic biochemical parameters, and histopathological examinations with SiNPs@AMF being more effective. The results obtained suggested that SiNPs@AMF significantly attenuated the cisplatin-hepatotoxicity, because it act as free radical scavenger and lipid peroxidation inhibitor.

Keywords: Hepatotoxicity –Liver –Oxidative stress- silica nanoparticles –Cisplatin- Amifostine

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INTRODUCTION

The liver is a crucial organ in the human body. It regulates functions including the synthesis of proteins, secretion of biochemical enzymes, and detoxification of xenobiotics [1].Nowadays, liver damage becomes a very common cause of various metabolic disorders and can lead to even mortality [2]. Liver damage or hepatic toxicity is referred as liver dysfunction which is often associated with exposure to toxins, over dosage of medicines or by some therapeutic agents [3]. The most promising results have been obtained with the organic thiophosphate compound – amifostine. Amifostine is a cytoprotective agent used in cancer chemotherapy and radiotherapy [4]. Amifostine is a thiophosphate cytoprotective agent developed as a radio protector [5].Also it is used to reduce the side effects associated with platinum-containing agents [6]. However, approximately 90% of the amifostine was rapidly cleared from plasma after 6 minutes after intravenous administration [7].The efficacy of its use for alleviating the side effects of chemotherapy and space travel is also limited by the extremely short half-life [8].Amifostine is generally well tolerated and is associated with transient side effects, including nausea, vomiting, a warm or flushed feeling and occasional allergic reactions. The most clinically significant toxicity is hypotension [9]. This article describes the preparation and characterization of nanoemulsion of amifostine silica nanoparticles [SiNPs@AMF] to reduce the side effects of amifostine. Moreover, the research study was extended to ameliorate the toxic effect of drugs without falling in new drug toxicity. Also, this nanoparticles model has the capability for change the unwanted properties of drugs. So, the circulatory half-life as well as bioavailability of drugs could be increased, while drug toxicity could be decreased [10]. This prompted us to consider if [SiNPs@AMF] could improve the drug stability and efficacy, thereby allowing low injection dosage for safety profile. On the other hand, there were no researches discussed the hepatoprotective effect of SiNPs@AMF. On this article, the study investigates the hepatoprotective effect of SiNPs@AMF against cisplatin induced hepatotoxicity in male Wister rats.

MATERIALS AND METHODS

Chemicals: 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 (AMF) were purchased from Sigma-Aldrich Chemical Company, St. Louis, MO, USA.

Animals: Wister strain male albino rats, weighing 150 ± 20 g, obtained from the Animal House, National Research Centre (NRC).The experiments were be carried out in accordance with guidelines and protocol approved by the Institutional Animal Ethics Committee.

Preparation of silica hollow nanoemulsion loaded with (AMF) 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 (AMF) 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 20 rats in each group. Rats were given saline I.P. (group 1) or Cis I.P (20 mg/kg BW) (group 2) or carrier I.P. 150mg/kg BW /day (group 3). This dose of Cis produces hepatotoxicity in rats. 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 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 (AMF) as a model drug:

The particles shape of the formed nanoemulsion of Si NPs loaded with and without (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: Livers were removed and homogenized for parameters estimationoflipid peroxidation by measuring the level of malondialdehyde (MDA) using the method of **Ruiz-Larrea et al [11]**,nitric oxide (NO) using Griess reagent, according to the method reported by **Montgomery and Dymock, 1961 [12]** and the arylesterase activity of paraoxonase using the method of **Higashino et al [13].**Serum alanine amino transferase (ALT), aspartate amino transferase (AST) and δ - Glutamyl transferase (δGT) were determined using kit was supplied from Spectrum Company. Determination of serum human laminin (LN) and tissue inhibitors of metalloproteinase1 (TIMP1) were performed by ELISA Kits which were purchased from Bioneovan Company**.**

Histological analysis: For microscopic evaluation livers 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 [**14].**

Statistical Analysis: The collected data were coded, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 22.0, IBM Corp., Chicago, USA, 2013. Descriptive statistics were done for quantitative data as minimum& maximum of the range as well as mean± SD (standard deviation) for quantitative parametric data. ANOVA Test for more than two independent groups with post hoc Tukey HSD test.

RESULTS

Nanoemulsion based on silica nanoparticles are chosen for encapsulation the model drug; (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 as prepared nanoemulsion occurred at neutral pH using such technique. Given below the full characterization of silica nanoemulsion with and without (AMF).

