



Protective Effect Of Drug Loaded Nanoparticles On Brain Toxicity In Experimental Animals

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Abstract

Salicylates are the group of chemicals that has salicylic acid as the parent compound and have the ability to treat inflammation, pain syndromes, brain and cardiovascular disorders. The present study was designed to investigate the protective effect of loaded sodium salicylates on nanoparticles to treat brain toxicity induced by cisplatin and decrease the drug side effects. Cisplatin 20mg/kg BW was given alone or in combination with sodium salicylate loaded on nanoparticles (Si-Sc-NPs) [100 mg/kg BW] for three weeks. The obtained results indicate that Si-Sc-NPs decrease oxidative stress markers such as malondialdehyde (MDA), nitric oxide (NO), and paraoxonase-1 (PON-1) activity in brain. There were also decreased in Monocyte Chemo-attractant Protein-1 (MCP-1) activities and Nuclear Factor kappa β (NF- κ) level of brain tissue while the Butyrylcholinesterase (BChE) activities of brain tissue increased after Si-Sc-NPs treatment. Histopathological results showed that cisplatin group showed several neurodegenerative changes, on the other hand, group treated with Si-Sc-NPs +Cis showed improvement in almost brain structure and mild pyknotic nuclei and apoptotic neurons were observed.

Key words: sodium salicylate, Nano emulsion, cisplatin, brain toxicity, MCP-1.

1. Introduction

In about 50 per cent of all patients with cancer, cisplatin is an important chemotherapeutic agent. Cisplatin is a platinum-based alkylating compound that reacts with DNA to form interstrand cross-links [1]. The cisplatin operating mechanism is regulated by cisplatin's association with DNA for adducts of DNA. As non-steroidal anti-inflammatory medications (NSAIDs), NSAIDs are used as agents to control inflammation sodium salicylates are also used [2]. The main action of sodium salicylate is the inhibition of Cyclooxygenase (COX) and prostaglandin and other eicosanoid synthesis mitigate pain, fever, and inflammation. Therapy containing COX inhibitors is well known to include a variety of side effects including gastrointestinal toxicity, an elevated amount of serum creatine, and hypercalcemia, interstitial nephritis, proteinuria, and acute renal failure (residential toxicity) [3]. In order to reduce possible side effects of the drug, sodium salicylates

were loaded on nanoparticles. Mainly nanoemulsions, lipid or polymer nanoparticles, and liposome are part of Nano delivery systems. The medicinal role of nanoparticles has been enhanced and the side effects minimized [4]. Owing in recent years to the use of particle vesicle structures as drug carriers for small and large molecules, a substantial amount of research has been performed in the Modern Drug Delivery System. Nanoemulsions are commonly used as intravenously delivered vessels of lipophilic treatment. On the other hand, the key aims of the other nanoparticles are to dramatically boost their potency and and their possible harmful adverse effects by modifying the natural bio destiny of active drug molecules in the body after intravenous administration [5]. So, in this study we elucidate a new way to get these treatments without falling in new drug toxicity. Based on the aforementioned obtained data, nanoemulsion of sodium salicylates

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silica nanoparticles [Si-Sc-NPs] offer a new approach in attenuating of drug induced brain toxicity.

2. Material and methods

2.1. Materials

2.1.1. Animals

The experiments were performed on 50 Wistar strain male albino rats, weighing 180 ± 20 g. Rats were obtained from the Animal House, National Research Centre (NRC). The rats were individually housed in clean polypropylene cages and maintained in a controlled temperature room with a 12 h light and a 12 h dark cycle. The rats were given a standard diet and water throughout the experimental period. The experiments were carried out in accordance with guidelines and protocol approved by the Institutional Animal Ethics Committee.

