



ORIGINAL ARTICLE

Neuroprotective effects of sonochemical-synthesized SiO₂ nanoparticles in vivo models of ischemic/reperfusion injury in stroke



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Received 30 June 2021; accepted 30 August 2021

Available online 6 September 2021

KEYWORDS

SiO₂;
Nanoparticles;
Ischemic/reperfusion;
NF-κB Signaling pathway

Abstract Cerebral ischemic injury is one of the debilitating diseases that showed inflammation plays an essential role in aggravating ischemic damage. After synthesizing silica nanoparticles (SiO₂ NPs) by sonochemical method, serum parameters in the presence of different concentrations of SiO₂ NPs are measured for toxicity assay. Rats were separated randomly into control, ischemia/reperfusion, and ischemia/reperfusion + SiO₂ NPs groups. Transient forebrain ischemia induced with bilateral occlusion of both common carotid arteries followed by 60 minutes of reperfusion. SiO₂ NPs were administered (500 mg/kg/day p.o.) 21 days before ischemia/reperfusion time. Animals sacrificed and frontal cortex and hippocampal tissues used to determine malondialdehyde (MDA) level, nitric oxide (NO), glutathione (GSH) levels, an essential antioxidant, superoxide dismutase (SOD), alterations in the level of cytokines, TNFα, IL-1β, MCP-1, and phosphor IκB. We also revealed the involvement of NF-κB downregulation by using western blotting. We reported on a histological investigation. The results showed that SiO₂ NPs with a diameter of around 50 nm in dose of 500 mg/kg didn't change the level of liver enzyme (including ALT, AST and ALP) and hematological parameters. 500 mg/kg SiO₂ NPs showed significant effects on remission of behavioral impairment. Ischemia/reperfusion oxidative injury in the rat hippocampus demonstrated a significant increase in MDA, TNFα, MCP-1, IL-1β, phosphor IκB, NO levels, and a significant decrease in GSH contents and SOD activities in the hippocampal tissue compared to the control group. Pretreatment of ischemic rats with SiO₂ NPs decreased the elevated levels of MDA, TNFα, MCP-1, IL-1β, phosphor IκB, and NO levels. A significant alteration observed in SOD activities and GSH content results between treated and untreated ischemia/reperfusion brains in rats. Decreased protein level of NF-κB also measured in SiO₂ NPs-treated animals. Untreated ischemia/reperfusion brains had significantly decreased in number of cells in CA1 hippocampus, never-

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Peer review under responsibility of King Saud University.



theless SiO₂ NPs increase the normal cell and decrease the neurodegeneration in hippocampus but it was not significant alteration. SiO₂ NPs reduced the damage caused by cerebral ischemia/reperfusion in rats and its molecular mechanism attributed to the downregulation of NF-κB signaling pathway.

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1. Introduction

Stroke is a significant cause of death or disability. Under permanent severe ischemia, brain cells cannot survive. Rechanneling occluded arteries through thrombolysis or thrombectomy is a common therapeutic strategy. Nevertheless, these procedures are associated with ischemia/reperfusion injury. Ischemia/reperfusion injury is often inevitable after stroke and can lead to different molecular cascades that break down the blood–brain barrier, which exacerbates brain damage (Pan et al., 2007; Fusco et al., 2019). Recent evidence showed how inflammation developed the side effect of brain injuries. It demonstrated that the leading causes of cerebral ischemia/reperfusion are the excessive formation of free radicals, cascading inflammatory reactions, overloading of calcium within neurocytes, and cytotoxic effect of excitatory amino acids. These factors are released quickly after brain injuries. The primary cause of cerebral injury exacerbation is acute inflammatory cascade reactions (Lin et al., 2016; Crack and Wong, 2008; Afshari et al., 2021).

In experimental stroke, models showed several transcription factors are activated. Nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1) were important transcript factors that regulate a huge number of inflammatory factors. NF-κB may be the most vital factor among the transcription which induce the initiation of inflammation and regulation of molecules related to inflammation (Harari and Liao, 2010; Kopitar-Jerala, 2015). Therefore, the downregulation of NF-κB has a vital role in inflammatory disease.

Silica nanoparticles (SiO₂ NPs) have hydrophilic nature, regular spherical morphology, and high specific surface area with high thermal and mechanical properties. Besides, they have been used in medicine because of lack of toxicity and biocompatibility (Downing and Jain, 2020). Recent studies showed SiO₂ NPs had the potential to target the brain (Oberdörster et al., 2004).

However, doubts remain regarding the effects of SiO₂ NPs on the inflammatory response during cerebral ischemia. This study investigated SiO₂ NPs on cerebral ischemia/reperfusion inflammatory response and surveyed anti-inflammatory mechanisms.

2. Material and methods

2.1. Experimental animals

Male Wistar animals were weighted 270–300 at eight weeks of age. These animals were housed in cages with free access to water and food and 12 h cycles of light/dark.

2.2. Synthesis of SiO₂ NPs

2.2.1. Sonochemical-mediated synthesis of SiO₂ NPs

SiO₂ NPs synthesized by the sonochemical-based method. In this method, two mM tetraethyl orthosilicate (TEOS: Si(OC₂H₅)₄) was dissolved in 100 mL deoxygenated distilled water. Afterward, ultrasonic irradiation (Ti horn, 25 kHz, five h under 1.8 atm of argon) was applied, and 12 mL ammonia was added dropwise to the SiO₂ precursor solutions at 50 °C, bubbling in the presence of N₂ aged for one h. The SiO₂ NPs were isolated from the solutions by centrifugation at 12,000 rpm for 15 min, washed with distilled water several times, then dried in an oven at 100 °C for two h (Kim et al., 2016).