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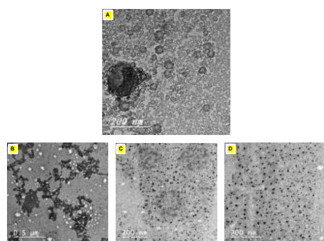


Figure 1: TEM images of (a) hollow structured porous nanoemulsion of silica nanoemulsion loaded with and without amifostine (AMF)

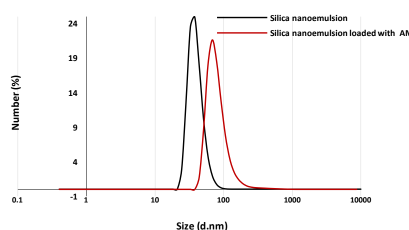


Figure 2: hydrodynamic size of the produced silica nanoemulsion (SiNPs) and (b) silica nanospheres encapsulated with (AMF) (SiNPs@AMF)

Characterization of the as-synthesized silica nanoemulsion loaded with and without (AMF)

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).

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).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 poly disparity 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: Figures (3, 4, 5, 6, 7) show a significant increases ($p<0.001$) in serum AST, ALT, γGT, serum LN and TIMP1 after induction with cisplatin compared to control group. On the other hand there was a significant decrease ($p<0.001$) in protected group (SiNs@AMF+ Cisplatin) compared to cisplatin group.

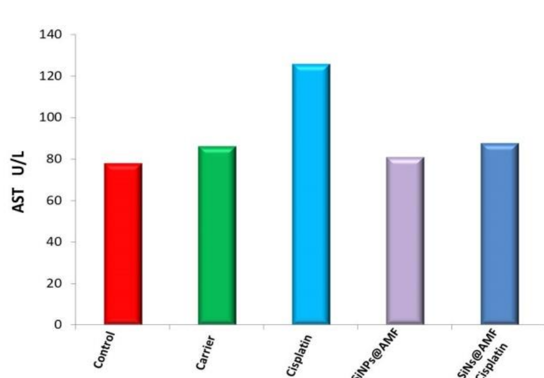


Figure (3): Mean value levels of AST in different studied groups.

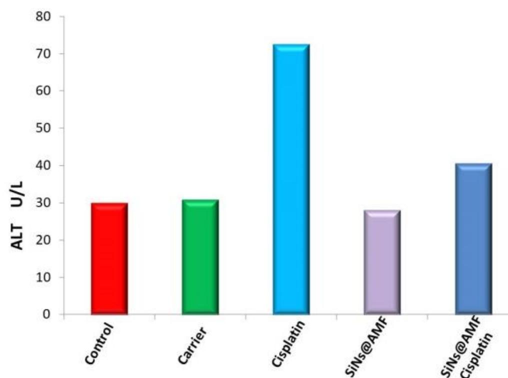
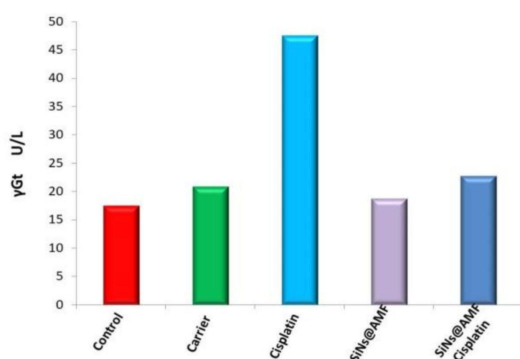


Figure (4): Mean value levels of ALT in different studied groups.



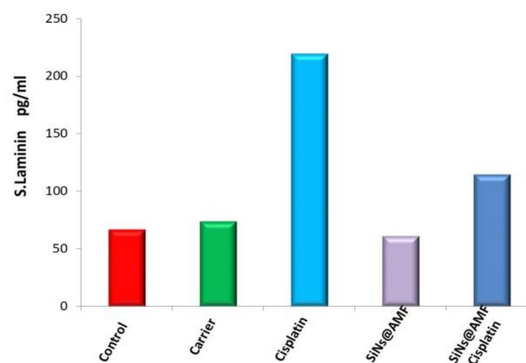


Figure (5): Mean value levels of γGt in different studied groups.

Figure (6): Mean value levels of s.laminin in different studied groups.

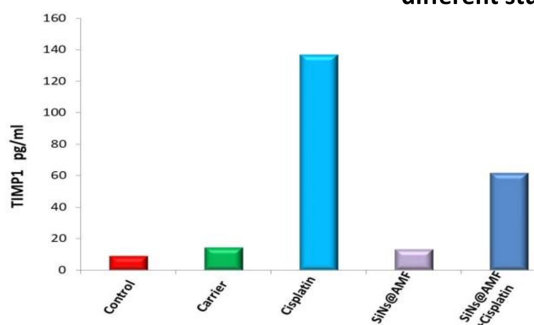
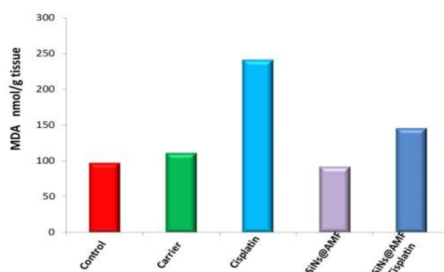


Figure (7): Mean value levels of serum TIMP1 in different studied groups.

