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Original Article

Cerium oxide nanoparticles impact on sepsis-induced cerebral injury: Deciphering miRNA / NF-κB/TLR signalling pathway

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Abstract

Sepsis is regarded as an inflammatory syndrome that consists of complex biochemical and pathophysiological dysregulation, brought on by endogenous factors in response to systemic infection. Sepsis can cause short- and long-term cerebral injury. Cerium oxide nanoparticles (CeO_2 NPs) have been reported to possess both anti-inflammatory and antioxidative properties. The current study investigated the potential role of cerium oxide nanoparticles in the management of sepsis-induced brain injury. To achieve this target, forty male albino rats were divided into 4 groups, ten rats each. Group (i) set as a shame group. Group (ii) set as shame group administrated CeO2 NPs. Group (iii) septic group treated with saline and Group (iv) septic group treated with CeO2 NPs. The sepsis model in rats was induced by cecal ligation and puncture (CLP). Results showed CeO2 NPs administration resulted in significant improvement in the survival rate of rats, suppression in serum sepsis biomarkers (CRP, ESM-1, PCT and D- dimer), amelioration of brain inflammatory mediators (TNF-α- IL-6, NF-kB and LTB4) as well as apoptotic markers (Cas-3 and BAX). Furthermore, immunomodulation of miRNAs expression (155,124 and 146a). These findings demonstrate a promising pivotal role of CeO2 NPs treatment in alleviating the deleterious effects induced by sepsis in the brain.

Keywords: Sepsis, Cecal ligation, Brain injury, Inflammatory mediators, Apoptosis, miRNAs

1. Introduction

Sepsis can be described as a medical condition where the body's response to an infection becomes imbalanced, leading to severe dysfunction of vital organs and posing a threat to life. It is classified as one of the world's leading causes of death (1).

Sepsis is marked by an intensification of the body's defences against microbes, leading to a systemic and intravascular inflammatory reaction in the bloodstream. This excessive response also causes significant and prolonged immune suppression, making the patient more vulnerable to additional infections and raising the risk of mortality (2). The governing entity of the World Health Organization responsible for making decisions has stepped up its efforts to prevent, diagnose, and treat sepsis because it acknowledges that the condition poses a serious risk to patient safety and global health (3).

Septic shock affects the brain, leading to a disrupted balance in the immune and inflammatory responses, as well as changes in the dynamics of blood flow within the cerebral region (4). Sepsis-related systemic inflammation entails neuroinflammatory cytokines elevation, resulting in BBB destruction and neurotransmission alteration. Sepsis boosts the migration of immune cells towards the brain, enabling the diffusion of pro-inflammatory cytokines across the blood-brain barrier (BBB). This process is characterised by the destruction of endothelial cells, loss of tight junction proteins, and heightened activity of metalloproteases (5). It also induces endothelial and vascular damage, as well as cerebral signal transmission disruption along with apoptotic neuronal cell death and degeneration (6).

The guidelines for sepsis treatment are divided into three key components. (i) hemodynamic stabilisation, (ii) infection management, and (iii) septic response modulation (7). Additional interventions concerning organ sup-

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port, including, corticosteroids, oxygen therapy, renal replacement therapy, mechanical ventilation, and hemodynamic support (8). While broad-spectrum antibiotics play a vital role in sepsis treatment, a significant challenge associated with their use is the development of pathogen resistance. This resistance poses a detrimental effect on sepsis outcomes and approximately doubles the fatality rates (9).

Due to their intrinsic potential to overcome bacterial resistance and pharmacokinetic optimization, the utilization of nanotechnology-based techniques is increasingly regarded as an attractive therapeutic choice to address the challenges associated with managing sepsis (5). Cerium oxide is a substance belonging to the lanthanide series of rare earth oxides, as classified in the periodic table. When cerium oxide reaches nanoscales, it can exhibit a variety of unique features. Cerium oxide nanoparticles, also known as nanoceria (CeO2 NPs), possess a small size that contributes to a greater surface area-to-volume ratio. This reduction in particle size leads to the creation of surface oxygen vacancies, which have the ability to exist on the particle surface in either the Ce3+ or Ce4+ state (10). Regenerating reduced CeO2 nanoparticles (CeO2 NPs) and eliminating reactive oxygen species (ROS) through this approach is advantageous. Additionally, CeO2 NPs are known to exhibit both catalase (11) and superoxide dismutase-mimetic activities (12).In addition, CeO2 NPs were reported to have anti-inflammatory properties and neuroprotective effects (13).