2.2.2. Transmission electron microscopy (TEM) analysis of SiO₂ NPs

The diameter, morphology, and distribution of the synthesized SiO₂ NPs by the sonochemical method explored by TEM (Phillips GM-30, Germany). One drop of the SiO₂ NPs suspensions was dropped on a copper mesh grid and dried at room temperature for this analysis.

2.2.3. Dynamic light scattering (DLS) analysis of SiO₂ NPs

The hydrodynamic radius of the synthesized SiO₂ NPs at room temperature was determined by DLS, using a Nicomp 380ZLS (Particle Sizing Systems, Port Richey, FL, USA) operating at 535 nm, 50 mW, a frequency of the 200 kHz, and scattering angle of 90°.

2.3. Experimental design

First, we evaluated the toxicity of SiO₂ NPs on liver cells and bone marrow. We administered SiO₂ NPs orally for 21 days in 100, 250, 500, 750, and 1000 mg/kg to determine the non-toxic dose. On day 21, after the last administration, a blood sample in 2 mL was taken from the central vein of the tail. Blood Sample complete blood count differentiation and aminotransferase test and alkaline phosphatase were analyzed. Flowchart of the procedure is shown in Fig. 1.

Then, the maximum non-toxic dose of SiO₂ NPs was administered to animals for 21 days orally. On day 21, a stroke was induced to rat by bilateral common carotid artery occlusion (BCCAO). In the BCCAO model, animals were anesthetized by ketamine (45 mg/kg, i.p.) and xylazine (8 mg/kg), then their necks incised to reveal the two common carotid arteries. Carotid arteries are covered with a nontraumatic clamp for 30 min after separation from the vagal nerves. Sixty

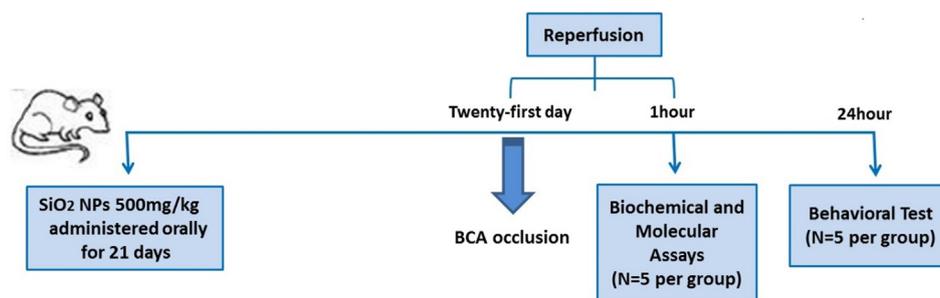


Fig. 1 flowchart of the experiment in this study.

minutes were allowed, and the clamp was removed. Twenty-four hours after surgery, behavioural tests were done on treated and untreated stroke groups. The blood sample was taken one hour after surgery. Then animals were decapitated and the entire brain isolated to analyze the acute molecular consequences of SiO₂ NPs on ischemic stroke. All experimental groups included five animals. Each goes through only one assessment. Control animals were administered only orally normal saline that was included in each experiment.

2.4. Behavioural test

We carried out a grid walking, hanging wire test, and an assessment of the neurologically modified severity score (mNSS) 24 h following BCCAO to detect post-stroke behavioural deficiencies and their enhancement through SiO₂ NPs pretreatment. The grid walking test intended to measure the animal feet at a 2.5 cm high grid floor (45 cm per 45 cm). Animals could move freely on the grid in a 1-minute trial. The number of full traps in which the animal's paw touched the floor counted, and two marks were given. One score was given if the animal pulled its foot quickly without touching the floor (S. Raza, xxxx; Curzon et al., 2011). In the hanging wire test, the rats' neuromuscular abnormality strength was measured. A wire cage lid with duct tape around the border is utilized for this test to keep the mouse from going off the edge. On top of the cage lid, the animal is placed. The lid is gently shaking three times to force the mouse to hold the wires. The lid is then rotated over and over again. The lid is secured at the height of 50–60 cm above a soft underlay, high enough to keep the mouse from jumping down but not high enough to inflict injury if it falls. The time it takes for a person to fall is measured. Time recorded before the animal fell. It took 90 s to cut off time (Gaur et al., 2011; OLIVÁN et al., 2015). We performed mNSS in rats to assess the functions of the engine, reflex, sensory, and balance. Higher scores show more severe injuries according to the methods described (Chen et al., 2008). Blinded researchers recorded and analyzed all behavioural experiments.

2.5. Cytokine levels

The animal's brains were dissected after decapitating rats by anesthesia. The hippocampus and frontal cortex homogenized in a buffer contain TRIS HCL, SDS, DTT, NP40, and glycerol. Then the solution was centrifuged for 5 min, and then the levels of IL-1 β and TNF- α in serum were detected with ELISA kits.

2.6. MDA level

Hippocampus and frontal cortex tissue samples were centrifuged for 5 min. Then 100 μ l of the supernatant along with 900 μ l of distilled water was added to the test tube, and 500 μ l of TBA reagent was placed in a boiling water bath for 60 min, after which the tubes were centrifuged again at 4000g for 10 min. Finally, the absorbance of the supernatant was read at 534 nm using a spectrophotometer (UV1600).

2.7. Oxidative stress measurements

All tissues were homogenized in the ice-cold buffer to determine SOD activity. Homogenized samples were centrifuged at 9000 μ g for 5 min at 4 $^{\circ}$ C, and a portion of the supernatant was immediately used for measuring SOD activity (Umg-1 protein) using the SOD assay kit (Sigma, Germany). In short, SOD activity is measured by the auto-oxidation method of pyrogallol.

A Glutathione Assay Kit was used to determine the glutathione level (Sigma-Aldrich, MI, USA). In short, in the 96 well-plate and thoroughly mixed (1.5 mg/mL DTNB, 6 U/mL glutathione reductase, and assay buffer), 10 mL liver homogenate and 150 μ l working solution were added. The plate was incubated for 5 min before 50 mL of NADPH solution (0.16 mg/mL) was added to each well. The absorbance spectrophotometrically was measured at 412 nm.