Figures (8, 9, 10) show that liver MDA and NO were significantly increase, while PON 1 was significantly decrease in cisplatin group in comparison with control, denoting the increase of oxidative stress. Otherwise, Liver MDA and NO were significantly decreased, but PON 1 was significantly increased in SiNPs@AMF protected groups in comparison with cisplatin group indicating the antioxidant potential of these supplemented agents.



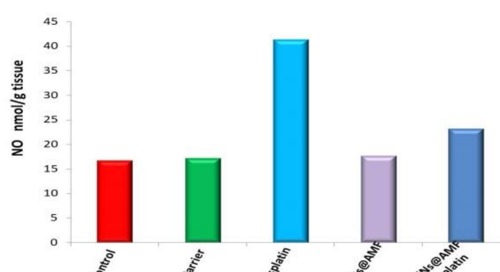


Figure (8): Mean value levels of MDA in different studied groups.

Figure (9): Mean value levels of NO in different studied groups.

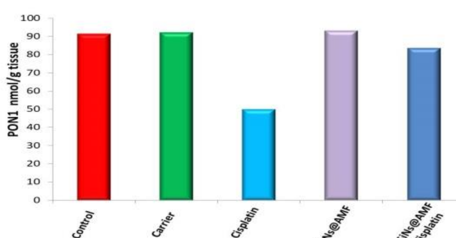


Figure (10): Mean value levels of PON1 in different studied groups.

Histopathological results: Normal structure of the hepatocytes, central vein, sinusoidal spaces and prominent nucleus were observed in the liver section of control rats (Fig. 11).Cisplatine exposure caused marked damage to hepatocytes in the form of degeneration, cytoplasmic vacuolation, centrilobular necrosis associated with mononuclear cells infiltration around central vein and the central vein was enlarged and congested. The sinusoid walls showed numerous Kupffer cells, hemorrhages with sinusoids dilatation, and nuclei are pyknotic with condensed chromatin (Fig. 12).In the group received carrier and SiNPs@AMF only showed nearly normal structure with few Kupffer cells and hemorrhage blood sinusoids (Fig.13 &14). SiNs@Ami and cisplatin showed moderated decrease in cisplatin-induced pathological alterations with few inflammatory cells, congestion central vein and blood sinusoids (Fig.15).

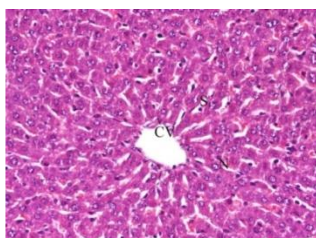


Fig.11. Photomicrograph of section from liver of control group (H & E X 400).

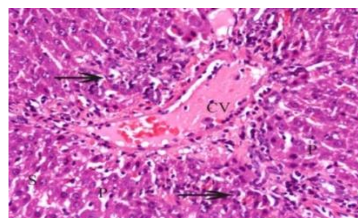


Fig.12. Photomicrograph of section from liver of cisplatin (H & E X 400).

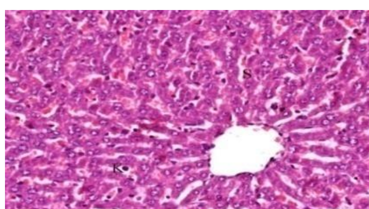


Fig.13. Photomicrograph of section from liver of Carrier group (H & E X 400).

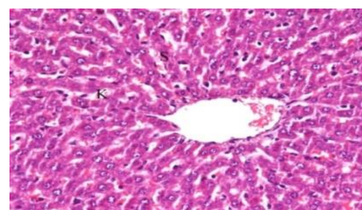


Fig.14. Photomicrograph of section from liver of SiNs@Ami group (H & E X 400).

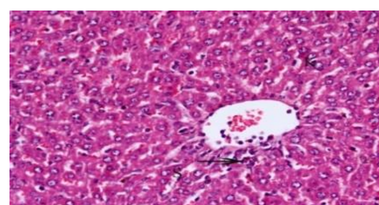


Fig. 15 :Photomicrograph of section from liver of SiNs@Ami + Cis group (H & E X 400).

