

ORIGINAL RESEARCH



Purpurogallin ameliorates sevoflurane-induced cognitive impairment and hippocampal neuroinflammation in neonatal mice

Yu Yao¹, Zhihong Li², Jingqi Zhang³, Jinzhao Wang^{4,*}, Baohua Wang¹, Fengjuan Wang¹

¹Department of Anesthesiology, Affiliated Hospital of Changchun University of Chinese Medicine, 130021 Changchun, Jilin, China

²Obstetrics and Gynecology Diagnosis and Treatment Center, Affiliated Hospital of Changchun University of Chinese Medicine, 130021 Changchun, Jilin, China

³Department of Massage, Affiliated Hospital of Changchun University of Chinese Medicine, 130021 Changchun, Jilin, China

⁴Department of Anesthesiology, Changchun Obstetrics-Gynecology Hospital, 130000 Changchun, Jilin, China

***Correspondence**

wjzh27@126.com
(Jinzhao Wang)

Abstract

Postoperative cognitive dysfunction contributes to memory impairment, which is involved in neurotoxicity, endoplasmic reticulum stress, calcium overload and hippocampal neuron apoptosis. Purpurogallin can mitigate ischemia-induced neuronal injury and microglial inflammation. However, whether purpurogallin affects cognitive dysfunction remains unclear. To investigate the efficacy of purpurogallin on cognitive dysfunction and explore the potential signal pathways, Sevoflurane (SEV)-induced model was established on mice, and water maze testing was performed to record escape latency, platform residence time, platform crossings and swimming speed analysis. The concentration of Interleukin (IL)-6, IL-1 β and Tumor necrosis factor (TNF)- α in hippocampus tissue were evaluated using Enzyme-linked immunosorbent assay (ELISA). The hippocampal neurons were analyzed using Nissl staining. The Iba-1 was evaluated using immunofluorescent assay (IFA), while the cell apoptosis was assessed using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. Western blots were performed to evaluate the expression of Ionized calcium binding adaptor molecule 1 (Iba1), integrin α -M (CD11b), Bax, integrin α -M (Bcl-2), p-65 and I κ B α , as well as the phosphorylation of p-65 and I κ B α . Purpurogallin at dose of 150 mg/kg significantly reduced escape latency in SEV-induced mice ($p < 0.001$), without affecting swimming speed. It increased time spent in the target quadrant and restored platform crossings reduced by SEV. Purpurogallin also mitigated the elevated IL-6, IL-1 β and Transforming growth factor alpha (TGF- α) levels caused by SEV in the hippocampus, preserved Nissl bodies, and reduced Iba-1 and CD11b expression ($p < 0.01$). It alleviated cell apoptosis by downregulating Bax and upregulating Bcl-2, while inhibiting p-65 phosphorylation and promoting I κ B α phosphorylation induced by SEV ($p < 0.05$). Purpurogallin effectively countered SEV-induced cognitive impairment, neuroinflammation, hippocampal injury, microglial activation and neuronal apoptosis through inhibiting nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) pathway, which facilitate purpurogallin to become a drug candidate for postoperative cognitive dysfunction.

Keywords

Cognitive dysfunction; Microglial activation; Neuronal apoptosis; NF- κ B pathway; Neuroinflammation; Purpurogallin

1. Introduction

Postoperative cognitive dysfunction (POCD) is marked by memory decline, abstract thinking impairment, disorientation, and reduced social engagement after surgery with anesthesia. It is influenced by factors like age, anesthesia type (notably, sevoflurane's potential neurotoxicity), surgery-related variables, and postoperative infection [1]. Currently, sevoflurane stands as a widely employed inhalational anesthetic in clinical practice [2]. However, it is associated with potential

harm to the maturation of the central nervous system and the function of learning and memory [3]. Excessive inhalation of sevoflurane exacerbates endoplasmic reticulum stress and calcium overload, accelerating the apoptosis of hippocampal neurons, ultimately intensifying cognitive dysfunction in mice [4].

