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Amifostine Silica Nanoparticles Characterization and Effect on Neuronal Damage in Cisplatin Treated Rats

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Abstract

The main obstacle to cisplatin's efficacy in cancer chemotherapy is neurotoxicity. The objective of this study was to create and characterize amifostine (AMF)-loaded silica nanoparticles (SiNPs@AMF), and investigate the cytoprotective effects of this nano-emulsion model of AMF against cisplatin-induced neurotoxicity in male albino rats. The nano-emulsion was prepared using cetyltrimethyl ammonium bromide (CTAB), castor oil (CAO), tetraethyl orthosilicate (TEOS), and amifostine (AMF) as extra stabilizing agent, surfactant, sources for silica, and a model drug, respectively. Transmission electron microscopy (TEM) and dynamic light scattering (DLS) were used to analyse the hydrodynamic average size and particle shape of the produced nano-emulsion of silica and SiNPs@AMF. It was evident that when compared to silica nano-emulsion, the prepared SiNPs@AMF slightly enhanced the particle size. Biomarkers of oxidative stress including malondialdehyde (MDA), nitric oxide (NO), paraoxonase-1 (PON-1) as well nuclear factor kappa B (NF-κB) were determined. Histopathological examination of brain was also done. Results indicated that MDA , NO and NF-κB levels were increased whereas PON-1 decreased following cisplatin injection.The administration of SiNPs@AMF protected against cisplatin-induced histopathological changes. Treatment with SiNPs@AMF returned these alterations to their original state. Our findings suggest that oxidative stress is involved in the neurotoxic effects of cisplatin and the cytoprotective action of SiNPs@AMF ameliorate the neurotoxic effect induced by cisplatin.

Keywords: Cisplatin, Oxidative stress, Neurotoxicity, Amifostine, Nanoparticles

1. Introduction

Chemotherapy is commonly used to treat a number of malignancies. The use and dose of platinum-based treatments (cisplatin), which were exceptionally efficient chemotherapeutic medications used to treat a variety of malignancies, were limited because of their toxicity on several systems, including neurotoxicity [1]. Cisplatin is thought to cause brain toxicity that is dose dependant [2].The blood-brain barrier (BBB) is highly permeable to cisplatin, which results in the degeneration of adult brain neurons [3].

Hydrogen peroxide and hydroxyl radicals are examples of the reactive oxygen species (ROS) that cisplatin produces. The produced free radicals interact with lipids, proteins and DNA causing lipid peroxidation and DNA damage [4].It was established that the two main pathways by which platinum-induced neurotoxicity led to neuronal death were oxidative stress and mitochondrial malfunction [5].

Amifostine (WR-2721) is a phosphorylated aminothiol prodrug that needs to be dephosphorylated in order to become WR-1065, which is active sulfhydryl metabolite and a free radical scavenger. It is dephosphorylated by alkaline phosphatase. Alkaline phosphatase levels are high in healthy tissues and low in tumour or cancerous cells. Once within the cell, WR-1065 scavenges free

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radicals, and protect the intracellular components [6, 7].

The dephosphorylated form of amifostine (WR-1065) functions by binding to toxic byproducts (produced by chemotherapy medicines and ionising radiation) and detoxifying it. Some of the cytoprotective mechanisms include the partial removal of preformed platinum-deoxyribonucleic acid (DNA) adducts, the scavenging of oxygen free radicals generated by ionising radiation, the donation of hydrogen from the free thiol to repair damaged target molecules, the binding of active species to alkylating and platinum agents, and the action of WR-1065 as an alternative target to DNA or ribonucleic acid (RNA) [8].

Numerous cytoprotectors function by preventing free oxygen radicals from damaging DNA either directly or indirectly. Superoxide (O2-) and the hydroxyl radical (OH.) are highly reactive oxygen radicals that can damage macromolecules including DNA and cause cell death [9, 10].

After 6 minutes of intravenous infusion, the plasma quickly removed over 90% of the amifostine. Its effectiveness in minimizing the side effects of chemotherapy is further constrained by the exceptionally short half-life[11].

Numerous investigations looked into how different compounds affected the neurotoxicity caused by cisplatin. Amifostine is generally well accepted, however in some persons, it may result in nauseousness, vomiting, a warm or flushed sensation, and allergic reactions. The American Society of Clinical Oncology (ASCO) recommends giving adults 910 mg/m2 of amifostine intravenously over a period of 15 to 30 minutes prior to the start of chemotherapy [12].Amifostine must therefore be given in large doses to fulfil its cytoprotective role. To measure the hydrodynamic size and particle shape of the produced nano-emulsion of hollow-structured porous silica and silica encapsulated with AMF, DLS and TEM techniques will be used. Moreover, the research study was extended to improve drug toxicity. The drug's solubility, biodistribution, immunogenicity, enzymatic degradation, renal filtration, and phagocytosis in the reticulo-endothelial system can all be changed using this nanoparticle model [13]. In turn, this can improve the drug's bioavailability and circulation half-life while lowering its toxicity [14].This prompted us to research whether amifostine-encapsulated hollow silica nanospheres (SiNPs@AMF) could increase medicine stability and efficacy while preserving a favourable safety profile.