DISCUSSION

Cisplatin intoxication is used as an experimental model for liver injury in the screening of hepatoprotective activity of nanoemulsion of amifostine silica nanoparticles (SiNPs@AMF). Administering cisplatin to rats noticeably increases serum ALT, AST and γ-GT levels which reflect hepatocytes cell membrane damage and enzymes leak from the hepatocytes. Our results are in agreement with studies of **Chengwei et al** [**15**] and **Hanan et al** [**16**]. They showed that increase in ALT, AST and γGT activities in the cisplatin group was related to liver cell damage and many other changes in the hepatic function and this notion was supported by histopathological examination of the liver. On the other hand, pretreatment with SiNPs@AMF significantly restores the changes of (ALT, AST and γGT),

thereby protecting membrane permeability, Indicating its cytoprotective effect. Also in this study, a significantly elevated levels of MDA noted in cisplatin administered rats indicated hepatic damage. MDA is the end product of lipid peroxidation and remarkable marker for oxidative stress [**17, 18**], thereby suggesting the increased release of reactive oxygen metabolites and consequent oxidative attack on polyunsaturated fatty acids in cisplatin group. Pretreatment with SiNPs@AMF prevented lipid peroxidation and returned the increased MDA to its normal level which could be likely to the radical scavenging antioxidant constituents. Also, administration of cisplatin reduced the levels of PON-1 in the liver tissue compared to the control group. PON-1 could be defined as an antioxidant enzyme [**19**] and exerts an antioxidant and anti-inflammatory actions in the liver and is considered a biomarker of liver diseases [**20**].On the other hand, in this study, pretreatment with SiNPs@AMF associated with a significant increase in PON-1indicating its antioxidant effect. In this study, we also found increased liver tissue NO by cisplatin. The increased tissue NO by cisplatin could be involved in the observed tissue damage via the formation of more reactive oxygen and nitrogen species by reacting with molecular oxygen. When present in high amounts, NO can react with the superoxide anion ($O_2^{\bullet -}$) resulting in the highly reactive peroxynitrite ($ONOO^-$) [**22**]. This highly toxic species reacts with GSH, lipids, proteins and DNA [**23**].This data was in agreement with previous studies of **(Reem et al., 2015)** that reported a significant increase in NO level after cisplatin treated rats compared to normal control animals [**24**].On the other hand, in this study, pretreatment with SiNPs@AMF associated with a significant decrease in liver NO compared to cisplatin group. The finding in the present study of the decrease in lipid peroxidation and NO after pretreatment with SiNPs@AMF might therefore indicate improved cell-redox state by the drug and/or a lower degree of tissue damage due to its antioxidant effect. In addition, the present results show that administration of cisplatin afforded a highly significant increase in LN level comparing with control groups indicate the ability of cisplatin to solubilize the lipid components and alter the ion channels of bio-membranes. While SiNPs@AMF exhibited a significant therapeutic effect on decreasing the levels of this marker indicating the effectiveness of SiNPs@AMF on hepatic regeneration after cisplatin damaged. In the present study results, TIMP-1 did not change in the control group. This supports the idea that the hepatic satellite cells (HSCs) were still in their quiescent state. However, those HSCs activated by the presence of cisplatin led to the high production of an extra cellular matrix (ECM) and consequently high levels of the TIMP1. SiNPs@AMF treatment successfully prevented the high synthesis of ECM and reduced the level of TIMP1 in comparison to cisplatin group. Most studies of human liver diseases and animal models of progressive fibrosis have showed that Liver fibrosis results from an imbalance of ECM deposition and degradation by matrix metalloproteinases (MMPs) and a TIMPs. It is characterized by the accumulation of collagen I, III and IV and other ECM proteins that disrupt normal hepatic architecture and impair liver functions [**24**]. The regulation of the enzymatic system involved in ECM degradation may be an important factor in liver fibrogenesis. So in our findings, hepatic increase in TIMP-1 in the cisplatin group can be explained as a consequence of the increased HSC apoptosis. The mechanism of hepatoprotection by SiNPs@AMF against cisplatin toxicity might be due to recon quest of the PON-1 level. The possible hepatoprotective activity of SiNPs@AMF against cisplatin-induced liver injury in rats may be due to its antioxidant activity as pointed out by protection against lipid peroxidation , reduced antioxidant levels and remodeling of the hepatocyte cellular system that preserves or sustains the normal liver function, shape and appearance, thereby minimizing free radical damage of hepatocytes.

CONCLUSIONS

The present study confirms the liver protective action of SiNPs@AMF against experimentally induced liver damage in rats. The Possible mechanism of hepatoprotective action of SiNPs@AMF may be due to its free radical scavenging activity as indicated by decrease in lipid peroxidation. Improved enzymatic biochemical parameters and histopathology observations also indicated recovered structural and functional integrity of the hepatic cells. This indicates that this model SiNPs@AMF may be used as an effective hepatoprotective agent.

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