Purpurogallin is a naturally occurring benzotropolone compound found in the nut galls of the *Quercus* genus. It has been demonstrated the ability to inhibit various enzymes, including glutathione S-transferase, xanthine oxidase, and catechol O-

methyltransferase. Furthermore, purpurogallin exhibits peroxyl radical scavenging activity, serving as a protective agent for ventricular myocytes, aortic endothelial cells, and red blood cells against peroxyl free radicals [5]. In the realm of neuroprotection, purpurogallin hinders the nuclear translocation of the p-65 subunit of nuclear factor- κ B (NF- κ B), effectively dampening the pro-inflammatory pathways activated in microglia [6]. Additionally, purpurogallin mitigates ischemia-induced neuronal injury and microglial inflammation by modulating the micro RNA (miR)-124-3p/Tumor necrosis factor receptor 6 (TNFR6)/NF- κ B pathway [7].

NF- κ B, a pivotal transcription factor, typically resides in an inactive state within the cytoplasm [8]. Activation occurs when its inhibitor, I- κ B, undergoes phosphorylation or ubiquitination, leading to NF- κ B's release into the nucleus. This transcription factor is implicated in inflammatory responses, neural differentiation, proliferation, and cognitive functions [9]. NF- κ B signaling pathway has been reported involving in inflammation, microglial activation and nervous system damage [3].

However, the precise role and mechanisms of purpurogallin in sevoflurane-induced cognitive dysfunction remain unclear. In this work, SEV-intoxicated mice were treated with purpurogallin, which served to the findings that purpurogallin alleviates SEV-intoxicated cognitive impairment, neuroinflammation, hippocampal injury, microglial activation and neuronal apoptosis through inhibiting NF- κ B pathway.

2. Materials and methods

2.1 Animal housing and administration

C57BL/6 mice were obtained from Shanghai Laboratory Animal Center (Shanghai, China), and raised in specific pathogens-free space provided with adequate food, drink and 12-h light/dark cycle. The young mice were divided into 4 groups, including control group, purpurogallin group (150 mg/kg PPG), sevoflurane group (SEV) and sevoflurane plus purpurogallin group (SEV + 150 mg/kg PPV), with 6 mice in each group. For intraperitoneally induction, the pups on postnatal 6, 9, 12 days were anesthetized with 3% sevoflurane (SEV) plus 40% oxygen for 2 h [10]. The group without sevoflurane induction were also provided with 40% oxygen for 2 h. During sevoflurane anesthesia, the chamber temperature for mice was kept in 37 °C, and the concentrations of sevoflurane and oxygen were monitored continuously using a gas analyzer. Subsequently, purpurogallin administration was performed *via* injecting intraperitoneally at the dose of 150 mg/kg [11]. The mice on postnatal 31 days were used for water maze trial, and then euthanized by cervical dislocation for dissecting hippocampus tissues as described previously [12].

2.2 Water maze testing

In water maze trials, the postnatal 31 days mice were navigated an opaque tank with a platform just below the water's surface, adding non-fat dry milk for opacity. Room lighting was kept indirect and water temperature remained consistent at 20–22 °C. Spatial acquisition training and testing sessions were

administered to mice four times over the initial four days. Mice were placed in a fixed position in the pool and given 60 seconds to locate the hidden platform. Escape latency and swimming speed were recorded and calculated through motion detection software. Subsequently, the platform was removed, and probe testing occurred on the fifth day. The number of platform crossings and the total time spent in the quadrant (original target platform) were recorded to evaluate learning and memory function during the 60-second session. All tests were carried out by a blinded operator. The data was recorded for escape latency, platform residence time, platform crossings, and swimming speed analysis.

2.3 Enzyme-linked immunosorbent assay (ELISA)

The concentration of IL-6, IL-1 β and TNF- α in hippocampus tissue were evaluated using ELISA kits including IL-6 (ab222503, Abcam, Cambridge, UK), IL-1 β (ab100705, Abcam, Cambridge, UK) and TNF- α (ab208348, Abcam, Cambridge, UK). In brief, the hippocampus tissue lysates were homogenized in cell lysate buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 10% Glycerol, 0.5% NP-40). 100 μ L lysate was added into ELISA well and incubated for 2 h followed by 100 μ L biotinylated antibody for 1 h. Then each well was reacted with streptavidin solution, 3,3',5,5'-Tetramethylbenzidine (TMB) development solution, and terminated by stop solution. The absorbance value was read at 450 nm.