2.1.2. Chemicals

Cetyltrimethyl ammonium bromide [CTAB, 99+%], tetraethyl orthosilicate [TEOS, 98], castor oil and Tween 80 was purchased from Across Co [Germany]. Ultrapure deionized [D.I.] water was generated using a Millipore Milli-Q plus system and was used for preparation and analysis. Cisplatin, Silica nanoparticles and sodium salicylate purchased from Sigma-Aldrich Chemical Company, St. Louis, MO, USA. Sodium hydroxide (NaOH) was purchased from Win-Lab Co. India. All chemicals are of analytical grade and used as received without further purification or modification.

PON-1, NO and MDA were purchased from biodiagnostics Company [Egypt] and was measured in brain tissue homogenate by spectrophotometer, BChE activity in the brain tissue supernatants was determined by BChE diagnostic kit (Chronolab, Barcelona, Spain). NF- κ B level was measured in the brain tissue supernatants using a commercially available ELISA kit (Glory Science, Del Rio, TX, USA). MCP-1 was measured by a commercially available Elisa kit (RayBio® ELISA Kits).

2.2. Methods

2.2.1. Experimental design

Rats were randomly divided into five equal groups, with ten rats in each group. Rats were given saline I.P. [group 1] or cisplatin I.P. [Cisplatin was been taken I.P. with dose 20 mg/kg body weight [6] to induce brain toxicity] [group 2] or carrier I.P. 100 mg/kg BW /day [group 3]. Some groups received I.P. injection of Si-Sc-NPs [100 mg/kg BW] either alone [group 4] or with cis [group 5]. The Si-Sc-NPs, was administered for three weeks alone before the injection of cisplatin and then were continued for one week. Animals were then euthanized by decapitation. Brain tissues were collected. Tissues were fixed in 10% neutral buffered formalin for

histopathological examination or homogenized for estimation of brain parameters.

2.2.2. Preparation of brain homogenate

Brain was removed quickly and placed in iced normal saline, blotted on filter paper and frozen at -80 °C. The frozen tissues were cut into small pieces and homogenized in 5 ml cold buffer 0.5 g of Na₂HPO₄ and 0.7 g of NaH₂PO₄ per 500 μ l deionized water pH= 7.4 then centrifuged at 4000 rpm for 15 minutes at 4 °C and the supernatant was removed for parameters estimation [37].

2.2.3. Chemical Synthesis of Silica nanoparticles loaded with sodium salicylate

Sodium salicylate nano drug was prepared using silica nanoparticles as starting material by a direct method. Firstly, the aqueous solution of sodium salicylate solution (2 mol/l) in was prepared in water contains 0.3 ml of Tween 80 under magnetic stirring for 20 min at room temperature. Secondly, silica nanoparticles solution was prepared by dissolving 0.3 g of silica nanoparticles in 25 ml of H₂O and kept under magnetic stirring for 15 min. After complete dissolution of silica nanoparticles, sodium salicylate solution coated with Tween 80 was added drop wise and kept under homogenization for 30 min. The pH was adjusted to 8.0 using an aqueous solution of NaOH (1.0 mol/l). At this point, milky solution was observed affirming the formation of emulsion which converted to nanoemulsion with the aid of homogenizer and ultra-sonication. Thus, the ultimate solution was submitted for homogenization for 30 min to enhance the dispersion of the synthesized nanoemulsion. Further technique such as ultra-sonication tool was used to improve the further dispersion for sodium salicylate nanoemulsion. For further comparison, silica nanoparticles nanoemulsion was prepared as mentioned before but without sodium salicylate. Finally, the produced nanoemulsions were kept in a bottle placed in a container and kept in dark for further analysis and characterization. The produced nanoparticles of Si loaded [Sc] was coded as Si-Sc-NPs.

2.2.4. Physical characterization for the formed nano-particles of Si and Si loaded SC as a model drug

Visualization of morphology and structure of the as-prepared nanoemulsion based on sodium salicylate loaded silica nanoparticles was investigated using transmission electron microscopy (TEM). The surface morphology of the as-synthesized silica nanoparticles with and without sodium salicylate was examined using scanning electron microscope (SEM; JSM-6360 LV; JEOL, Tokyo, Japan). The

morphology of internal phase of emulsions was characterized by optical microscopy (CX41 RF, Olympus, Japan).