In the present study, the objective was to investigate the potential impact of CeO2 nanoparticles (CeO2 NPs) on cerebral injury induced by sepsis. This investigation was conducted using an experimental rat model of sepsis triggered by cecal ligation and puncture.

2. Material and Methods

2.1. Animals

Forty male Wister rats (200± 50 g) were recruited for this study. Animals were taken from the National Research Centre's Animal House Colony in Cairo, Egypt. They were housed in conventional cages with unrestricted access to pellet food and water, maintained at a temperature of 25 ± 1 ℃, under an independent ventilation system, and subjected to a 12-hour light/12-hour dark cycle. All animals were given human care as per the guidelines of Egypt's National Research Centre's Ethical Committee, which follows the recommendations outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) (Ethical approval No. sci1432307001).

2.2. Materials

Samples of cerium oxide nanoparticles (CeO2 NPs) with an average diameter of approximately 20 nm, containing 10 wt% in water, were acquired from Sigma-Aldrich (St Louis, MO, USA). For the experiments, the CeO2 NPs samples were diluted in saline. Particle suspensions were prepared immediately before use and thoroughly mixed by vortexing to ensure a well-mixed suspension prior to each installation.

2.3. Sepsis induction

Caecal ligation and puncture (CLP**)** surgery was able to replicate the symptoms of clinical polymicrobial sepsis (14). Experimental animals were intraperitoneally anesthetized using pentobarbital (30 mg/kg), and a rat sepsis model was induced through the CLP method. All rats underwent surgery after a 12-hour fast during which they were only allowed to drink water. Following standard surgical cleaning and disinfecting, a 2cm incision was performed along the midline of the abdomen to expose the cecum. Subsequently, the cecum was ligated at the ileocecal junction. Using an 18-gauge needle, the cecum was punctured once, allowing the fecal contents to enter the peritoneum through gentle squeezing. After that, the abdominal cavity was sealed and the intestines were placed back into the abdomen. Rats in the sham group underwent laparotomies where the cecum was not perforated or ligated. After the surgical procedure, all rat groups were administered a subcutaneous injection of a resuscitation solution consisting of 0.90% sodium chloride (30 mL/kg).

2.4. Experimental design

Animals were assigned into four groups (n=10): (i) Sham group: rats received vehicle (0.1 saline). (ii) Sham + CeO2 group: rats received 3.5 mg/kg of CeO2 NPs in 0.1 saline. (iii) CLP group: rats received vehicle (0.1 saline). (i) CeO2 NPs group: rats were subjected to CLP procedure followed by CeO2 NPs treatment (3.5mg/kg iv 0.1 saline) (according to Lee et al., 2013) (15). Vehicle and CeO2NPs were administered 24 hours after the surgery once daily for 6 days via tail vein injection

2.4.1. Survival rate

The survival of the rats in all four groups was closely monitored 24 hours for a total of seven days in standard cages with enough food and water.

2.4.2. Sampling

At the end of the experimental period, anaesthesia was induced in the research animals by administering a sodium thiopental injection (50 mg/kg). Subsequently, blood samples were collected from the animals via retro-orbital haemorrhage. The sera were then separated and kept at -20 °C. Following the experimental procedures, the rats were decapitated, and their brains were dissected and rinsed with isotonic saline. One portion of the brain tissues was preserved at -80°C for gene expression analysis, while the remaining portion was homogenized with 0.1 M phosphate buffer saline at pH 7.4, resulting in 10% w/v final concentration. The homogenate was then centrifuged at -4°C and 3000xg for 15 minutes. The resulting supernatant was kept for subsequent biochemical tests.

2.4.3. Serum sepsis biomarkers

Serum levels of Procalcitonin (PCT), Endothelial cell-specific molecule (ESM-1), C-reactive protein (CRP) and D-Dimer were assessed by quantitative enzyme-linked immunoassay technique (ELISA) (Biosource, Inc., California, USA) according to the manufacturer's protocols

2.4.4. Inflammatory and apoptotic mediators

Brain tumour necrosis factor-alpha (TNF-α), Interleukin-6 (Il-6), human leukotriene B_4 (LTB_4), nuclear factor kappa B (NF-kB), Caspase -3 (CAS-3) and Bcl2 associated X protein (BAX) concentrations were estimated using ELISA (R&Dsystems, Inc. CA, USA)

Table 1. Primers of target genes.