2.4 Nissl staining

The hippocampus tissues of mice were air-dried, dehydrated in 75% ethanol, and then immersed in Nissl staining solution (n21479; Thermo Fisher, Waltham, MA, USA). Afterward, the glass slide was rinsed gently with tap water, made transparent with xylene, and sealed with neutral gum. The sections were observed under the microscope.

2.5 Immunofluorescent assay (IFA)

The hippocampus tissues of mice were fixed with 4% paraformaldehyde for 15 min and permeabilized with 0.1% Triton X-100 for 15 min at room temperature. The cells were stained with primary antibody against Iba-1 (17198, Cell Signaling Technology, Danvers, MA, USA; 1:100) for 1 h followed by incubation with Alexa Fluor™ 488 conjugated goat anti-rabbit IgG (A-11008, Thermo Fisher; 1:1000) for 45 min at room temperature. The cells were incubated with 4',6-diamidino-2-phenylindole (DAPI; D9524, Millipore Sigma, St. Louis, MO, USA) for 5min. After washes twice, the stained cells were observed under the fluorescence microscope (T100, Nikon, Tokyo, Japan), the captured images were analyzed using ImageJ software (National Institute of Health, ImageJ 1.54, Bethesda, MD, USA).

2.6 Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay

The hippocampus of mice was analyzed using TUNEL Assay Kit (ab66110, Abcam, Cambridge, MA, USA). Briefly, the tis-

sues were fixed with formaldehyde, incubated with proteinase K solution for 5 min at room temperature and refixed with formaldehyde. Then the samples were incubated in DNA labeling solution for 60 min at 37 °C followed by incubation with antibody solution for 30 min at room temperature. Finally, the RNase A solution was used to incubate samples for 30 min, and the signal was analyzed using fluorescent microscopy.

2.7 Western blot assay

The total protein was extracted using lysis buffer (25 mM Tris-HCl pH 7.4, 250 mM NaCl, 50 mM KCl, 10% Glycerol, 0.5% NP-40). The lysates were subjected to gel separation and transferred to Nitrocellulose membrane followed by overnight incubation at 4 °C with the primary antibodies listed in Table 1. After incubation with horseradish peroxidase-conjugated goat anti-rabbit IgG at room temperature for 1 h and washing for three times with TBST buffer, the target bands were visualized with Electrochemiluminescence (ECL) reagents (PE0010, Solarbio, Beijing, China). The relative intensity of each band was measured using ImageJ software and normalized to β -actin [13].

2.8 Quantification and statistical analysis

The data is portrayed as the mean \pm standard deviation (SD) derived from three biological replicates. To ascertain variances between pairs of groups, unpaired *t*-tests were conducted, while comparisons involving multiple groups were evaluated through a two-way analysis of variance (ANOVA) using GraphPad Prism 8.0 software (Dotmatics, Boston, MA, USA).

3. Results

3.1 Purpurogallin improves SEV-induced cognitive dysfunction

To investigate the effect of purpurogallin on cognitive dysfunction, water maze trials were performed to analyze the activities of SEV-intoxicated mice. The action trajectories of mice in different groups were recorded and analyzed (Fig. 1A). The findings indicated that while purpurogallin did not affect cognitive impairment in control mice, it significantly reduced escape latency in SEV-induced mice (Fig. 1B). Swimming speed remained unaffected by both purpurogallin treatment and SEV induction (Fig. 1C). Furthermore, purpurogallin treatment increased the time spent in the target quadrant (Fig. 1D) and restored the decreased number of platform crossings induced by SEV exposure (Fig. 1E). A novel objective recognition test was conducted 24 hours after SEV, both with and without purpurogallin treatment. The overall distance traveled showed no significant difference (Fig. 1F), indicating that SEV did not induce motor function impairment. However, the recognition index was observed to decrease in the SEV group compared to the Control group. Remarkably, purpurogallin treatment rescued the decreased recognition index following SEV treatment (Fig. 1G). Collectively, these results suggested that purpurogallin has the potential to improve SEV-induced cognitive dysfunction.