The current study was therefore designed to alleviate the toxic effects of repeated drug doses over a long period of time, as well as to assess the potential of amifostine encapsulated nano-emulsion (SiNPs@AMF) as a new neuroprotector model against cisplatin-induced neurotoxicity.

2. Materials and methods

2.1. Chemicals

Without additional purification, all reagents were used in their original form. Decane (purity greater than 98%), hydrochloric acid (HCl; 32% aqueous solution), ammonia solution (20%), sodium chloride (NaCl), and ethanol (purity greater than 99.5%) were used as received. Tetraethyl orthosilicate (TEOS) (purity greater than 99%), ammonia solution (20%), sodium chloride (NaCl), and ethanol (purity greater than 99.5%) were All of these chemicals were purchased from the American Sigma Aldrich Co. A Millipore Milli-Q Plus system was used to produce ultrapure deionized (D.I.) water. Amifostine and cisplatin were purchased from Sigma-Aldrich Chemical Company in St. Louis, Missouri, in the United States.

2.2. Experimental Animals

A total of 60 adult male *Wistar* albino rats weighing about 180 ± 20 g were obtained from Animal House Colony, National Research Center, Giza, Egypt. Animals were maintained on standard lab diet in artificially illuminated and temperature-controlled room free from any chemical contamination at the Animal House Lab, NRC, Dokki, Cairo, Egypt. All animals were received human care in compliance with the guidelines of the Animal Care and Use Committee of the National Research Centre, Dokki, Cairo, Egypt.

2.3. In vivo Experimental design

After 1 week of acclimatization, the animals were divided into six groups (10 rats per group). Group 1 received saline intraperitoneally (IP) (negative control), group 2 received a 150 mg/kg/day intraperitoneal (IP) injection of silica nano-emulsion (SiNPs) (Carrier) , group 3 received cispatin (12 mg/kg/day) IP for 3 consecutive days (positive control), group 4 received amifostine (150

mg/kg/day) IP (three times a week) before the injection of cisplatin(for 3 consecutive days), group 5 received silica nano-emulsion (SiNPs@AMF) IP (150 mg/kg/day) alone (three times a week) for one month, group 6 received IP injection of (SiNPs@AMF) (150 mg/kg three times a week) for three weeks alone before the injection of Cisplatin (for 3 consecutive days) and then were continued for one week.

2.4. Amifostine-loaded silica hollow nano-emulsion serves as a model medication preparation

Oil/water emulsions were used to create the silica nano-emulsion, and high-speed ultrasonication was used to disperse the resulting nano-emulsion. Decane (the oil phase) comprises 20% (w/w) Tetraethyl orthosilicate (TEOS) in this procedure, while Brij 96V (5% w/w) is employed as a surfactant in 5 mL of decane. The mixture was initially blended for 10 minutes while being vigorously magnetically stirred. The oil phase was then gradually supplemented with the aqueous phase (NaCl, 10 mL at 10 mM), and stirring was maintained continuously for an additional 5 minutes. Then, the whole solution was sonicated for 5 minutes using an ultrasonication device. To keep temperature, the sonicated solution was kept in an ice bath during sonication to prevent the temperature from rising above 25 °C [13].

Amifostine was added to the aqueous phase (NaCl) for loading the medication (100 mg) before being added to the oil phase. The created nano-emulsion combination was stored in the freezer for analysis [13].

2.5. Physical characterization of the formed nanoparticles of Si and SiNPs@AMF as a model drug

Utilizing a transmission electron microscope, the particle shape of the the nano-emulsion was measured (TEM, JEOL, JEM-2100, Tokyo, Japan). The nano-emulsion sample was sonicated for 15 minutes prior to TEM analysis, after which it was deposited onto copper-coated grids and allowed to air dry. Then, using dynamic light scattering (DLS) with nano-ZS, the average size and polydispersity index (PDI) of the silica nano-emulsion were assessed (Malvern Instruments, Malvern, UK). Using ZS, the surface charge was also described in terms of zeta potential. We can learn information about the stability of the created nano-emulsion from the zeta potential. A value of greater than 20 mV is regarded as suitable for creating stable nano-emulsions.

2.6. Biochemical Analysis

After the animals were killed on the 30th day, the brains were swiftly removed, quickly put in cold normal saline, quickly blotted on filter paper, and quickly frozen at -80^{o}C. The frozen tissues were divided into small pieces and homogenized in 5 ml of cold buffer (0.5 g of Na_2HPO_4 and 0.7 g of NaH_2PO_4 in 500 ml deionized water, pH = 7.4). The centrifuged samples were then centrifuged at 4000 rpm for 15 minutes at 4^{o}C, and the supernatant was collected for parameter estimation.