2.2.5. Histopathological analysis

Brain sections will be excised, and then fixed in 10% neutral buffered formalin. Tissues will be then processed for paraffin embedding, subsequent serial sectioning, and stained with hematoxylin/eosin (H&E) to allow the assessment of brain morphology in different treatment groups. Tissues were observed from every tenth serial section; morphometric measurements were performed using "LaicaQwin 500" image analyzer computer system (Cambridge, England) [7].

2.2.6. Statistical analysis

Data are presented as mean \pm SE. Differences between groups were evaluated using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test using SPSS software (SAS Institute Inc., Cary, NC). Differences were regarded as statistically significant at a probability value of less than 0.05.

3. Results

3.1. Physical characterization of the synthesized nanoemulsion

For confirmation the preparation of nanoemulsion, TEM, SEM, and optical microscope were used to clarify the surface morphology and particle shape of the formed Si-Sc-NPs. It was observed that, Si-Sc-NPs were successfully prepared in nanoform as observed in Figure 1 (A, B). The two images of TEM were taken at two different magnifications (200 nm and 100 nm) to clarify the size and shape of the formed nanoemulsion. Moving to the surface morphology, SEM was utilized in order to examine the surface of silica nanoemulsion and Si-Sc-NPs. Figure 1 (C) showed the morphological structure of silica nanoemulsion which exhibit agglomerate particles due to the absence of dispersion medium 6 during examination. On the other hand, SEM image (Figure 1 D) displayed very small particles presented on the surface of silica nanoparticles which attribute to the existence of sodium salicylate. For further confirmation, optical microscope Figure 1(E, F) was carried out to clarify the shape and size of Si-Sc-NPs at two different magnifications respectively.

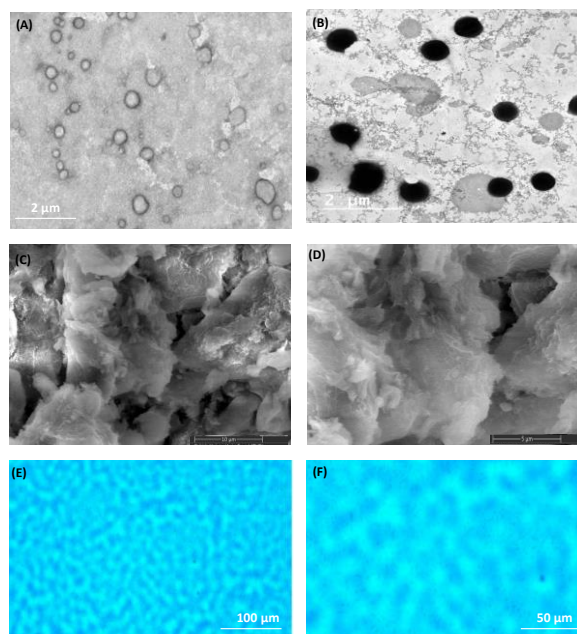


Figure (1): (A, B) TEM, (C, D) SEM and (E, F) optical microscope of sodium salicylate loaded butane tetracarboxylic acid at different magnifications.

3.2. Biochemical investigations results

3.2.1. The effect of Si-Sc-NPs on brain oxidative stress markers in different groups under study

Table (1) showed that the mean level of brain PON-1 activity, MDA and NO has no significance in carrier group and Si-Sc-NPs group when compared with control group. On the other, brain PON-1 activity in cisplatin group significantly decrease ($p < 0.05$) when compared with control group (table 1, figure 2). On the contrary, brain MDA and NO level in cisplatin group has increased significance ($p < 0.05$) when compared with control group (table 1, figure 2). Also, brain PON-1 activity significantly increases ($p < 0.05$) in Si-Sc-NPs +Cis group when compared with cisplatin group (table 1, figure 2). On the other hand, brain MDA and NO level significantly decreases ($p < 0.05$) in Si-Sc-NPs +Cis group when compared with cisplatin group (table 1, figure 2).