3.2 Purpurogallin ameliorates SEV-induced neuroinflammation and hippocampal injury

Purpurogallin's modulation of neuroinflammation has been investigated. Initially, ELISA kits were employed to assess the concentrations of IL-6, IL-1 β and TGF- α in mouse hippocampal tissue. The findings indicated that purpurogallin treatment did not exert any notable impact on IL-6, IL-1 β and TGF- α compared to the mice in control group. Notably, SEV intoxication significantly elevated the concentrations of IL-6, IL-1 β and TGF- α in the hippocampus, which were alleviated by purpurogallin treatment at the dose of 150 mg/kg (Fig. 2A). Additionally, hippocampal injury was also evaluated using Nissl staining. In comparison to the control group, the hippocampal neurons exhibited significant damage following SEV treatment, evidenced by a reduction in Nissl bodies and disordered arrangement of granule cell layer and pyknotic neurons. However, the administration of purpurogallin effectively mitigated the neuronal damage caused by SEV, leading to an increase in the number of Nissl bodies (Fig. 2B). Hence, purpurogallin demonstrates its potential in alleviating SEV-induced neuroinflammation and hippocampal injury.

3.3 Purpurogallin attenuates SEV-induced microglial activation

To investigate the effect of purpurogallin on microglial activation, microglia/macrophage-specific calcium-binding protein Iba-1 was evaluated in SEV-induced mouse. Compared to control group, purpurogallin treatment did not result in a significant change in the levels of Iba-I expression in hippocampal tissue. However, SEV intoxication led to an elevated expression of Iba-I, which was notably reduced upon administration of purpurogallin at a dose of 150 mg/kg (Fig. 3A). Similar findings were observed in western blot assays, in which purpurogallin exhibited no significant effect on Iba-I levels compared to control group, but effectively decreased Iba-I expression in SEV-intoxicated mice (Fig. 3B). Furthermore, western blots data revealed that purpurogallin also reduced CD11b production in SEV-intoxicated mice (Fig. 3C). Consequently, purpurogallin demonstrates an attenuating effect on SEV-induced microglial activation.

3.4 Purpurogallin attenuates SEV-induced neuronal apoptosis

The effect of purpurogallin on neuronal apoptosis has been investigated using TUNEL assay, which demonstrated that cell apoptosis was enhanced by SEV intoxication, which was alleviated by treatment with 150 mg/kg purpurogallin (Fig. 4A). Additionally, two apoptosis related markers Bax and Bcl-2 were assessed using western blots. The data suggested that Bax was upregulated by SEV intoxication, the expression of which was attenuated by purpurogallin; Correspondingly, purpurogallin increased Bcl2 production compared to SEV-intoxicated mice (Fig. 4B). These data collectively support the conclusion that purpurogallin alleviates SEV-intoxicated neuronal apoptosis.

TABLE 1. Antibodies information.

Protein	Cat. No.	Manufacturer	Dilution
Iba-1	17198	Cell Signaling Technology, Danver, MA, USA	1:1000
CD11b	93169	Cell Signaling Technology, Danver, MA, USA	1:1000
Bax	50599-2-Ig	ProteinTech, Rosemont, IL, USA	1:3000
Bcl-2	26593-1-AP	ProteinTech, Rosemont, IL, USA	1:2000
p-p65	3033	Cell Signaling Technology, Danver, MA, USA	1:1000
p-65	8242	Cell Signaling Technology, Danver, MA, USA	1:1000
p-I κ B α	2859	Cell Signaling Technology, Danver, MA, USA	1:1000
I κ B α	4812	Cell Signaling Technology, Danver, MA, USA	1:1000
β -actin	20536-1-AP	ProteinTech, Rosemont, IL, USA	1:5000