Micro RNA	Forward	Reverse	Acession NO.
miR-155	GACTGTTAATGCTAATCGTGATAG	GTGCAGGGTCCGAGGTATTC	NC_000021
miR-124	TCTCTCTCCGTGTTCACAGC	ACCGCGTGCCTTAATTGTAT	NC_000008
miR-146-a	CAGTGCGTGTCGTGGAGT	GGGTGAGAACTGAATTCCA	NC_000005
U6	GCTTCGGCAGCACATATACTAAAAT	CGCTTCACGAATTTGCGTGTCAT	NC_076931

2.4.5. Sepsis-related microRNA gene expression

2.4.5.1. Total RNA extraction and cDNA synthesis

The Trizol® Reagent (Invitrogen, Germany) kit was employed for the extraction of total RNA from rat brain tissues. The isolation procedure was followed exactly as directed by the manufacturer. The isolated RNA from brain tissue was subjected to reverse transcription, converting it into complementary DNA (cDNA). The reverse transcription reaction was conducted in a 20 µl volume following the guidelines provided by the Revert Aid TM First Strand cDNA Synthesis Kit (Takara Biotechnology Co., Ltd., Dalian, China). The resulting PCR products containing the cDNA were stored at 4 °C until further utilization in quantitative real-time PCR (qRT-PCR) experiments.

The examined gene expression was determined using a StepOne™ Real-Time PCR System (Applied Biosystems, USA) to quantify the cDNA copies in male rats. The specific primer sequences for the genes used are provided in Table 1. The relative quantification of the target genes compared to the reference gene (U6) was calculated using the $2^{-\Delta\Delta CT}$ method.

2.5. Statistical analysis

Data obtained were presented as mean ± standard error of the mean (SEM). Data analysis was performed using GraphPad Prism version 8, employing one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for significance comparison at a threshold of $P<0.05$. Animal survival was assessed using the log-rank test and Kaplan-Meier analysis.

3. Results

3.1. Effect of CeO2 NPs treatment on CLP septic animals' survival

The survival rate of septic animals was improved following CeO2 nanoparticles treatment as Compared to control sham animals, CLP -animals showed several shock-related symptoms such as diarrhoea, piloerection, lethargy and little or no spontaneous movement in comparison with control and sham groups. Death started 24 hours following CLP and by day 6 the mortality rate reached 100% in the CLP untreated group. However, septic animals treated with CeO2 nanoparticles exhibited markable improvement in their alertness and frequency of food and water consumption additionally, administering CeO2 nanoparticles to septic animals boosted animal survivability by up to 85%.Neither the sham group nor the sham + CeO2 NPs group showed any mortality (Fig.1).

3.2. Effect of CeO2 NPs administration on serum sepsis markers in CLP-induced sepsis in rats

CLP induced a significant elevation in serum levels of sepsis biomarkers; CRP (592%), ESM-1 (540%) (Fig. 2A), PCT (183%) and D-dimer (400%) (Fig. 2B) as compared to sham control group. Otherwise, CeO2 NPs administration in CLP septic rats resulted in significant attenuation in septic markers level comparable with CLP group.

3.3. Effect of CeO2 NPs administration on brain inflammatory mediators in CLP-induced sepsis in rats

Results revealed that the levels of brain TNF-α, IL-6, LTB_4 and NF-kB were markedly elevated following CLP (300%, 550%, 180% and 147% respectively) as compared to the Sham group. After CeO2 NPs treatment, the levels of these markers in the brain were significantly suppressed compared to the CLP group (Fig. 3A-3B).

3.4. Effect of CeO2 NPs administration on brain apoptotic markers in CLP-induced sepsis in rats

The septic rats group depicted a significant increment in brain content of apoptotic markers; CAS-3 (233%) and

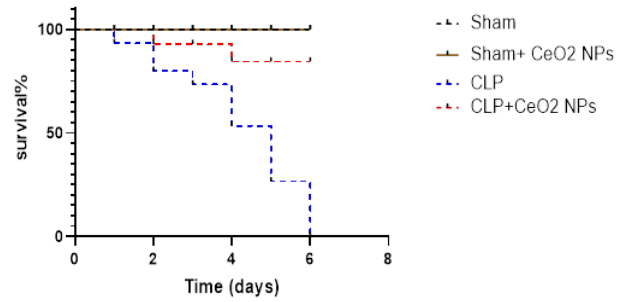

Fig. 1. Kaplan –Meier survival plot following CeO2 NPs treatment in CLP- septic rats. Sham group: sham control, Sham +CeO2 NPs group: sham + cerium oxide nanoparticles, CLP group: cecal ligation and puncture induced sepsis group, CeCO2 NPs group: cerium oxide nanoparticles (3.5 mg/kg) was administered 24 hours after sepsis induction and continued for 6 consecutive days via tail vein. n=10 rats/group.