3.2.2. The effect of Si-Sc-NPs on brain BChE, MCP-1 and NF- κ B tissue activities in different groups under study

Table (2) showed changes in the brain BChE, MCP-1 and NF- κ B activities in different groups under study, which the mean level of brain BChE, MCP-1 and NF- κ B showed no significance in carrier group and Si-Sc-NPs group when compared with control group (figure 3).

Table 1 :The biochemical changes of brain PON-1 activity (kU/l), MDA (nmol/g) and NO (nmol/g) levels in different groups under study:

Group	PON-1 (kU/l)	MDA (nmol/g)	NO (nmol/g)
Control	14.01± 0.35	20.04± 0.53	20.50± 0.66
Carrier	14.23± 0.47	19.45± 0.26	20.74± 0.50
Cis	8.40± 0.21 *	32.60± 0.57*	32.01± 0.37*
Si-Sc-NPs	13.40± 0.25	19.22± 0.63	22.025± 0.23
Si-Sc-NPs +Cis	11.19± 0.08 **	26.52± 0.47*#	26.33± 0.96*#

Values are expressed as means ± SE. T test was significant at P < 0.05. *P < 0.05 vs. control group, #P < 0.05 vs. cis group.

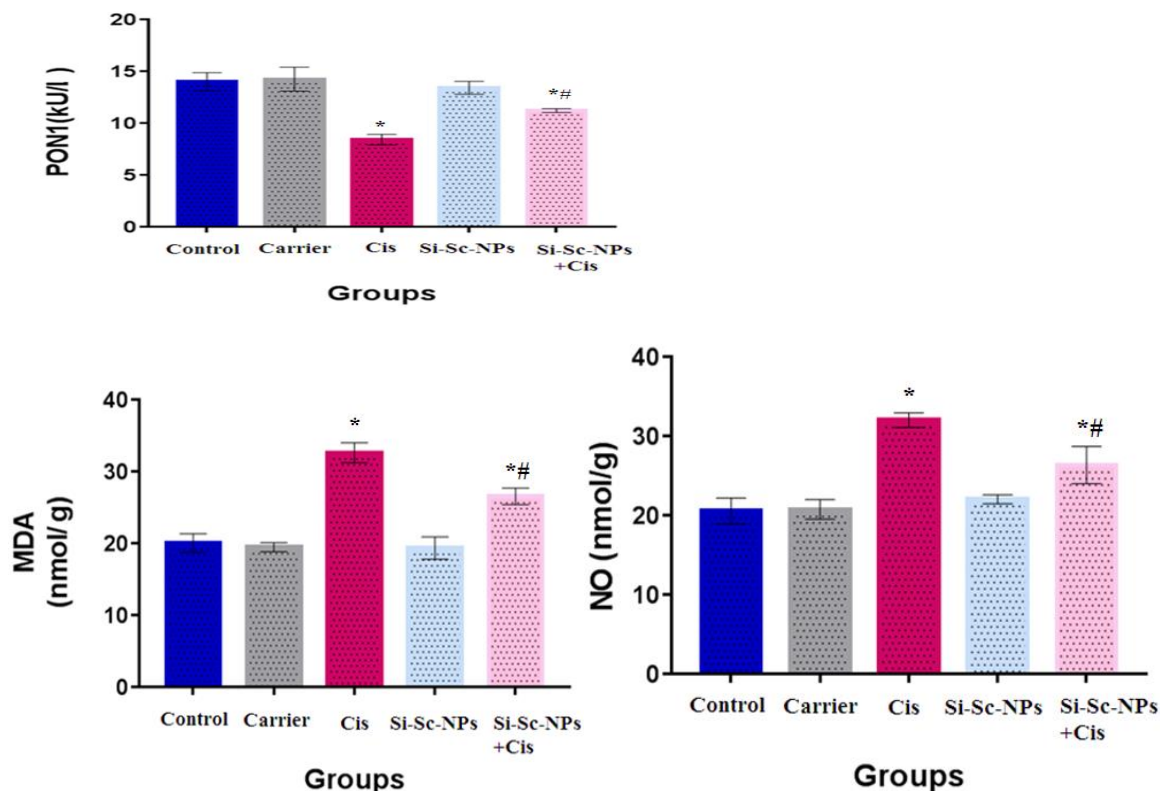


Fig. 2. Mean level of brain PON-1 activity, MDA and NO level in different groups under study.

Table 2: Changes in the activity of brain BChE (U/l), MCP-1 (pg/ml) and NF-kβ (U/l) levels in different groups under study:

Group	BChE (U/l)	MCP-1 (pg/ml)	NF-kβ (U/l)
Control	236.43± 2.42	325.15±19.13	2.29± 0.091
Carrier	242.83± 2.04	347.31± 16.57	2.30± 0.06
Cis	124.23± 2.97*	556.54± 14.77*	9.15± 0.42*
Si-Sc-NPs	243.17± 3.18	344.33± 12.77	2.23± 0.043
Si-Sc-NPs +Cis	195.09± 1.53*#	465.88± 10.32*#	6.57±0.12*#

Values are expressed as means ± SE. T test was significant at P < 0.05. *P < 0.05 vs. control group, #P < 0.05 vs. cis group.