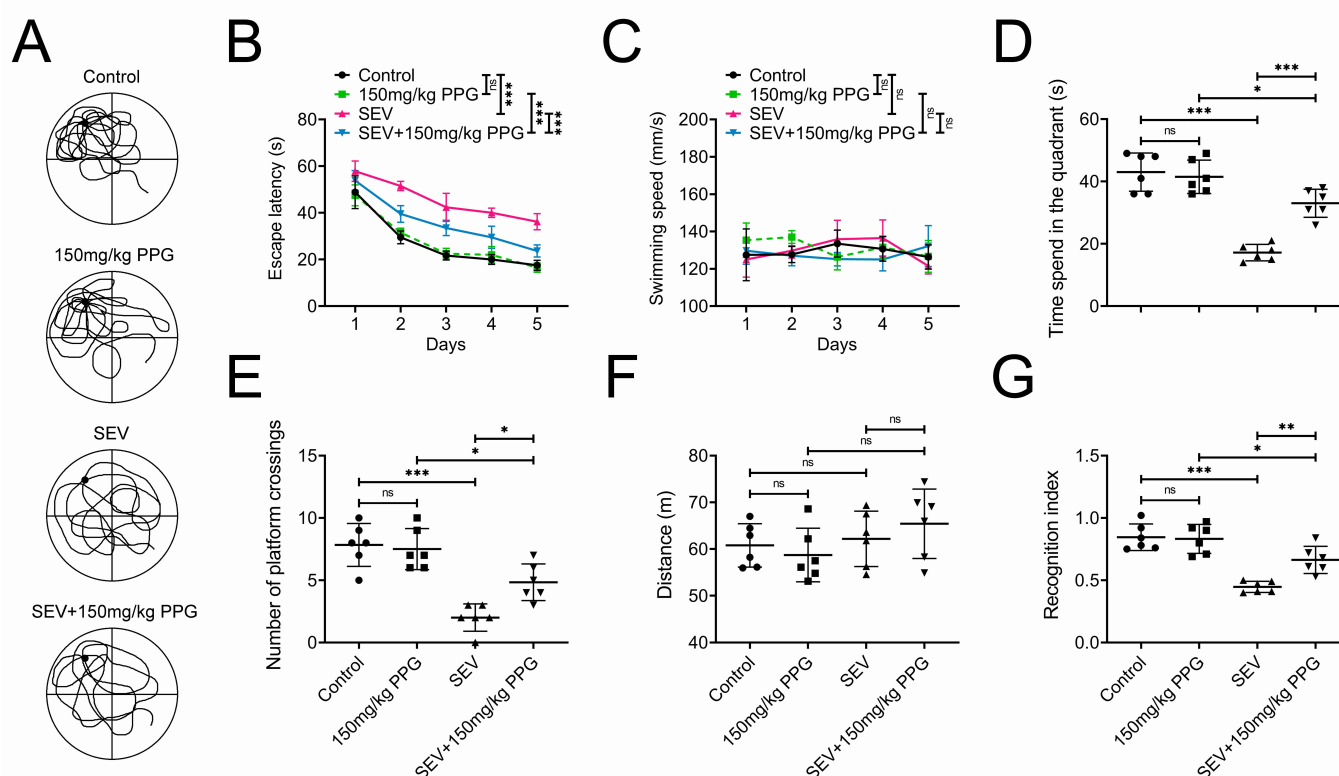


FIGURE 1. Purpurogallin improves SEV-induced cognitive dysfunction. (A) The action trajectories of mice. (B) The escape latency of mice. (C) Swimming speed analysis of mice. (D) The time spent in the target quadrant. (E) The number of platform crossings. (F) The overall distance traveled in a novel objective recognition test. (G) The decreased recognition index following SEV induction was rescued by purpurogallin treatment. The data from three repeated experiments were used for the statistical analysis. Error bar, mean \pm SD; ns, non-significant; * p < 0.05; ** p < 0.01; *** p < 0.001. SEV, Sevoflurane; PPG, Purpurogallin.

3.5 Purpurogallin inhibits the NF- κ B pathway

NF- κ B pathway was reported to be involved in purpurogallin's function, in this work, the modulation of NF- κ B pathway was studied. Western blots were performed to evaluate the protein expression and phosphorylation of p-65 and I κ B α . Notably, neither SEV-intoxication nor purpurogallin treatment alone had a significant impact on p-65 protein levels (Fig. 5A).

However, p-65 phosphorylation was increased by SEV intoxication, and this effect was attenuated by purpurogallin treatment (Fig. 5B). In the case of I κ B α , its phosphorylation was promoted by SEV intoxication, which was declined by purpurogallin treatment (Fig. 5C). Furthermore, I κ B α expression was downregulated by SEV intoxication but increased by purpurogallin (Fig. 5D). In summary, purpurogallin plays a role in inhibiting NF- κ B pathway in SEV intoxicated mice.