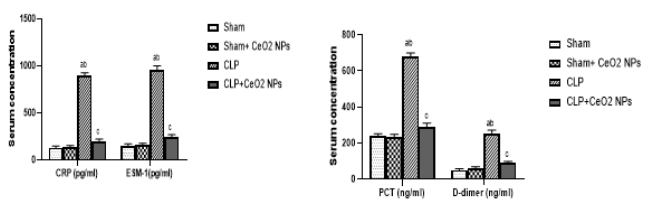

Fig. 2. Effect of CeO2 NPs administration on serum sepsis markers in CLP – septic rats (A) CRP: C-reactive protein, ESM-1: Endothelial cell-specific molecule-1.(B) PCT: Procalcitonin, D- dimer. Data were expressed as the mean ± SEM (n=10 rats/group).[a]$P<0.05$ vs Sham, [b]$P<0.05$ vs Sham +CeO2 NPs, [c]$P<0.05$ vs CLP. Sham group: sham control, Sham +CeO2 NPs group: sham + cerium oxide nanoparticles, CLP group: Cecal ligation and puncture induced sepsis, CeCO2 NPs group: Cerium oxide nanoparticles (3.5 mg/kg) were administered 24 hours after sepsis induction and continued for 6 consecutive days via tail vein .

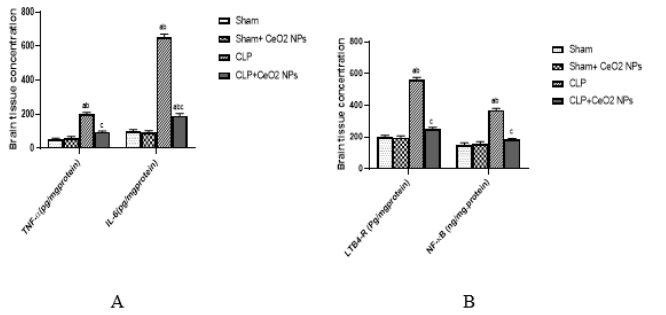

Fig. 3. Effect of Ceo2 NPs administration on brain inflammatory mediators in CLP–septic rats (A) TNF-α: Tumor necrosis alpha, IL-6: Interleukin-6 .(B) LTB4: Human leukotriene B4, NF-kB: Nuclear factor kappa B. Data were expressed as the mean ± SEM (n=10 rats/group).[a]P<0.05 vs Sham, [b]P<0.05 vs Sham +CeO2 NPs, [c]P <0.05 vs CLP. Sham group: sham control, Sham +CeO2 NPs group: sham + cerium oxide nanoparticles, CLP group: cecal ligation and puncture induced sepsis, CeCO2 NPs group: cerium oxide nanoparticles (3.5 mg/kg) were administered 24 hours after sepsis induction and continued for 6 consecutive days via tail vein.

BAX (140%) versus the Sham group. However, treating rats with CeO2 NPs significantly inhibited apoptotic markers in comparison with CLP –the septic group (Fig.4).

3.5. Effect of CeO2 NPs administration on brain miRNA expression in CLP-induced sepsis in rats

The rats group that underwent CLP showed significant upregulation in the expression of miR155 (200%) (Fig.5A), associated with significant downregulation in miR124 (Fig.5B) and miR146-a (Fig.5C) expression (-60% and 54% respectively) as compared to the sham group. On the contrary CLP group treated with CeO2 NPs resulted in restoration in the expression of the aforementioned miRNAs.

4. Discussion

Sepsis is a severe condition associated with a high rate of mortality, prompting numerous studies aimed at discovering new therapeutic approaches to mitigate its adverse outcomes (16). The cecal ligation and puncture (CLP) animal model has been frequently employed in exploring innovative strategies for sepsis management (17). In this model, the punctured cecum serves as a continual source of bacteria, and the endotoxin, a major component of bacterial cell walls, initiates multiple pathophysiological processes in gram-negative sepsis. These processes include excessive free radicals generation and a robust inflammatory response, resulting in various degrees of organ dysfunction (18).In the present study, septic rats showed a high mortality rate that reached 100% in 6 days. The signs of sepsis including mental depression, lethargy and reduced activity after CLP surgery were demonstrated, indicating the establishment of a CLP-induced sepsis rats model.