2.7. Determination of lipid peroxidation

Malondialdehyde (MDA) levels in brain tissue homogenate were assessed for lipid peroxidation using the Ruiz-Larrea et al., 1994 technique [15].

2.8. Determination of Nitric oxide

Using the Haagen and Brock method [16], nitric oxide (NO) levels in the brain tissue were measured spectrophotometrically. This approach was based on measuring the level of endogenous nitrite as a sign of nitric oxide production.

2.9. Determination of Paraoxonase -1

Using the Higashino et al. 1972 approach [17], the arylesterase activity of paraoxonase was quantified spectrophotometrically in brain tissue.

2.10. Determination of Nuclear Factor Kappa B

By following the manufacturer's instructions, an NF-κB kit (SUNLONG Biotech Co., Ltd.), catalogue number SL0537Ra, was used to test the level of NF-κB in brain tissue.

2.11. Statistical analysis

Statistical analyses were performed using SPSS version 19.0 (IBM Corp, NY, USA). All data were expressed as mean±SEM. Statistical significance of differences among the different studied groups was evaluated by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. All p-values were two-sided and a p-value <0.05 was taken as a criterion for a statistically significant difference.

3. Results

3.1. Characterization of SiNPs@AMF

TEOS and Brij 96V were utilized as the precursor and surfactant, respectively, to fabricate the silica

nano-emulsion. The purpose of the surfactant is to prevent agglomeration of the produced nanoparticles. As is well knowledge, nanoparticles are tiny and have a large surface area. Small particles prefer to collide with other nanoparticles in the solution and combine with other smaller particles to generate larger particles because they are so small. Consequently, the presence of surfactant can serve as a shield for these particles, preventing their fusion or collision. It was initially allowed to stand for two months without showing any signs of phase separation or precipitation. Moreover, the colour of the nano-emulsion is uniform. Additionally, silica nanoparticle shape was evaluated using TEM.As shown in Figure 1 (a, b), the silica particles have been formed and exhibit a spherical shape with a clear cavity (faint color). These cavity particles were filled with amifostine when silica nano-emulsion was used as a carrier for the drug (Figures 2 a, b). It can be confirmed by the change in the color of the particles. The color of drug-loaded silica nanoparticles changed to deep black, indicating drug encapsulation inside the cavity of silica nanoparticles.

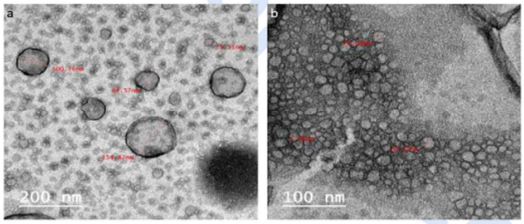

Figure 1: (a,b) TEM of silica nano-emulsion at two different magnifications

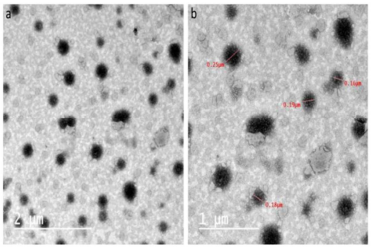

Figure 2: (a,b) TEM of drug loaded silica nano- emulsion at two different magnifications

For further affirmation, DLS was used to assess the average hydrodynamic size of silica nanoparticles and drug-loaded silica nano-emulsion. It can be clearly seen that the average hydrodynamic size of silica nanoparticles is 79 nm with a polydispersity index of 0.5, illustrating that the particles were homogeneous and uniform (Figure 3a). Meanwhile, the average size of drug-loaded silica nano-emulsion increased to 123 nm with PDI 0.1 (Figure 3b). The increments in particle size after drug loading are reasonable and attributable to the physical adsorption of drugs on the surface of silica nanoparticles, which cause an enlargement in the size of the whole particles. However, the PDI was enhanced to 0.1, indicating that the drug-loaded silica nanoparticles are more homogeneous than silica nanoparticles. As mentioned in the experimental section about the importance of zeta potential, it was depicted that the value of zeta potential for amifostine-loaded silica nano-emulsion was -20.9 mV (Figure 4), which affirmed the stability of the nano-emulsion even after storage for a long time (2 months), as depicted by our naked eye.

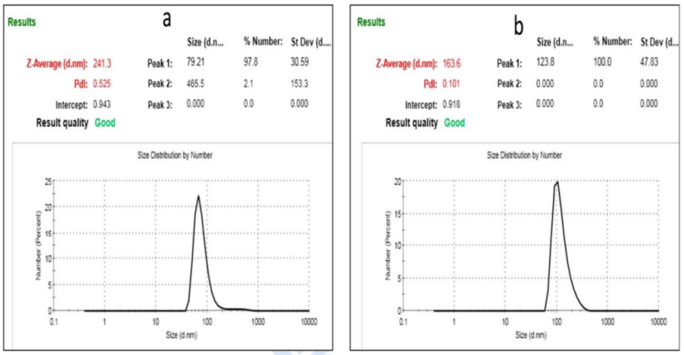

Figure 3: Particle size analyzer of (a) silica nano-emulsion and (b) amifostineloaded silica nano-emulsion