2.8. Nitric oxide

Nitrite level, the rapid oxidation metabolite of NO based on the Griess reaction method, was measured using hippocampus and frontal cortex homogenate supernatant. In short, a mixture of 50 μ l of supernatant with 100 μ l of Griess reagent (Sigma, USA) was incubated for 30 min at room temperature and, according to the manufacturer's instructions, absorbance was measured at 540 nm by the ELISA reader (Taha, 2003).

2.9. Western blot measure NF- κ B protein

Isolated animal brain tissue was crushed and homogenized in a microtubule with 1 mL of RIPA (Radioimmunoprecipitation Assay) buffer (Abcam, Cambridge, UK) and 1 percent protease inhibitor (Sigma) in a homogenizer device after rinsing with a cold PBS buffer. The supernatant was collected after centrifugation for 15 min at 13000 rpm, and the protein concentration was determined by a BCA kit (Thermo, Pierce,

USA). 60 μ g total protein electrophoresed at a constant voltage of 80 V for 35 min and then 120 V for 45 min on 10 percent polyacrylamide gel. The electrophoresed proteins were then transferred to the Polyvinylidene Fluoride (PVDF) paper, and monoclonal antibodies were incubated at 4 $^{\circ}$ C overnight with NF- κ B (Cell Signaling, USA) and beta-actin (β -actin). PBST solution containing 0.1 percent Tween-20 was used to wash the membrane, and then HRP-Rabbit anti-rat secondary antibody (1:10000) was incubated and re-washed against the primary antibody for 190 min on the shaker. Finally, the presence of NF- κ B protein in the samples was investigated by adding chemiluminescence ECL (GE Healthcare, Uppsala, Sweden) to the PVDF paper. The negative control sample (without primary antibodies) was also electrophoresed with the same protein concentration. The results of Western blot test were evaluated by Image-Pro plus 6.0 software (Media Cybernetics, Bethesda, MD, USA).

2.10. Nissl staining and histological examination

Histological examination was done 4 days after ischemia/reperfusion. The animals were anesthetized by intraperitoneal injection of ketamine (45 mg/kg), and xylazine (8 mg/kg) and their brains were perfused by heparin (1 unit/g bodyweight). Paraformaldehyde 0% and 4% in 1 M phosphate buffer (pH = 4.7) were tissue stabilized through the heart. After removing the brain from the skull, the samples were kept in 4% paraformaldehyde for 3 days and prepared. Paraffin blocks were fabricated in coronal sections for Nissl staining (Histo Line Laboratories Srl, Milano, MI, Italy). Nissl staining of coronal sections in thickness of 5–10 μ m in the distance of 2.3 mm was cut from the back of the leaf and placed on gelatin glass slides, and dried in air. After staining the slides with violet crystal 1%, samples were taken by Leica DM2000 Light microscope (Leica DM2000 Light microscope). Wetzlar, Germany) was examined with a magnification of 400%. Pyramidal cell in the CA1 hippocampus was counted by ImageJ software version 1.40 g (Wayne Rasband, NIH, USA). Only neurons with clear senses and senses were measured (Alipanahzade et al., 2012; Azad et al., 2011).

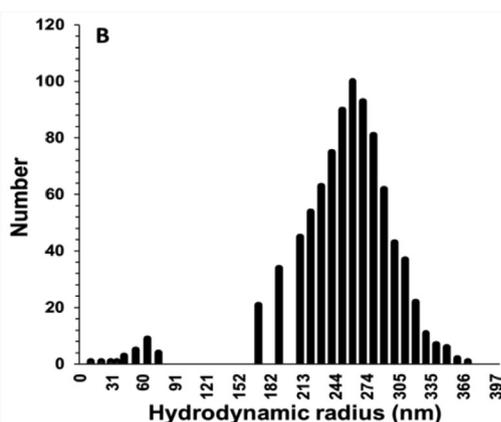
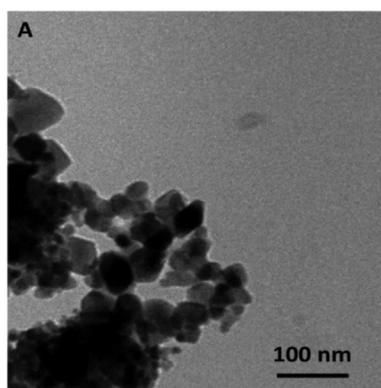


Fig. 2 (A) TEM microraphs of synthesized SiO₂ NPs through sonochemical methods. (B) The Hydrodynamic radius distribution of the SiO₂ NPs determined by the DLS study.

2.11. Statical analysis

The analysis was conducted using Graphpad Prism 8 (version 8.0; GraphPad, San 142 Diego, CA, USA). All the Value expressed as mean \pm SEM. Value differentiation was compromised by one-way, and two-way ANOVA followed by the Tukey test. Significantly considered $p < 0.05$.

3. Result

3.1. TEM analysis of SiO₂ NPs

The SiO₂ samples at the nanoscale were analyzed with TEM to explore the presence of SiO₂ NPs. As shown in Fig. 2, the non-aggregated grains of SiO₂ NPs successfully fabricated through the sonochemical method. Also, the average size of synthesized SiO₂ NPs was 25.9 \pm 11.07 nm, and they were spherical primarily (43%) or cubical (34%) or hexagonal (17%), as shown in Fig. 2A. DLS study was also done to measure the hydrodynamic radius and zeta potential of synthesized SiO₂ NPs. As depicted in Fig. 2B, it indicated that the hydrodynamic radius and zeta potential of SiO₂ NPs were about 261.32 \pm 47.61 nm (PDI: 0.191) with a zeta potential value of about -37.24 mV, indicating the good colloidal stability of synthesized Nanoparticles.