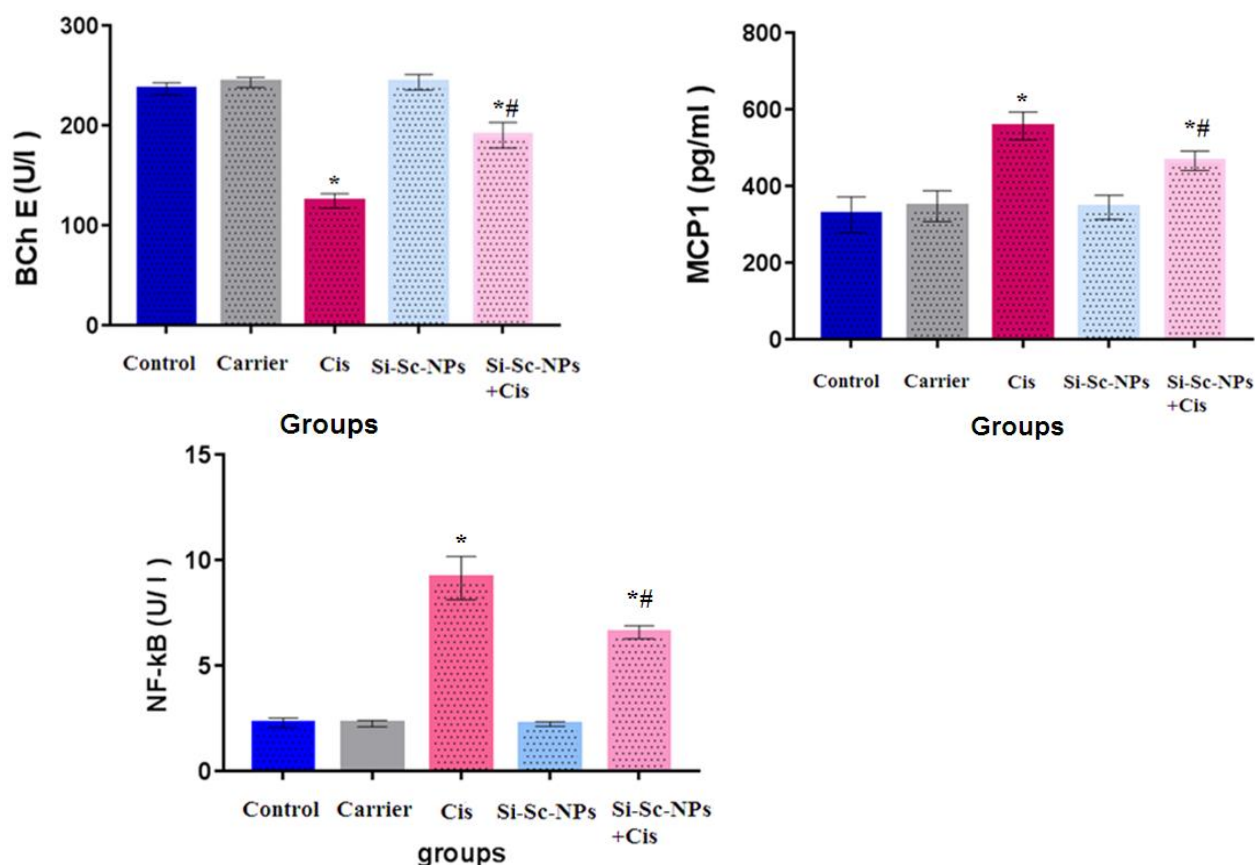


Fig. 3. Brain BChE activities, MCP-1 and NF-k β level in the different groups of experiment.

3.3. 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 4 (A). Also section from carrier group showed the brain tissues appeared nearly normal structure Figure 4 (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 4 (C).

In the group received Si-Sc-NPs revealed nearly normal with few pyknotic nuclei Figure 4(D). However in the group treated with Si-Sc-NPs +Cis showed improvement in almost brain structure and mild pyknotic nuclei and apoptotic neurons were observed Figure 4 (E). In the group received Si-Sc-NPs revealed nearly normal with few pyknotic nuclei Figure 4(D). However in the group treated with Si-Sc-NPs +Cis showed improvement in almost brain structure and mild pyknotic nuclei and apoptotic neurons were observed Figure 4 (E).

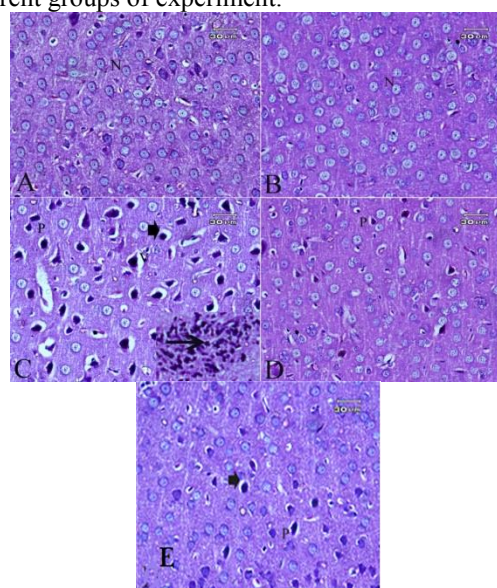


Fig. 4. Photomicrograph of brain section stained with H&E. A. Control group B. Carrier group C. Cisplatin group D. Sc-NPs group E. Sc-NPs +Cis group.(H& E X 400).

4. Discussion

Encapsulation in the required carrier increased drug efficacy, depending primarily on their functional classes. The goal of the present study was to explore

the protective effect on nanoparticles of cisplatin-induced brain toxicity and to decrease the drug side effects of loaded sodium salicylates. So silica nanoparticles were chosen to bear the proposed model medication due to its potential to encapsulate. The use of Tween 80 was aimed at spreading and avoiding the concentration of the sodium particles. Si-Sc-NPs is found to be of very small size under 50 nm, demonstrating the prediction for efficiency in nanoemulsion developed for medical purposes. The Si-Sc-NPs are formed with very small size and good distribution can be clearly seen. The data obtained for Si-Sc-NPs from optical microscopy are in accordance with TEM and SEM data which revealed the successful preparation of nanoemulsion with well distribution and small size thanks to the presence of Tween 80 and dispersion tools; homogenizer and ultrasonication.