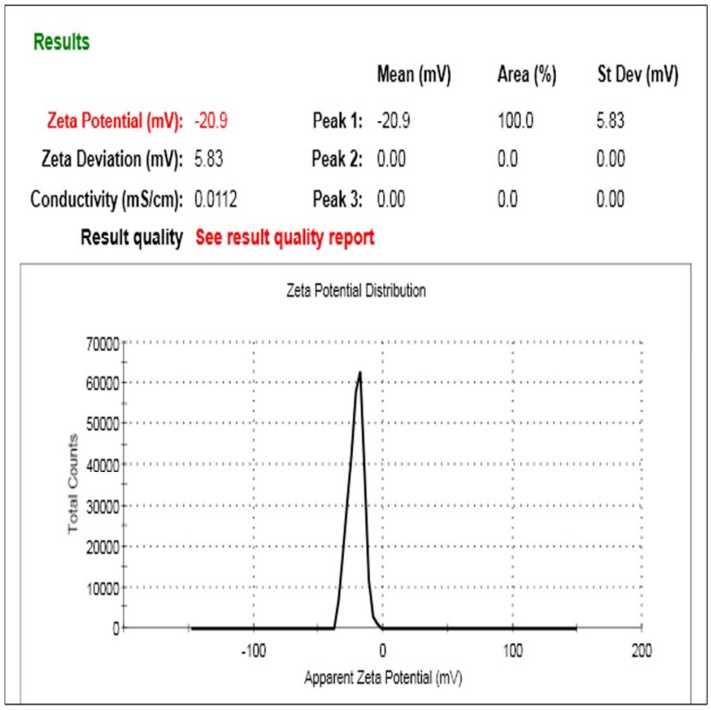

Figure 4: zeta potential of amifostine loaded silica nano-emulsion

3.2. Biochemical results

3.2.1. The effect of SiNPs@AMF on brain Malondialdehyde (MDA)

When compared to the control group, the MDA level in the Cisplatin group showed significant increase by 107.08% (41.79 ± 0.29 vs. 20.18 ± 0.34 nmol/g tissue; $p < 0.05$).Treatment with amifostine (Amifostine+ Cisplatin and SiNPs@AMF + Cisplatin) resulted in – 27.75% and – 41.58% decrements in malondialdehyde level respectively (30.19 ± 0.26 and 24.41± 0.36 vs. 41.79 ± 0.29 nmol/g tissue; $p<0.05$). There was no significance between the control and SiNPs@AMF groups (Table 1, Figure 5).

3.2.3. The effect of SiNPs@AMF on brain Paraoxonase-1 (PON1)

Cisplatin injections caused a significant decrease in PON-1 activity by – 40.62% compared with the saline control value (8.33 ± 0.23 vs. 14.03 ± 0.10 kU/l; $p < 0.05$). PON-1 activity increased by 31.33%, 66.14% following Amifostine treatment (Amifostine+ Cisplatin and SiNPs@AMF + Cisplatin) (10.94 ± 0.23 and 13.84 ± 0.15 vs. 8.33 ± 0.23kU/l; $p < 0.05$). There was no significance between the control and SiNPs@AMF groups (Table 1, Figure 7).

3.2.4. The effect of SiNPs@AMF on brain Nuclear factor kappa B (NF-κB)

Following Cisplatin injections, there was a significant increase in brain NF-κB by 275.56% (8.30± 0.69 vs. 2.21±0.22 ng/ml; $p < 0.05$) compared with the control group. Treatment with amifostine (Amifostine+ Cisplatin and SiNPs@AMF + Cisplatin) resulted in – 19.27% and – 35.78% decrease in brain NF-κB(6.70± 0.55 and 5.33±0.75 vs. 8.30±0.69 ng/ml; $p < 0.05$). There was no significance between the control and SiNPs@AMF groups (Table 1, Figure 8).

Table (1): Brain Malondialdehyde (MDA), Nitric Oxide (NO), Paraoxonase-1 (PON1) and Nuclear factor kappa B (NF-κB) in different studied groups

Parameters / Groups	MDA (nmol /g. tissue)	NO (µmol/g. tissue)	PON1 (kU/L)	NF-κB (pg/ml)
Control	20.18 ± 0.34	20.58 ± 0.34	14.03 ± 0.10	2.21±0.22
Carrier	19.45 ± 0.24	20.74 ± 0.33	14.23 ± 0.22	2.37±0.20
Cisplatin	41.79 ± 0.29*	32.07 ± 0.30*	8.33 ± 0.23*	8.30± 0.69*
Amifostine+ Cisplatin	30.19 ± 0.26#	27.1 ± 0.33#	10.94 ± 0.23#	6.70± 0.55#
SiNPs@AMF	19.42 ± 0.45	21.25 ± 0.27	13.84 ± 0.15	2.22±0.28
SiNPs@AMF + Cisplatin	24.41± 0.36#	23.68 ± 0.25#	13.11 ± 0.25#	5.33±0.75#