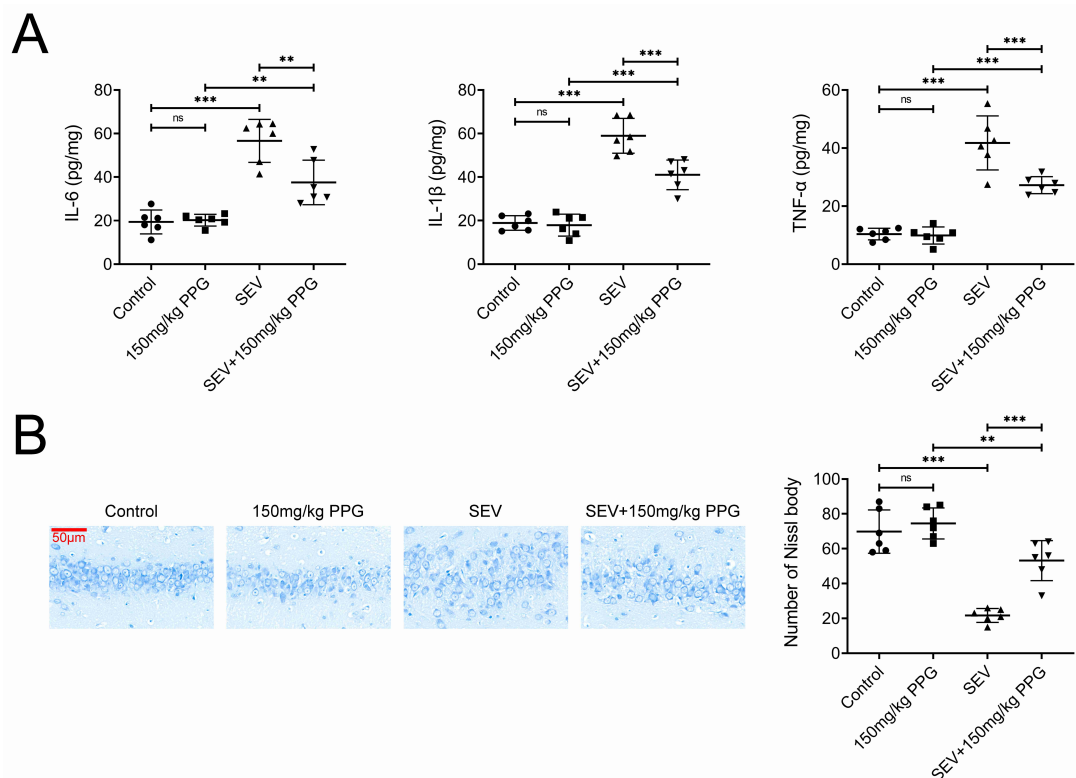


FIGURE 2. Purpurogallin ameliorates SEV-induced neuroinflammation and hippocampal injury. (A) ELISA analysis of the concentrations of IL-6, IL-1 β and TNF- α in mouse hippocampal tissues. (B) Nissl staining of hippocampal tissues. All the experiments were repeated at least three times. Error bar, mean \pm SD; ns, non-significant; ** p < 0.01; *** p < 0.001. SEV, Sevoflurane; PPG, Purpurogallin; IL-6, Interleukin 6; IL-1 β , Interleukin 1 β ; TNF- α , tumor necrosis factor α .