3.2. Toxicity dose of SiO₂ NPs

We administered 100, 250, 500, 750, and 1000 mg/kg SiO₂ NPs orally and investigated the effect on cell blood count, aminotransferase and alkaline phosphatase enzyme in rats. Table 1 showed the maximum dose of SiO₂ NPs, which didn't change the blood count significantly, was 500 mg/kg. In Fig. 3., 1000 mg/kg SiO₂ NPs significantly increased ALT ($P < 0.01$) and ALP ($P < 0.05$).

3.3. Effect of SiO₂ NPs on behavioural remission after stroke

We evaluate the effect of pretreatment of the SiO₂ NPs on improving motor, reflex, sensory and balance after stroke.

Table 1 Laboratory finding after administration SiO₂ NPs (* P < 0.05, ** P < 0.01, One way-ANOVA, post-hoc Tukey).

Hematological parameters	Control	SiO ₂ NPs 100 mg/kg	SiO ₂ NPs 250 mg/kg	SiO ₂ NPs 500 mg/kg	SiO ₂ NPs 750 mg/kg	SiO ₂ NPs 1000 mg/kg
WBC(K/ μ L)	8.44 \pm 0.37	8.31 \pm 0.61	8.98 \pm 0.83	9.18 \pm 0.93	10.38 \pm 0.53*	10.86 \pm 0.57*
RBC(M/ μ L)	8.14 \pm 1.00	8.08 \pm 0.30	0.56 \pm 8.34	0.91 \pm 8.28	0.81 \pm 7.78	0.39 \pm 7.18
Hb(gr/dL)	14.19 \pm 0.37	13.92 \pm 0.58	13.91 \pm 0.14	13.62 \pm 0.53	13.34 \pm 0.86	12.22 \pm 0.63*
Hct(%)	38.28 \pm 0.82	39.1 \pm 1.00	38.7 \pm 0.90	37.92 \pm 0.99	37.3 \pm 0.51	36.92 \pm 0.45
MCV(fL)	48.25 \pm 0.30	48.54 \pm 0.88	48.46 \pm 0.41	48.04 \pm 0.54	47.7 \pm 0.46	47.15 \pm 0.82
MCH(pg)	18.14 \pm 0.20	18.18 \pm 0.66	18.04 \pm 0.46	18.02 \pm 0.37	17.48 \pm 0.43	17.06 \pm 0.20*
PLT(K/ μ L)	768 \pm 10.20	762 \pm 12.1.00	742 \pm 15.70	732 \pm 20.50	706 \pm 21.70**	709 \pm 15.70**
NEU(%)	28.14 \pm 0.27	28.28 \pm 0.20	28.5 \pm 0.30	28.48 \pm 0.34	28.8 \pm 0.15	28.69 \pm 0.13
LYM(%)	67.2 \pm 0.82	67 \pm 0.89	67.63 \pm 1.40	69 \pm 0.82	70 \pm 1.67	75.33 \pm 0.31*

Abbreviation: WBC(K/ μ L)White blood cell, RBC(M/ μ L)Red blood cell., Hb(gr/dL) Hemoglobin., Hct(%)Hematocrit., MCV(fL)Mean corpuscular volume., MCH(pg)Mean corpuscular hemoglobin., PLT(K/ μ L)Platelet., NEU(%)Neutrophils ., LYM(%)Lymphocyte, Mean \pm SEM.

As shown in Fig. 4, footfall (F (2, 2) = 1.425, p = 0.0198) (Fig. 4A), severity of neurologic (F(2, 2) = 1.246, p = 0.0014) (Fig. 4B) and hanging wire test score (F(3, 3) = 1.207, p = 0.041) (Fig. 4C) were significantly different in untreated and treated animals. Post hoc Tukey test showed footfall (P < 0.05) and severity of neurological score (p < 0.01) significantly decreased in of SiO₂ NPs treated compared to untreated groups. Treated animals with SiO₂ NPs showed improvement in hanging wire score compared to untreated animals in post-hoc Tukey test (p < 0.05).

3.4. Effect of SiO₂ NPs on oxidative stress after stroke

MDA, GSH, SOD, and NO concentrations were computed to indicate oxidative stress in rat brains. One-way ANOVA analysis showed MDA level, GSH, and SOD level were significantly different from the control group in the frontal cortex and hippocampus. As shown in Fig. 5A, Post-hoc Tukey test

represents MDA level was significantly higher than the control group. Still, no significant difference was seen between the stroke group and the treated animal in the frontal cortex. However, in the hippocampus, MDA level significantly decreases in the treated group compared to untreated. The level of GSH and SOD was significantly decreased after stroke compare to control group in frontal cortex (p(GSH) < 0.01, p (SOD) < 0.001) and hippocampus (p(GSH) < 0.001, p (SOD) < 0.001) (Fig. 5B and 5C). Treatment with SiO₂ NPs only increased the level of the SOD significantly in the frontal cortex compared to untreated rats (p < 0.05) (Fig. 5B and 5C). The level of NO significantly increased after stroke in the frontal cortex (p < 0.001) and the hippocampus (p < 0.001) compared to the control group (Fig. 5D). NO level had significantly decreased after treatment in the hippocampus (p < 0.01) (Fig. 5D).

3.5. Effect of SiO₂ NPs on inflammatory cytokine after stroke

The inflammatory cytokines level after stroke is shown in Fig. 6A, B, C. Stroke significantly increased the level of TNF- α , IL-1 β and MCP-1 in frontal cortex (p(TNF- α) < 0.001, p(IL-1 β) < 0.001 and p(MCP-1) < 0.01) and hippocampus (p(TNF- α) < 0.001, p(IL-1 β) < 0.001 and p(MCP-1) < 0.01) compare the control group. SiO₂ NPs treated group had a significant decrease in TNF- α (p < 0.05), IL-1 β (p < 0.01), and MCP-1 (p < 0.05) levels in the hippocampus compared to the untreated group.