All data are represented as mean ± standard error (SEM).Range test P <0.05 was considered statistically significant.*P<0.05 versus control group, #P<0.05 versus cisplatin group

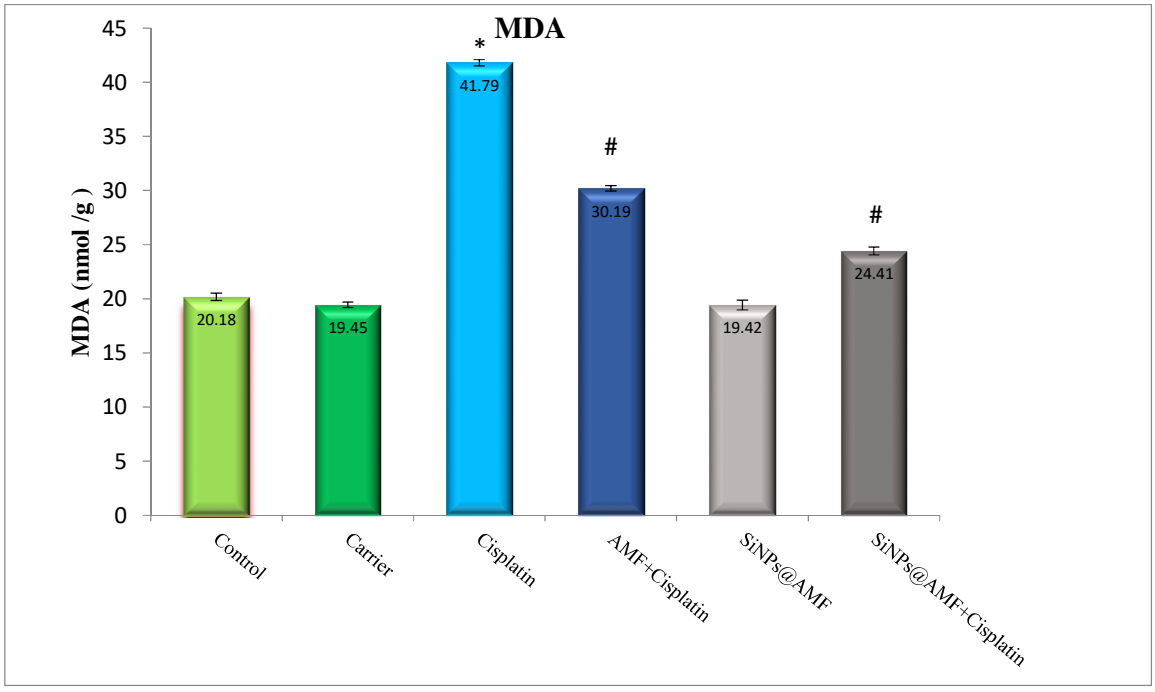

Figure (5): Malondialdehyde (MDA) levels of brain tissue in different studied groups. Range test P <0.05 was considered statistically significant. *P<0.05 versus control group, #P<0.05 versus Cisplatin group