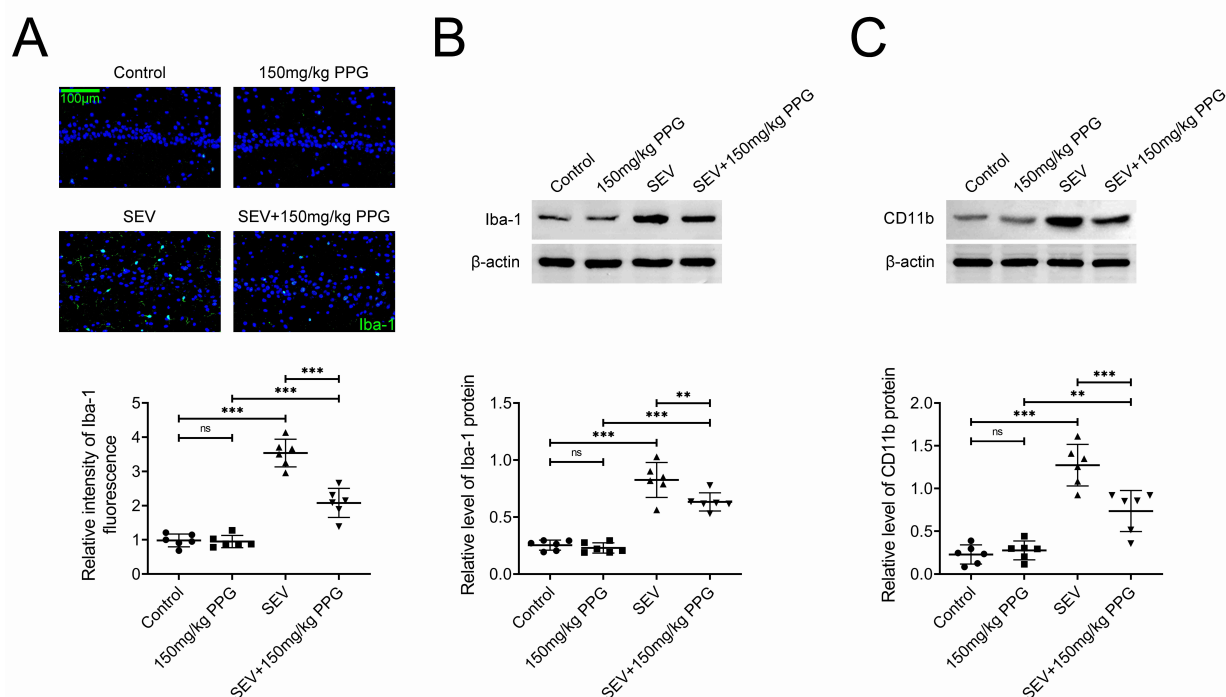


FIGURE 3. Purpurogallin attenuates SEV-induced microglial activation. (A) IFA analysis of Iba-1 in hippocampal tissues. (B) Western blot assays of Iba-1 expression in hippocampal tissues. (C) Western blot analysis of CD11b production in SEV intoxicated mice. Three repeated experiments were used for analysis. Error bar, mean \pm SD; ns, non-significant; ** p < 0.01; *** p < 0.001. SEV, Sevoflurane; PPG, Purpurogallin; Iba1, Ionized calcium binding adaptor molecule 1; CD11b, Integrin α M.