3.6. Effect of SiO₂ NPs on the expression of pNF- κ B protein and Phospho-I κ B α level

We used western blot to show the anti-inflammatory of SiO₂ NPs mediated by pNF- κ B protein. As shown in Fig. 7A, a significant difference seen in expression pNF- κ B (F (4, 4) = 1.255, p = 0.03) protein. The treated group with SiO₂ NPs compared to the untreated had a significantly lower level of pNF- κ B (p < 0.05) protein. SiO₂ NPs treated groups had a significant decrease in Phospho-I κ B α (p < 0.05) levels in comparing untreated groups (Fig. 7B).

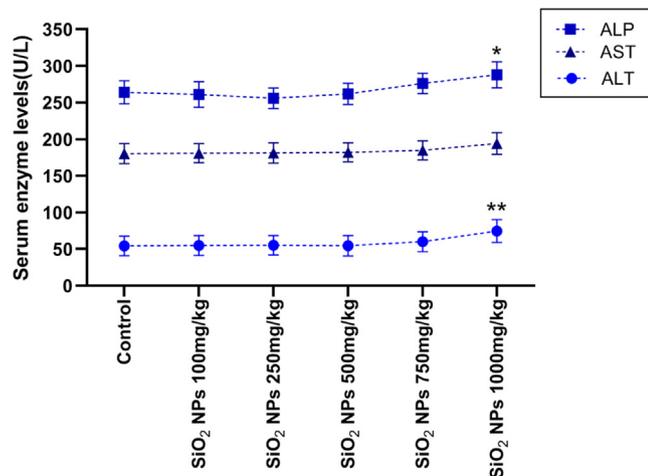


Fig. 3 Effect of different doses of SiO₂ NPs on the liver enzyme. 1000 mg/kg significantly increased the level of ALP and ALT compared to the control group. (* P < 0.05, ** P < 0.01, One way-ANOVA, post-hoc Tukey).

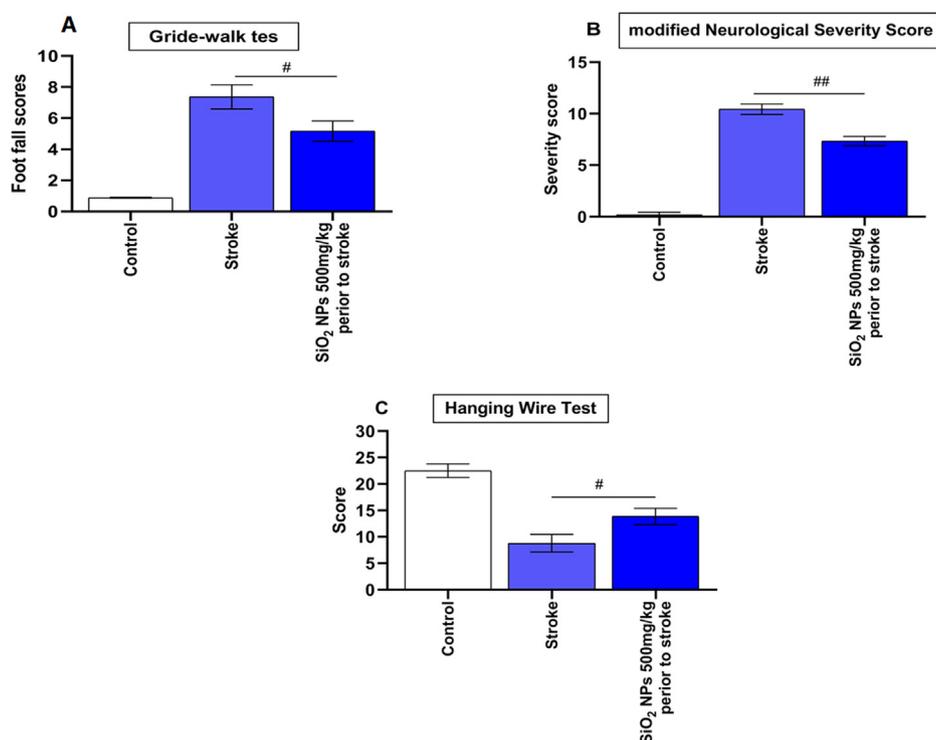


Fig. 4 Effect of SiO₂ NPs on Grid-walk and hanging wire tests and neurologic severity scores. (A) SiO₂ NPs treated animals showed a significant decrease in the Grid-walk test a day post-stroke. (B) SiO₂ NPs treated animals showed a significant decrease in clinical scores a day post-stroke. (C) Hanging wire test scoring also showed significant improvement in rats receiving SiO₂ NPs (# $p < 0.05$, ## $p < 0.01$, one-way ANOVA).

3.7. Effect of SiO₂ NPs on the histological change in the hippocampus

In the control group, all cells in the hippocampus were healthy, and the nucleus was wholly distinct and darker than the cytoplasm (Fig. 8A, B, C). Many cells were seen in the CA1 region. In the untreated stroke group, the number of cells decreased significantly and was darker than the control group cells in the CA1 region. The shape of cells changed from round to spindle-shaped and triangular in the control group with undetected and degenerated nuclei. In the stroke group treated with SiO₂ NPs, a slight increase in the number of healthy cells in the hippocampus was observed with reduced neurodegeneration, which was not statistically significant (Fig. 9). They had lower cell death and higher cell density than the untreated ischemic group.