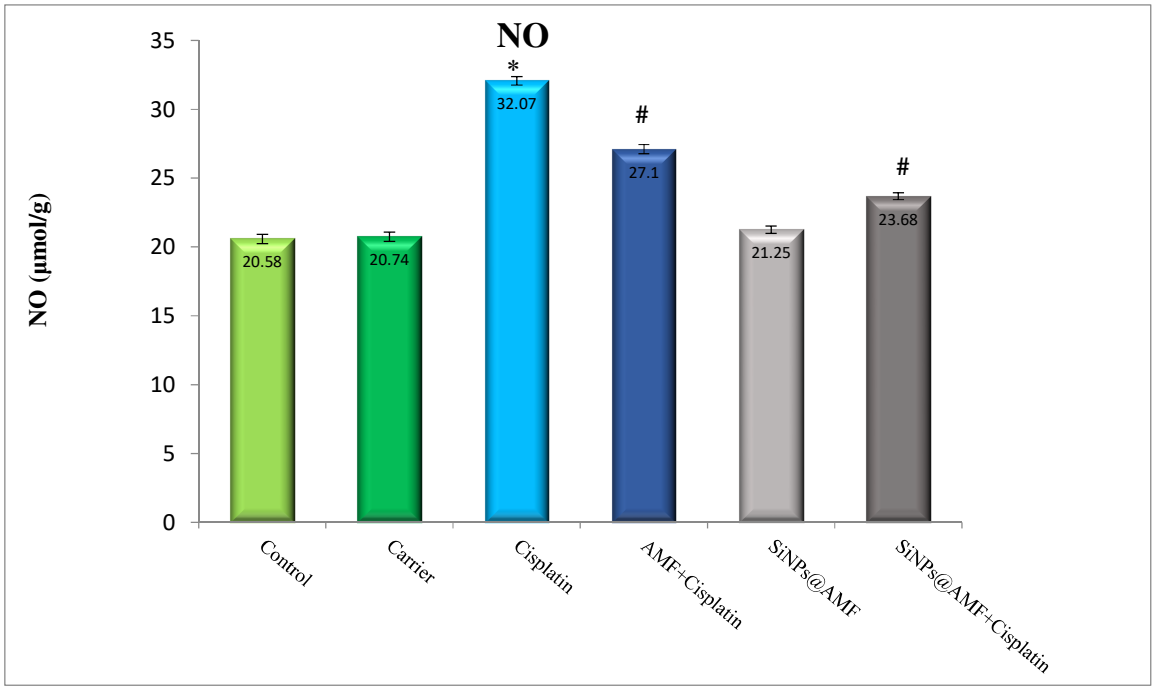

Figure (6): Nitric oxide (NO) levels of brain tissue in the different groups of experiment. Range test P <0.05 was considered statistically significant.$^{*}P$<0.05 versus control group,$^{\#}P$<0.05 versus cisplatin group

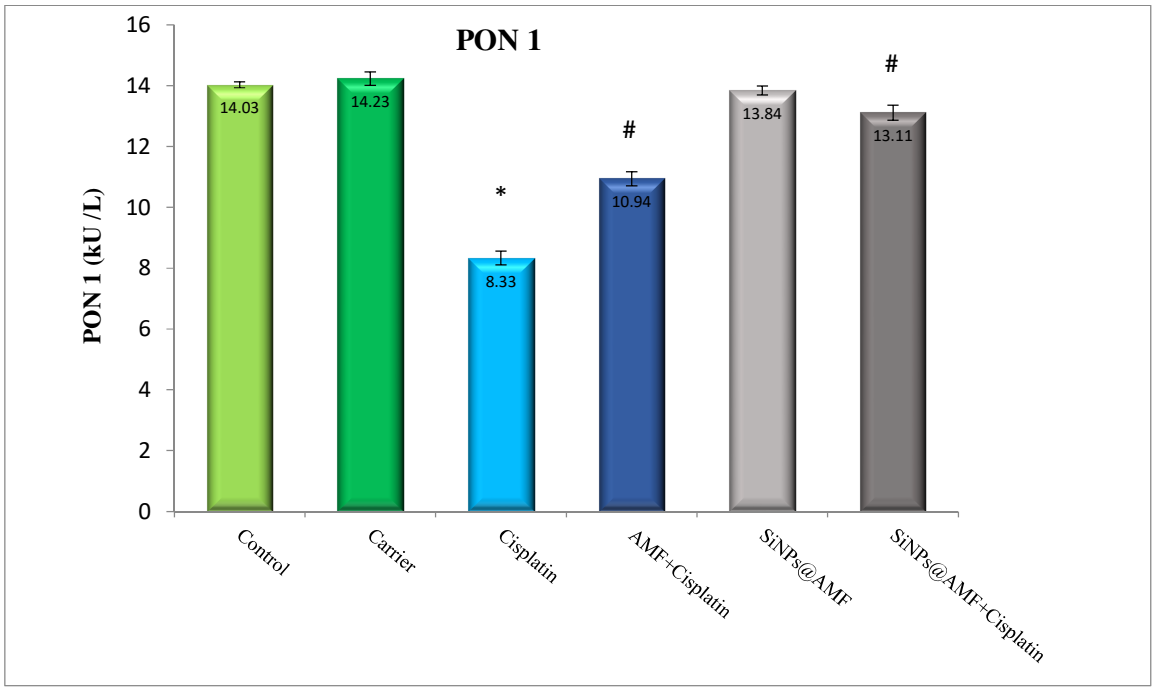

Figure (7): Paraoxonase 1(PON-1) levels of brain tissue in different studied groups. Range test $P \leq 0.05$ was considered statistically significant.$^{*}P$<0.05 versus control group, $^{\#}P$<0.05 versus cisplatin group