Cisplatin administration quality is often hampered by its side effects. Several studies have reported that cisplatin causes the development of the several side-effects ROS; oxidative stress, one of the main pathways implicated in cytotoxicity of cisplatin [8]. The amount of super oxide anions and hydroxyl radicals increases Cisplatin induce oxidative stress. Excessive ROS can destroy cellular protein, lipids, and DNA under oxidative stress conditions, as well as regulation of survival signals [9, 10]. Cisplatin entry into tubular cells induces oxidative stress, followed by inflammation and subsequent cell apoptosis and necrosis which induces renal dysfunction due to cisplatin, the excessive development of ROS due to damage caused by DNA and mitochondria also inhibit antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) [11].

Cisplatin cytotoxic action is analogous to that of alkylating agents, when entering the cell, the chloride ion dissociates, and leaving a reactive complex that reacts with water and then interacts with the DNA by forming covalent bonds, preferably at the N7 position of adenine and guanine [12].

Cherian et al., [13] reported that the production of MDA is used as a biomarker to measure the level of oxidative stress in an organism. MDA results from lipid peroxidation of polyunsaturated fatty acids. ROS has very short life span, so it is not easy to detect them. Nevertheless, ROS-related tissue destruction could be observed by the final product of lipid peroxidation, such as MDA [14]. Our current hypotheses suggest that MDA levels increased after administration of cisplatin were a consequence of cisplatin cytotoxicity and development of oxidative stress. Measurement of the concentration of lipid peroxidant products and antioxidants may determine the level of tissue harm. Gupta et al., [15] reported that MDA levels were controlled to normal by

sodium salicylate dosage. In the present analysis, we demonstrate that MDA return to decreased level after Si-Sc-NPs treatment when compared with cisplatin group.

NO is a large molecule of signs found in the nervous system. NO exhibits cytoprotective properties at lower concentrations, like antibacterial and antiviral, whereas the cytotoxicity of the cytoxin is large [16, 17]. NO may not itself be harmful to neurons because it is the production of NO₂ and N₂O₃ or ONOO – nitrosylation and/or degradation of proteins and sulphide essentially. This happens when NO is produced overly long-lasting [18]. At these higher concentrations, NO is involved in the pathogenesis of various neuropsychological diseases, including neuroinflammatory diseases, schizophrenia, epilepsy, neurodegenerative diseases, and stroke [17]. Also Collin [19] said that High levels of NO react to ROS and lead to peroxynitrite formation, which is highly reactive and leads to oxidative damage to the brain and protein nitration.

Becerril et al. [20] reported that during inflammation followed by cisplatin administration, no formulation is increased. In this analysis we looked at increases in NO groups. In comparison with the control group, NO levels in the cisplatin group have risen. Our results agreed with Ji et al. [21] who reported that the ability of sodium salicylates to up-regulate plant antioxidant defenses, reduce ROS, nitric oxide, nitric oxide synthase. Our researchers reported that NO level significantly decreases in Si-Sc-NPs +Cis group when compared with cisplatin group.

PON-1 is a wide-ranging hydrolytic enzyme able to defend against lipid oxidation [22]. The enzyme belongs to a class of paraoxonases that also contains isoforms PON-2 and PON-3. PON-1 also hydrolyzes aromatic esters such as the activity of phenyl acetate and various aromatic lactones (activity of lactonase). It is synthesised in the liver and released into the blood, binding to HDL and preventing oxidation of lipoproteins [23]. In patients with Alzheimer's and other dementias and selfishness the function of PON-1 declines.

PON-1 has an antioxidant role and is discontinued by increased oxidative stress, which could explain reduced enzyme activity in patients with these neurological conditions [18]. Moreover and in agreement with previous work, we observed significant inhibition of PON-1 activity in the brain of rats treated with the cisplatin. Our researchers noted that PON-1 activity decreased during cisplatin administration due to oxidative stress; on the other hand, the antioxidant properties of sodium salicylates cause PON-1 activity significantly increases in Si-Sc-NPs +Cis group when compared with cisplatin group.