In septic rats, serum biomarkers related to sepsis such as CRP, ESM-1, PCT, and D-dimer exhibited a notable increase. This elevation is triggered by bacterial infection and participates in regulating the production of essential immune effector molecules (19). CRP is an acute-phase reactant protein that displays an increased expression during the inflammatory process and helps macrophages eradicate bacteria by interacting with their phospholipid

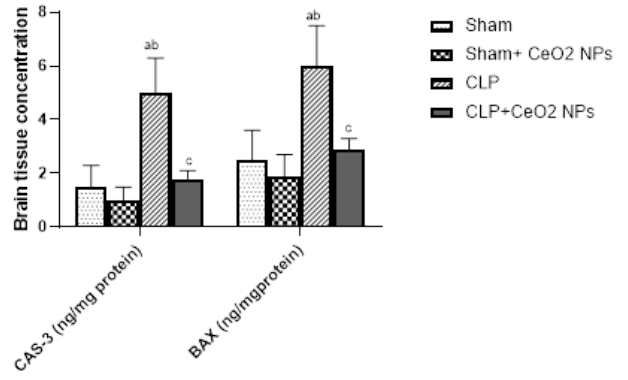

Fig. 4. Effect of Ceo2 NPs administration on brain apoptotic markers in CLP-septic rats CAS-3:Caspase-3 and BAX: Bcl2 associated X protein. Data were expressed as the mean ± SEM (n=10 rats/group). [a]P<0.05 vs Sham, [b]P<0.05 vs Sham +CeO2 NPs, [c]P <0.05 vs CLP. Sham group: sham control, Sham +CeO2 NPs group: sham + cerium oxide nanoparticles, CLP group: cecal ligation and puncture induced sepsis, CeCO2 NPs group: cerium oxide nanoparticles (3.5 mg/kg) were administered 24 hours after sepsis induction and continued for 6 consecutive days via tail vein.

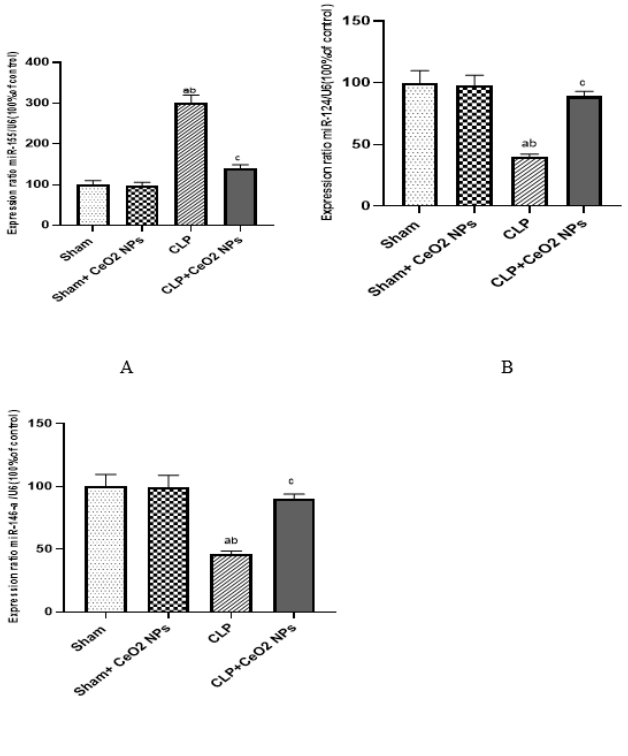

Fig. 5. Effect of Ceo2 NPs administration on brain miRNA expression in CLP – septic rats miR-155: microRNA 155, miR124: microRNA 124 and miR146-a: microRNA 146-a. Data were expressed as the mean ± SEM (n=10 rats/group). [a]P<0.05 vs Sham, [b]P<0.05 vs Sham +CeO2 NPs, [c]P <0.05 vs CLP. Sham group: sham control, Sham +CeO2 NPs group: sham + cerium oxide nanoparticles, CLP group: cecal ligation and puncture induced sepsis, CeCO2 NPs group: cerium oxide nanoparticles (3.5 mg/kg) were administered 24 hours after sepsis induction and continued for 6 consecutive days via tail vein.