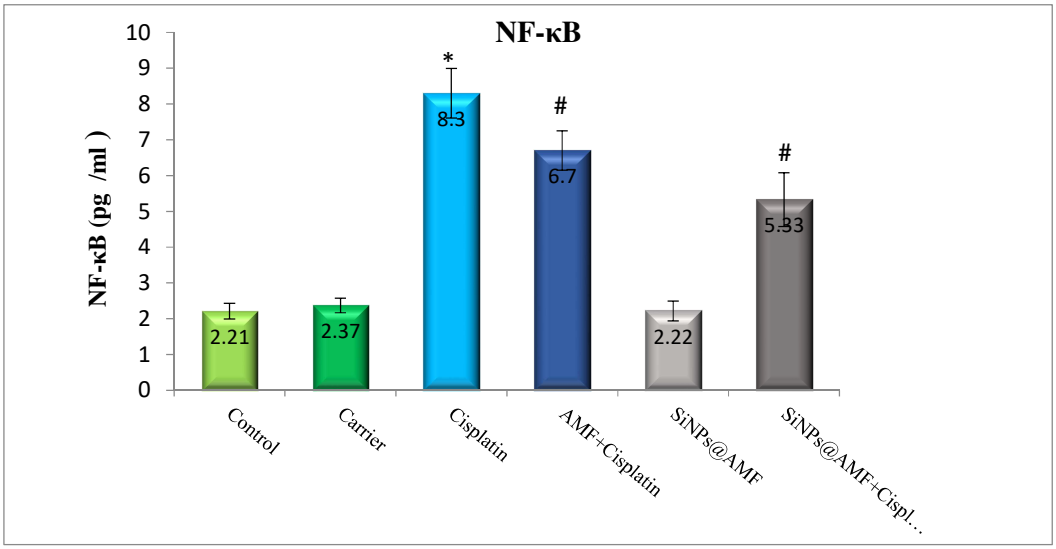

Figure (8): Nuclear factor kappa B (NF-κB) levels of brain tissue in the different groups of experiment. Range test P <0.05 was considered statistically significant. $^{*}P$<0.05 versus control group, $^{\#}P$<0.05 versus cisplatin group

3.2. Histopathological results

The section of the brain tissues from the control group, showed cortical neurons are arranged in neat rows with abundant cytoplasm and round basophilic nuclei (Figure 9, A). Also section form carrier group showed the brain tissues appeared nearly normal structure (Figure 9 B). However, light microscopic examination from brain of cisplatin group showed several neurodegenerative changes. These changes include perineuronal vacuolation, eosinophilic cells, shrunken neurons with pyknotic nuclei, apoptotic cells and signs of gliosis (Figure 9, C).

Rats treated with the amifostine exhibited normal appearance of brain tissues as nearly similar to that of control rats with some pyknotic nuclei (Figure 9, D). Microscopic investigation of brain sections of rats-treated with SiNPs@AMF showed healthy neurons with minimal neurodegenerative changes and some pyknotic nuclei were seen (Figure 9, E). Examination of sections of rat-treated with SiNPs@ AMF &Cisplatin showed the neurons more or less like normal. Slight degeneration with some pyknotic nuclei and apoptotic neurons were seen (Figure 9, F).

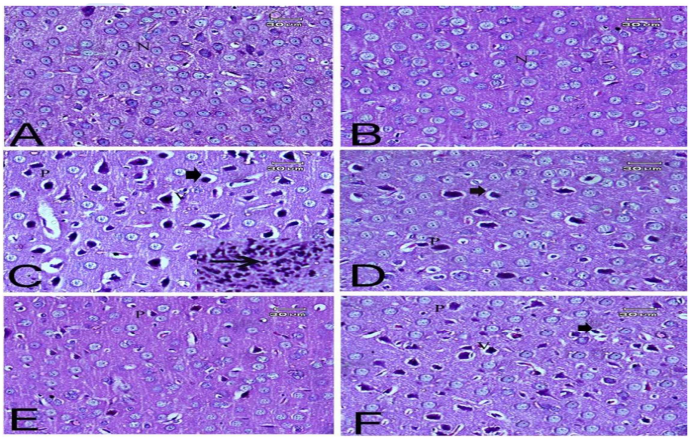

Figure (9): Representative photomicrographs of brain sections from different groups. (A) Control group (B) Carrier group (C) Cisplatin group (D) Amifostine + Cisplatin group (E) SiNPs @ AMF group (F) SiNPs @ AMF + Cisplatin group.

3. Discussion

The main barrier to cisplatin's effectiveness as a cancer chemotherapy drug is neurotoxicity. The main processes causing cisplatin-induced neurotoxicity are thought to be oxidative damage and inflammation [18, 19]. Reactive oxygen species (ROS) produced by the short- and long-term administration of CP have been shown to cause oxidative stress, which is accompanied by an increase in lipid peroxidation and a decrease in the activity of antioxidant enzymes in tissues [20, 21]. The accumulation of cisplatin in the cerebral tissue, which causes oxidative damage, is thought to be the cause of the oxidative stress brought on by CP in the neurological system [22].The disruption of the mitochondrial electron transport chain caused by cisplatin has been reported to cause mitochondrial energy dysfunction, the production of ROS, and oxidative stress, all of which have been identified and connected to the complications of cisplatin [23, 24].