4. Discussion

Our result showed that oral administration of SiO₂ NPs improved behavioural impairment after ischemia/reperfusion stroke. In ischemia/reperfusion stroke brain, neutrophil migration and microglial activation facilitated inflammatory cascades. The inflammatory cascades begin the neuroinflammation process, which then causes more cellular damage and neuronal function loss (Hailer, 2008). A Previous report showed silica nanoparticles deposited in the frontal cortex and hippocampus (Wu et al., 2011), and our result demonstrated SiO₂ NPs reduced cytokines in the hippocampus of

stroke animals. Increment of viable cells in the hippocampus and decrement of neurodegeneration after administration of SiO₂ NPs suggested that the therapeutic effect of SiO₂ NPs on ischemia/reperfusion stroke may conduct through an anti-inflammatory effect.

Although silica nanomaterials had been extensively researched, there is consensus on silica toxicity as hemolytic potential and cytotoxicity. (Möller and Bein, 2019). In contrast, amorphous silica has been considered by the International Agency for Research on Cancer (IARC) as an ingredient that has insufficient evidence of carcinogenicity and is generally accredited as safe by the food and drug administration (FDA) for inclusion in food additives and packaging. It means that high levels of human exposure stand without any risk affirmation (Kim et al., 2018). SiO₂ nanoparticles have been used as an imaging agent and therapeutic delivery cargo since the 2000s (Baeza et al., 2016). Further, to assess the potential for accumulation-based toxicity, the route of administration and chronic exposure is essential and more research is needed in this area (Fu et al., 2013). Our result showed oral administration of 500 mg/kg SiO₂ NPs for 21 days had no hemolytic and cytotoxic effect.

The potential to generate reactive oxygen species (ROS) is present in silica nanoparticles. Although overproduction of ROS causes oxidative stress and toxicity, low ROS levels play a role in regulating the physiological functions of living organisms. Moderate levels of ROS can extend the long life of living species. (Obata et al., 2018). Kim et al. showed silica nanoparticles by altering the ROS and oxygen balance represent the diminish in pro-inflammatory of M1 macrophage and incre-

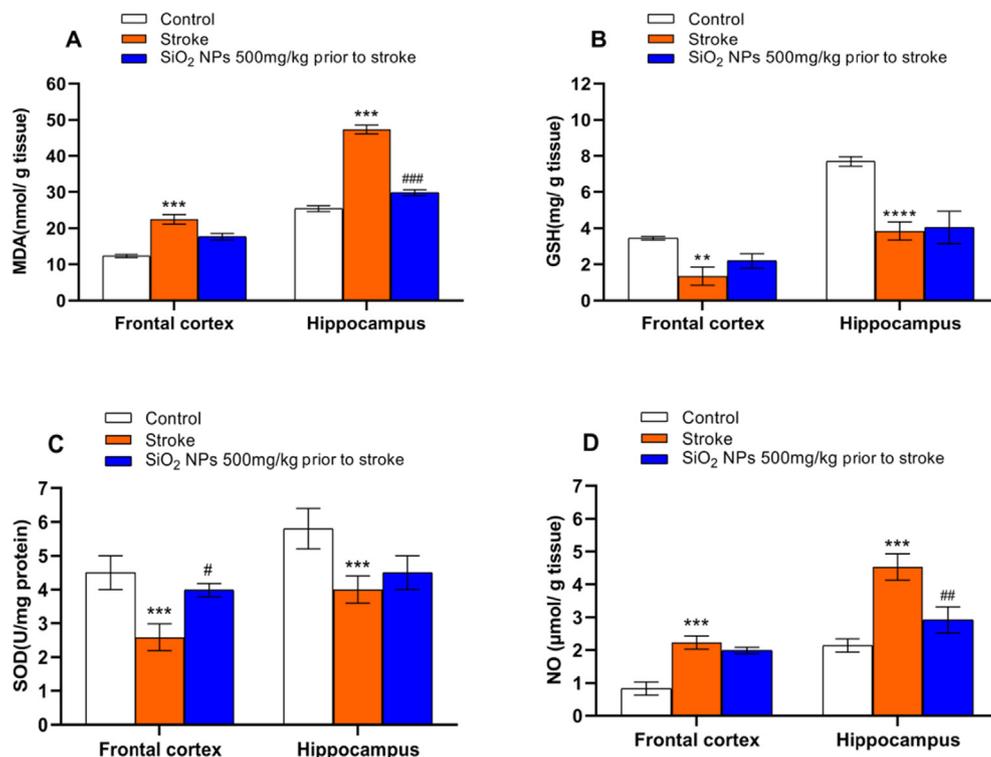


Fig. 5 Effect of SiO₂ NPs treatment on the oxidative stress factor. MDA (A) and NO (D) significantly increased, and GSH (B) and SOD (C) significantly decreased after stroke in the frontal cortex and hippocampus compared to the control group. (***) $p < 0.001$, **** $p < 0.0001$ one-way ANOVA test, post-hoc Tukey) (A) MDA was significantly decreased in the hippocampus of treated animals compared to untreated. (B) Treatment had no significant effect on GSH level in the frontal cortex and hippocampus. (C) SOD level was significantly increased in treatment compared to the untreated group. (D) The treated group had a significantly lower level of NO compared to the untreated group. (# $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, **** $p < 0.0001$, one-way ANOVA test).

ment the anti-inflammatory of M2 macrophage in treatment of rheumatoid arthritis (RA) (Kim et al., 2019).

Inflammation plays an essential role in stroke pathophysiology. Serial cellular events include infiltration of circulating immune cells and activation of brain-resident immune-responsive cells, including microglia and astrocytes, and stroke seen in endothelial cells. (Zhang et al., 2005; Impellizzeri et al., 2019; Khalilzadeh et al., 2018). The initial injury was exacerbated by the subsequent activation of the inflammatory NF- κ B signalling pathway (Shih et al., 2015). NF- κ B occurs in an inactive form by binding to the suppressor protein of the I κ B (inhibitor of nuclear factor kappa B) group (Wang et al., 2012). We found in this study that ischemia/reperfusion injury caused activation of Phospho-I κ B α . SiO₂ NPs significantly reduced the protein levels of Phospho-I κ B α and inhibited phosphorylation and degradation induced by ischemia-reperfusion. Concerning the mechanism underlying attenuation by SiO₂ NPs of ischemia/reperfusion induced downregulation of NF- κ B.