constituents (20). Cytokines including IL-6 and TNF-α promote its production, which occurs mostly in the liver (21). It has a decent prognosis value as high CRP levels are related to the severity of the illness (22). Also, decreased CRP levels may suggest a favorable response to antibiotic treatment (23). ESM-1 is released by the activated

endothelial cells, indicating endothelial damage, and is linked to sepsis severity and prognosis (24). Scherpereel et al. (25) revealed that elevated ESM-1 plasma levels have been observed in pathological circumstances such as sepsis. ESM-1 expression is influenced by inflammatory mediators such as IL-1β, TNF-α, and interferon-γ. ESM-1 plays a role in inflammation by interacting with LFA-1 (lymphocyte function-associated antigen 1), which in turn reduces the adherence of leukocytes to intercellular adhesion molecule 1 (ICAM1). This suggests that ESM-1 may have a negative impact on leukocyte extravasation, the process by which leukocytes exit blood vessels and enter tissues during inflammation. PCT has gained popularity as a biomarker for sepsis due to its relative specificity for bacterial infections (26). PCT is a precursor of the hormone calcitonin produced by C cells in the thyroid gland and other neuroendocrine cells and normally, it is undetectable in the serum of healthy individuals. However, in the presence of bacterial infection, proinflammatory cytokines stimulate CALC-1 gene expression which is responsible for PCT synthesis, in various cells throughout the body (27). However, due to the fact that most cells do not have the ability to convert PCT into calcitonin, PCT enters the bloodstream, leading to an increase in blood levels (26). Similarly, elevated serum D-dimer level which reflects both thrombin generation, as well as fibrinolytic system suppression has been identified as a potential prognostic and effective predictor of mortality in sepsis (28).

Sepsis is a multifactorial disease resulting in an imbalance in the body's proinflammatory and anti-inflammatory pathways. Monocytes and macrophages, which are part of the innate immune system, express pathogen recognition receptors (PRRs) such as Toll-like receptors (TLRs), with TLR4 being particularly notable. These PRRs recognize pathogen-associated molecular patterns (PAMPs) and initiate nuclear factor κB (NF-κB) signalling pathways. Once these pathways are activated proinflammatory cytokines such as TNF-α, IL-1, and IL-6 are released mediating inflammation (29). T and B lymphocytes are the primary mediators of the adaptive immune response. To regulate and prevent an excessive inflammatory response following activation, CD4+ Th1 and Th17 cells secrete proinflammatory cytokines such as interleukin-17 (IL-17), interferon-γ (IFN-γ), and granulocyte-macrophage colony-stimulating factor (GM-CSF). On the other hand, Th2 and Treg cells secrete anti-inflammatory cytokines such as IL-4 and IL-10 (30). When these two processes are out of balance, a flood of inflammatory mediators is released, causing organ damage. The brain is an important organ that is susceptible to being attacked during sepsis involving mitochondrial dysfunction, blood–brain–barrier (BBB) damage, oxidative stress, neuroinflammatory response, and neuronal apoptosis in the brain tissue (31). Animals model of systemic sepsis demonstrated a rise in pro-inflammatory cytokines and chemokines in the brain. Additionally, sepsis causes a rise in astrogliosis and complement activation in the animal brains, indicating persistent neuroinflammation (32). This study coincides with the current results which revealed a significant increment in brain inflammatory mediators: TNF-α, IL-6, NF-kB as well as LTB4. TNF-α is crucial in sepsis because it may start a systemic inflammatory response (33). Furthermore, IL-6 has been associated with sepsis induced by CLP (cecal ligation and puncture), and studies have proved that selective inhibition of IL-6 trans signalling can enhance the survival rate. Moreover, cytokine cascade response in sepsis was significantly influenced by IL-6 and TNF-α (34). In addition, the significant amount of bacterial cell wall released interacts with innate immune cells via binding to Toll-like receptors (TLRs) combined with CD14 leading to stimulation of the NF-kB pathway and the release of a significant amount of inflammatory mediators (35). LTB_4 is an effective neutrophil chemoattractant, inflammatory lipid mediator, and promoter of reactive oxygen species, which damage tissues (36). LTB4 concentrations are increased during sepsis and may be a factor in sepsis-induced injuries, septic shock, and septic mortality (37). Notably, increased LTB4 during sepsis contributes to vascular endothelial disorders (38). LTB4 also helps activate NFκB-dependent production of proinflammatory cytokines and induces both acute inflammatory responses and the maintenance of chronic inflammation (39).