BChE is a serine enzyme present in most organs and plasma [24]. Cholinesterase (ChE) is 2 primary groups. The first is the actual AChE, concentrated in the membrane of the cortex, muscle and erythrocytes. The second is BCHE, which is synthesised in the liver but also is present in the intestine, lung, and kidney, and in plasma in large quantities [25]. The neurotransmitter acetylcholine is hydrolyzed into choline and acetic acid in humans, but with different specialties. This neurotransmitter is found in the neural membrane post-synaptic, the myonic junction, an autonomic ganglia and the end of the post-ganglionic nerves [18]. People suffering from BChE are asylum-based, but are more susceptible to suxamethonium and mivacurion relaxants of the muscles [26]. There is not a clear explanation of the biochemical role of BChE. Heroin can hydrolyze and act as a naturally occurring detoxification enzyme. This human enzyme's hereditary defect has no clear physiological effects [27].

Haghnazari et al. [28] reported that Decline in the activity of cholinesterases can induce oxidative stress and inflammation. Our researchers noted that oxidative stress caused by cisplatin administration resulted in decline in the mean value level of BChE activities in cisplatin group.

MCP-1 is a powerful chemocyte-catching factor, which leads to blood monocyte recruitment at sites of inflammations and tumours [29]. MCP-1 is formed by a host of various cell types, including monocytes, lymphocytes and fibroblast cells, endothelial cells and epithelial cells [30]. MCP-1 is up-regulated for infectious agents, oxidative radicals and host cell-released pro-inflammatory mediators. Both TNF- α and IL-1 β can stimulate MCP-1 production [31]. Humanes et al. [32] reported that In the inflammatory cascade of organs like the kidneys, cisplatin can control many cytokines (e.g., MCP-1 and IL-6). Our observations showed that inflammation caused by cisplatin administration results in elevation in the MCP-1 level in cisplatin group. On the other hand, Si-Sc-NPs +Cis group showed drop in the MCP-1 level due to the anti-inflammatory effect of sodium salicylates.

NF- κ B is a protein complex that regulates DNA, cytokine production and cell survival transcriptions [33]. NF- κ B is an important control factor of cellular responses, since NF- κ B belongs to the group of primary transcription factors for "fast-acting" activation, i. e. transcription factors in cells in an inactive state that requires no new protein synthesis. [34]. NF- κ B is kept inactivated in the cytosol by binding to an inhibitory subunit I κ B- α in the non-stimulated condition. It is released from its inhibitory subunit whenever the cell is stimulated by inflammatory and toxic signals, translocates to the nucleus and activates the transcription of genes encoding proinflammatory mediators and proinflammatory cytokines [18].

Known inducers of NF- κ B activity are highly variable and include ROS, TNF α , IL-1 β , LPS, isoproterenol, cocaine, and ionizing radiation [35]. Zhou et al. [36] said that several pro-inflammatory mediators, such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 cause NF- κ B transcription.

Consistent with the previous observations, we noted that cisplatin administration results in ROS production in the rat brain, which triggers NF- κ B production in high level in cisplatin group. Due to the inhibitory effect of sodium salicylates on cyclooxygenase enzymes, Si-Sc-NPs +Cis group showed depression in NF- κ B level.

Our histopathological investigations said that light microscopic examination from brain of cisplatin group showed several neurodegenerative changes. These changes include perineuronal vacuolation, eosinophilic cells, and shrunken neurons with pyknotic nuclei, apoptotic cells and signs of gliosis. On the other hand, the group treated with Si-Sc-NPs +Cis showed improvement in almost brain structure and mild pyknotic nuclei and apoptotic neurons were observed.

Conclusion

Sodium salicylates loaded on silica nanoparticles has protective effect on treating brain toxicity induced by cisplatin and decreasing the drug side effects.

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