Delayed nerve cell death after ischemia/reperfusion has been demonstrated in sensitive areas of the central nervous system, such as the CA1 region of the hippocampus (Pulsinelli and Brierley, 1979). Many studies have been performed on the cause of ischemic cell death, but so far, no definitive function has been found that causes delayed neuron death after ischemia. Numerous studies have reported that neurogenesis occurs after ischemia in the CA1 region of the hippocampus

(Mori et al., 2009). Conventionally neurogenesis does not appear in the brain's nerve tissue, but in ischemic model animals, new neurons are produced in the sub-ependymal sub-region (Yamashita et al., 2006). New neurons that have developed in the subgranular region of the hippocampus differentiate into granular neurons and become mature neurons (Morris, 1984). Brain nerve cells need oxygen; therefore, adult neurons are more vulnerable to ischemia (Bendel et al., 2005). Pyramidal cells in the CA1 region of the hippocampus are susceptible and react rapidly to pervasive ischemia (Kirino, 1982). These pyramidal cells play a crucial role in learning, and spatial memory and their destruction can cause disorders in this area (Eckenhoff and Rakic, 1988). Tissue lesions due to ischemia result from pathophysiological events that lead to an increase in glutamate concentration, which in turn can stimulate glutamate receptors, especially NMDA, which leads to increased intracellular calcium and eventually causes cellular death (Choi, 1992).

Inflammation plays an essential role in stroke pathophysiology. Serial cellular events include infiltration of circulating immune cells and activation of brain-resident immune-responsive cells, including microglia and astrocytes, and stroke seen in endothelial cells. (Zhang et al., 2005). Glial cells, specifically microglia, are involved in acute inflammation, mediated by NF- κ B activation (Godínez-Rubi et al., 2013). Cytotoxic molecules such as NO, oxygen radicals, and cytokines were reported to play a crucial role in releasing microglia (Shi

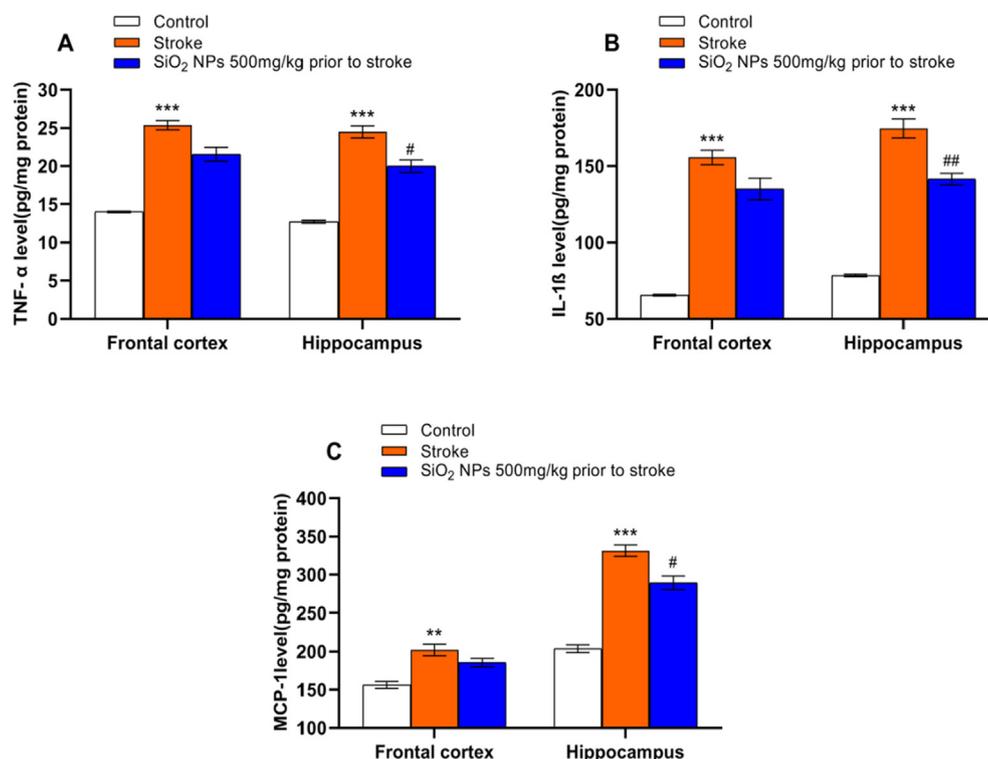


Fig. 6 Effect of SiO₂ NPs on inflammatory cytokines level. Stroke significantly increased the level of TNF- α (A), IL-1 β (B), and MCP-1 (C) compared to the control group (** $p < 0.01$, *** $p < 0.001$, one-way ANOVA test, post-hoc Tukey). SiO₂ NPs significantly decreased the levels of TNF- α (A), IL-1 β (B), and MCP-1 (C) compared to untreated groups. (# $p < 0.05$, ## $p < 0.01$, One-way ANOVA test).