Neuronal apoptosis also plays a role in sepsis-related brain damage, in addition to inflammatory toxicity. The current data showed significant elevation in brain cas-3 and BAX apoptotic biomarkers in CLP septic rats. Neuroinflammation during sepsis induces oxidative stress and mitochondrial dysfunction (40), which may cause neurons and glial cells to apoptosis. Numerous stimuli, such as cytokines and inflammation, trigger mitochondria-dependent apoptosis by inducing proapoptotic to antiapoptotic protein ratio disruption (41). Pan et al. (42) reported that sepsis induces apoptosis which leads to direct cell destruction as well as an inflammatory cascade called pyroptosis which is mediated by Cas-3. When caspase-3 is activated, it causes cell pyroptosis and produces inflammatory mediators. Moreover, it has been reported that CLP septic rats exhibited elevated levels of Bax and reduced levels of Bcl2 in hippocampal and cortical cells. This effect was associated with the activation of the P38-mitogen-activated protein kinase (P38-MAPK) pathway and the involvement of a mitochondria-dependent apoptotic pathway (32).

Microglia, a specialized long-term resident macrophage, is one of the main resident cells of the CNS that cause neuroinflammation. Microglia contribute to the preservation of brain homeostasis by existing in a "quiescent" or "resting" state (43). Microglia, in order to detect and respond to a diverse range of danger-associated molecular patterns (DAMPs), pathogen-associated molecular patterns (PAMPs), and other molecular signatures, microglia express an array of pattern recognition receptors, cytokine receptors, and neuronal receptors. This response sets off signalling that results in microglial activation (44). Microglia can develop into either M1 (pro-inflammatory) or M2 (anti-inflammatory) phenotypes, depending on the sort of signals that activate them (45).

MiRNAs (microRNAs) are small non-coding RNA molecules that regulate post-transcriptional genes by suppressing gene expression through interference with target mRNA translation or stability (46). Additionally, miRNA dysregulation has been linked to clinical symptoms and the severity of sepsis (47). In the present study, brain miR155 expression was significantly upregulated. However, there was a significant downregulation in miR124 and miR146-a brain expression. Chen et al. (48) reported an increment in miR-155 expression during the early stages of sepsis, and this increase was found to be positively correlated with the progression and severity of the disease.

MiR-155 is a central proinflammatory mediator of the central nervous system, triggered by microglia in response to Toll-like receptor (TLR) signalling dependent on NF-κB (49). Conversely, miR-124 is an anti-inflammatory miRNA that is primarily expressed in the brain. It is involved in the regulation of neuronal function as well as mediating immune system activity within the CNS (50). Han et al.(47) reported that low expression of miR124 in cultured microglia during neuroinflammation in the early stage of AD was detected. The downregulation of miR-124 in microglia is associated with a shift away from the resting state of the microglia and polarizing towards the M1 phenotype leading to increased inflammatory mediators and the progress of neuroinflammation (51). MiR-146a is an additional anti-inflammatory miRNA that is expressed in neurons, microglia, and astrocytes (52). Wang et al. (33) reported that septic subjects demonstrated lower plasma miR-146a levels than healthy patients and miR-146a expression was also suppressed in blood leukocytes from sepsis. Deficiency in miR-146a triggers inflammatory signalling within the central nervous system (CNS) upon stimulation by NF-κB, resulting in an excessive neuroinflammatory response (52).

Cerium oxide (CeO_2) nanoparticles have been reported to possess both anti-inflammatory and antioxidative activities suggesting that these particles may show positive effects in the treatment of inflammatory disorders (53). Without the use of antibiotics, fluid resuscitation, or any other pharmaceutical intervention, treatment of CLP rats with CeO2 NPs dramatically increased animal survival by up to 85% in the current research. These findings corroborated those of Selvaraj et al. (54), who found that CeO2 nanoparticles treatment significantly reduced animal death, hypothermia induced by sepsis, and increased animal survivability, with evidence of decreased serum free radicals inflammatory mediators levels.

The present data demonstrated that the elevated levels of circulating CRP, ESM-1, PCT and D-dimer in septic rats were significantly lowered following CeO2 NPs administration. The observed reduction in sepsis markers is probably due to the anti-inflammatory effects and potential auto-regenerative antioxidant properties of CeO2 NPs (55). Amiri et al. (56) studied the effect of CeO2 NPs administration on CRP level during the wound healing process and demonstrated that CeO2 NPs significantly depressed the level of endogenous intoxication markers and consequently CRP level, which could be attributed to antioxidant, antimicrobial, growth factor restoration, ROS reduction, SOD restoration, Catalase reduction properties of CeO2NPs. Moreover, CeO2 NPs have been reported to maintain endothelium homeostasis by attenuating endothelial oxidative injury induced by H2O2 via exerting antioxidant, antiapoptotic and anti-inflammatory effects on neuronal cells (57), thereby decreasing ESM-1, PCT, and D-dimer secretion which are upregulated by proinflammatory mediators including (IL)-1β, (TNFα), and IFNγ (58).