According to Cherian et al., 2019 [25], the generation of MDA is considered a biomarker to evaluate an organism's level of oxidative stress. Taking into consideration the high content of polyunsaturated membrane lipids in the brain, together with its reduced antioxidant mechanism and its high metabolic rate, the brain is considered one of the most vulnerable organs to oxidative stress [26, 27]. Polyunsaturated fatty acids undergo lipid peroxidation, which produces MDA. Since ROS have a relatively short life span, it is difficult to identify them. Nevertheless, ROS-related tissue destruction could be observed by the final product of lipid peroxidation, such as MDA [28]. Our current hypotheses suggest that increased brain MDA levels after administration of cisplatin were a consequence of cisplatin cytotoxicity and the development of oxidative stress. The current findings support prior research by demonstrating that cisplatin treatment generates a significant increase in the tissue level of MDA, a highly reactive molecule that serves as a useful oxidative stress marker [29].By implying a rise in reactive oxygen metabolites and, as a result, an oxidative attack on polyunsaturated fatty acids. Our findings further suggest that in the Cisplatin group, pretreatment with SiNPs@AMF reduce brain MDA levels.It is believed that the main cause of this is due to its capacity to act as an antioxidant and free radical scavenger.

Our findings indicated that cisplatin injection resulted in a marked increase in brain NO, which is consistent with previous observations [30].The signalling molecule NO is synthesised from L-arginine via the action of the enzyme nitric oxide synthase (NOS), which exists in constitutive endothelial (eNOS) and neuronal (nNOS) isoforms and a third inducible isoform (i.e., iNOS) activated by ROS and cytokines. There is evidence that the increase in NO is linked to neurodegeneration through the formation of more reactive nitrogen species, such as peroxynitrite ($ONOO^-$), capable of oxidation and nitration of protein tyrosine residues and nitrosylation of thiols. In this context, iNOS is the source of excessive NO release by microglia and astrocytes during brain inflammation, infection, and trauma [31].This study found that cisplatin also elevated NO levels in brain tissue, which by reacting with molecule oxygen to produce more reactive oxygen and nitrogen species, may have contributed to the tissue damage observed. High levels of NO can interact with the superoxide anion ($O2^{\bullet-}$) to create the incredibly reactive peroxynitrite (ONOO-) [32, 33].

Contrarily, pretreatment with SiNPs@AMF was associated in this study with a substantial decrease in brain tissue NO compared to the Cisplatin group. The decrease in lipid peroxidation and NO after pretreatment with SiNPs@AMF could indicate that the medicine improves cell redox state and/or reduces tissue dysfunction due to its antioxidant activity.

This study also showed that the administration of cisplatin caused a marked decrease in brain PON-1, which is in agreement with previously reported studies. Reduced enzyme activity in individuals with certain neurological disorders could be explained by PON-1's antioxidant function, which is interrupted by increased oxidative stress [34].In addition, and in agreement with other researches, we observed significant inhibition of PON-1 activity in the brain of rats treated with cisplatin due to oxidative stress. The decrease in PON1 activity observed in brain cells after induction with Cisplatin in the current study could be due to decreased synthesis by intoxicated cells, and the recovery in PON1 after pretreatment with SiNPs@AMF could be due to a decreased level of oxidative stress and/or a consequence of neuroprotection.

Nuclear factor-kappa B (NF-B) is an essential factor that stimulates the transcription of pro-inflammatory genes and hence plays a key role in inflammation. It has been discovered that intrapretonial injection of cisplatin is connected with

activation of NF-B, which initiates the overproduction of pro-inflammatory cytokines [35].

Nuclear factor kappa B (NF-κB) is activated by oxidative damage from cisplatin, which encourages the production of pro-inflammatory cytokines like TNF-α [36].The current data showed that the transcription factor NF-κB was found at a higher level in the cisplatin group compared to the control group. After pretreatment with SiNPs@AMF, there were significantly fewer NF-κB increases in brain tissue, indicating that Cisplatin-induced neuroinflammation and apoptosis are greatly diminished. The present results showed that systemic administration of cisplatin is associated with NF-κB activation, which then results in an excess of pro-inflammatory cytokines [37, 38].

The findings of the current study showed that administering the [SiNPs@AMF] model reduced the oxidative stress caused by CP and the alterations in brain tissue lipid peroxidation caused by CP injection; it functions both directly and indirectly as an antioxidant. It may remove ROS like singlet oxygen, hydrogen peroxide, peroxyl radicals, superoxide anion, and peroxyl radicals. As a result, it protects cells against cisplatin treatment (cytoprotector).All of these findings suggest that silica nanospheres encapsulated in amifostine (SiNPs@AMF) have improved endocytosis as a small particle behaviour and that they are gradually converted to their metabolites in acidic lysosome organelles.

5. Conclusion

In conclusion, the present findings suggest that [SiNPs@AMF] could efficiently ameliorate the neurotoxicity induced by cisplatin (CP). This was mediated by the potential antioxidant, anti-inflammatory effects of [SiNPs@AMF]. These effects were reflected in the ability to improve the histopathological changes caused by CP in the cerebral cortex. Therefore, [SiNPs@AMF]could not only prevent the adverse effects of CP but could also act as an antitumor agent. Accordingly, [SiNPs@AMF]could be used with CP to prevent its toxicity and increase its efficiency as antitumor. This in turn may help in reducing the dose of CP without affecting its potency.

6. Funding

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7. Acknowledgments

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8. Conflicts of Interest

The authors declare no conflict of interest.

9. References

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