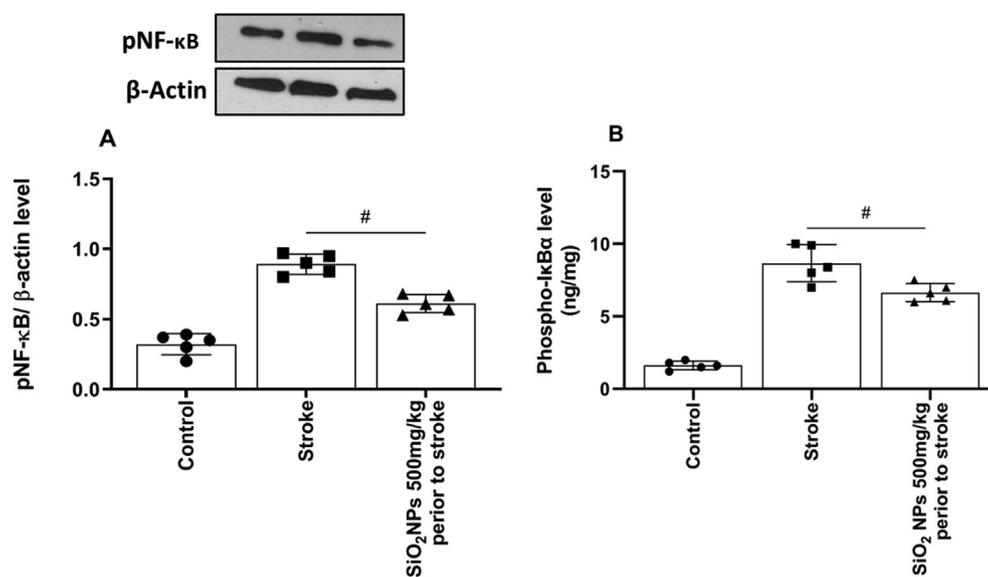


Fig. 7 Effect of SiO₂ NPs on expression of pNF- κ B and Phospho-I κ B α protein in western blot. pNF- κ B (A) and Phospho-I κ B α (B) protein expression level significantly decreased in treated animals (# $p < 0.05$, One-way ANOVA, post-hoc Tukey).

et al., 2019). In the present study, ischemia/reperfusion injury showed a significant increase in pro-inflammatory cytokines, TNF- α , IL-1 β , MCP-1, and ROS. SiO₂ NPs treatment reduced elevated levels of MDA, TNF- α , MCP-1, IL-1 β and NO. A

significant alteration between treated and untreated ischemia/reperfusion brains in rats was observed in SOD operations and GSH content results.

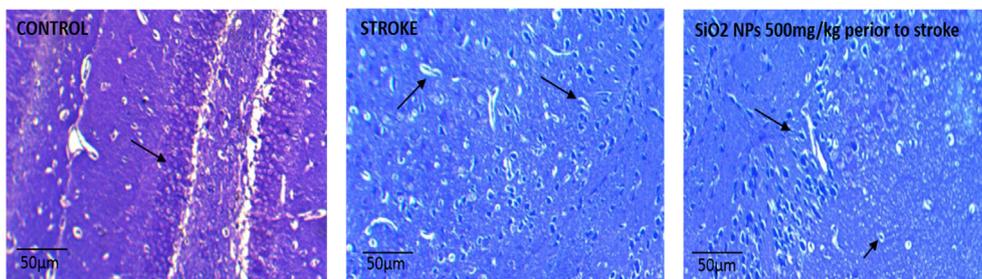


Fig. 8 Photomicrograph of the CA1 region of the rat hippocampus in the experimental groups by Nissl staining Zoom $\times 4$ (A) Control group (healthy neurons and healthy nuclei and euchromatin). (B) Stroke group (necrotic, degenerate and pyknotic cells with heterochromatin nucleus). (C) SiO₂ NPs 500 mg/kg + Stroke (decrease of necrotic cells).

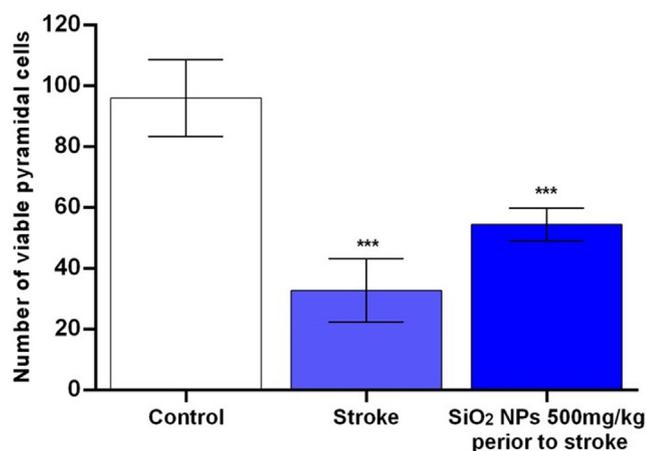


Fig. 9 Effect of SiO₂ NPs on number of CA1 pyramidal cells in rat models of ischemic/reperfusion injury in stroke *P-value ≤ 0.05 , statistically different from control group. Data are mean \pm SD.

5. Conclusion

In conclusion, our study showed that SiO₂ NPs administration significantly reduced the effect of cerebral ischemic/reperfusion stroke in a rat model. The neuroprotective effects of SiO₂ NPs appear to be associated with the increment of viable cells and the decrement of neurodegeneration in the CA1 hippocampus. This effect is associated with inhibition of NF- κ B activation and consequent suppression of inflammatory responses. SiO₂ NPs had varied ranges of use and needed more investigation on its properties. Our study had some limitations so for future investigation we suggest to focus on ROS and their precursor.

Ethics approval and consent to participate

All animal use procedures were carried out under the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China, with the approval of the Ethics Committee in our university.

Consent for publication

The authors declare consent for application

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

CRedit authorship contribution statement

Chengcheng Cui: Conceptualization, Methodology. **Dayong Shen:** Conceptualization, Methodology. **Dandan Zuo:** Conceptualization, Methodology. **Xinchun Ye:** Supervision, Validation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Dr Xinfeng Liu and Haotao Li for their assistance in discussion and data analysis of this manuscript.

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