According to our findings, the administration of CeO2 nanoparticles (CeO2 NPs) led to a significant decrease in brain proinflammatory cytokines such as TNF-α, IL-6, NF-kB, as well as LTB4. These results are in line with a study by Selvaraj et al. (54), which demonstrated that CeO2 NPs treatment reduced the secretion of TNF-α, IL-6, IL-1β, and HMGB1 induced by LPS (lipopolysaccharide). Carzasta et al. (58) reported that TNF-α is responsible for LTB4 production and release from endothelial cells. So, its depletion by CeO2 NPs treatment participates in the suppression of LTB4 level. Furthermore, CeO2 nanoparticles (CeO2 NPs) were found to reduce LPS-induced iNOS (inducible nitric oxide synthase) in cultured macrophages. The mechanism underlying cytokine and NO production regulation in macrophages during sepsis is believed to involve increased intracellular levels of reactive oxygen species (ROS) (59). CeO2 NPs treatment showed a tendency to decrease cellular ROS induction in macrophage cells and mitigate mitochondrial membrane potential damage caused by LPS-induced sepsis (54). Inflammatory gene expression is primarily regulated by NF-kB and MAPK (60). The transcription of NF-kB is controlled by the phosphorylation and subsequent degradation of IkB-α (61). CeO2 NPs treatment demonstrated inhibition of LPS-induced IkB-α degradation, leading to reduced translocation of NF-kB and diminished binding of NF-kB to DNA, thereby attenuating NF-kB transcriptional activation (54).

Our results demonstrated that CeO2 NPs treatment resulted in significant abrogation of brain apoptotic markers (Cas-3 and Bax) in septic rats. The findings presented align with previous research conducted by Kyosseva et al. (62), which similarly demonstrated that CeO2 nanoparticle administration reduced caspase-3 cleavage and the Bax/Bcl-2 ratio in septic rats. Furthermore, the nanoparticle treatment exhibited the ability to protect the liver from apoptosis triggered by sepsis. The anti-apoptotic effects of CeO2 NPs could be attributed to alleviating ROs generation and oxidative stress-induced mitochondrial damage (63), increasing ATP/ADP ratio, elevating mitochondrial membrane permeability, blocking calcium channel associated with mitochondrial depolarization which plays a crucial role in cell apoptosis (64).

MiRNAs are crucial in the emergence and progression of numerous neurological diseases. Treating CLP-septic rats with CeO2NPs resulted in significant downregulation in miR- 155 associated with upregulation in miR -124 and miR-146a expression. Zingale et al.(65) reported that uncontrolled neuroinflammation induces excessive glial cell activation which produces proinflammatory cytokines that simultaneously stimulate NF-κB /TLR-dependent biogenesis of miR-155 (66). CeO2 NPs have been reported to attenuate I_kB-α degradation and NF-kB/p65 trans-location from the cytoplasm to the nucleus hence reducing NF-kB transcriptional activation and interfering with NF-κB /TLR-dependent biogenesis of miR-155 (54). While the increase in miR124 expression negatively regulates multiple components of the TLR signaling, including TLR6, MyD88, and TNF-α, indicating an underlying negative feedback loop between miR-124 and TLRs signalling to mitigate excessive inflammation (67). Also, miR146-a expression was increased. The upregulation of miR-146-a induces negative feedback of NF-κB signaling and directly targets TLRs and their downstream effectors, IRAK1 and TRAF6 (68). Thus preventing inflammatory cell infiltration and cytokine production (69).

5. Conclusions

The current study elucidates the potential therapeutic mechanism of Cerium oxide nanoparticles against CLP sepsis-induced brain injury. These positive effects were mediated through enhancing animal survival, suppressing serum septic biomarkers, inhibiting brain inflammatory

mediators, curbing brain apoptotic markers and modulating brain miRNA expression. These findings suggest that CeO2 NPs could become a promising agent in ameliorating sepsis-induced brain insult.

Conflict of interest
The authors have has no conflicts with any step of the article preparation.

Consent for publication
The authors have read and approved the final manuscript for publication.

Ethics approval and consent to participate
Animals were used in the present research. (Ethical approval No. sci1432307001).

Availability of data and material
The data that support the findings of this study are available from the corresponding author upon reasonable request

Authors' contributions
Abdulaal and Helmi designed the experiment , Salem conducted the experiment , Abdulaal and Helmi analysed the data , Salem and Hamza wrote the manuscript and all the authors revised the manuscript and approved it

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