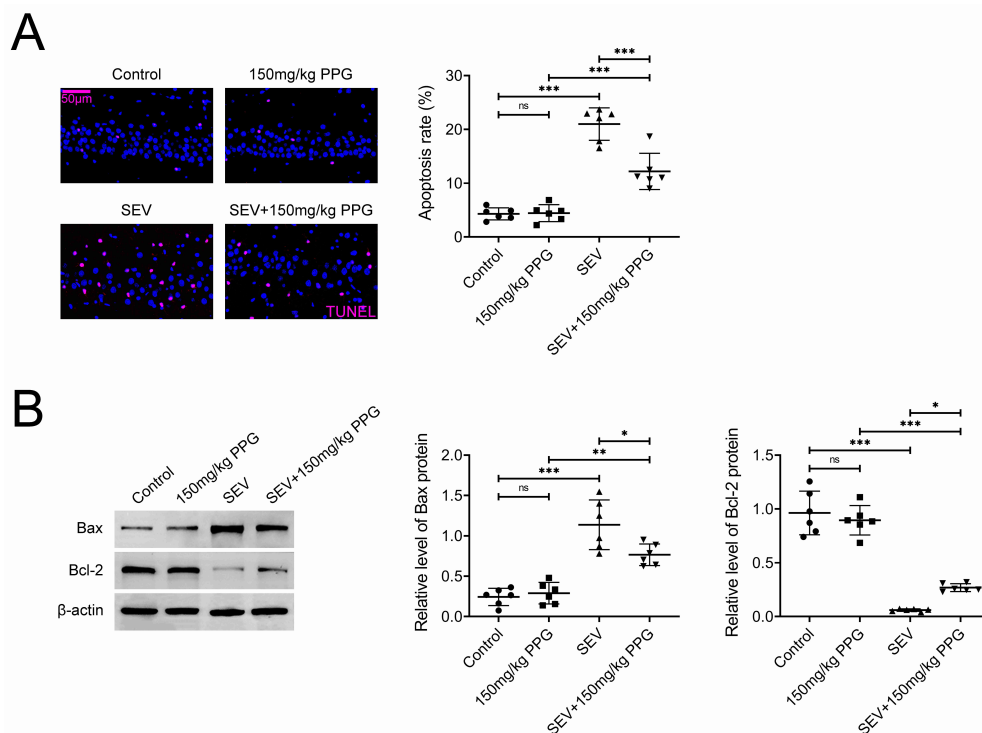


FIGURE 4. Purpurogallin attenuates SEV-induced neuronal apoptosis. (A) TUNEL analysis of effect of purpurogallin on neuronal apoptosis. (B) Western blot analysis of the modulation of purpurogallin on Bax and Bcl-2 expression. The data from three independent replications were used for the analysis. Error bar, mean \pm SD; ns, non-significant; * p < 0.05; ** p < 0.01; *** p < 0.001. SEV, Sevoflurane; PPG, Purpurogallin; Bcl-2, B-cell lymphoma 2.

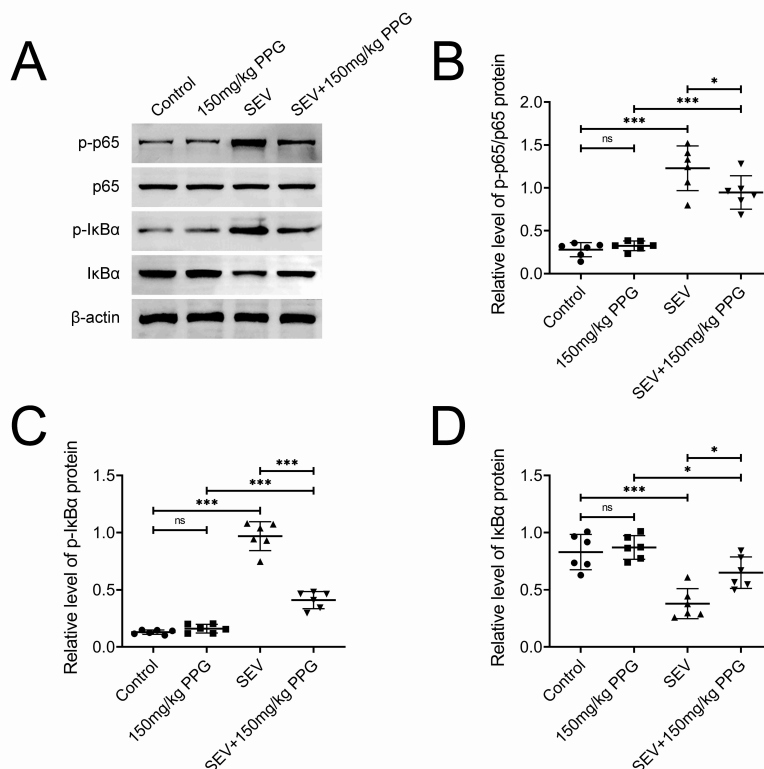


FIGURE 5. Purpurogallin inhibits the NF- κ B pathway. (A) Western blot analysis of the protein expression and phosphorylation of p-65 and I κ B α in hippocampal tissues of mice with SEV induction or purpurogallin treatment. (B) Relative level of phosphorylated p-65 (p-p65) to p-65. (C) Relative level of phosphorylated I κ B α (p-I κ B α). (D) Relative expression level of I κ B α protein. The data from three independent replications were used for the analysis. Error bar, mean \pm SD; ns, non-significant; * p < 0.05; *** p < 0.001. SEV, Sevoflurane; PPG, Purpurogallin.

4. Discussion

Cognitive dysfunction always contributes to memory impairment, thinking disorders, disorientation, and a reduced capacity to engage in social activities. The etiology of cognitive dysfunction involves central nervous system, endocrine system, and immune system dysfunction, influenced by factors including age and anesthesia choice, notably sevoflurane, which can induce neurotoxicity, endoplasmic reticulum stress, calcium overload, and hippocampal neuron apoptosis, exacerbating cognitive dysfunction [4]. Purpurogallin has demonstrated ability to mitigate ischemia-induced neuronal injury and microglial inflammation [7]. However, it remains to be clarified whether purpurogallin affects cognitive dysfunction. In the present study, SEV-induced model was established on mice, and the data illustrated that purpurogallin improves SEV-induced cognitive dysfunction. Previous reports indicated that purpurogallin inhibits the nuclear translocation of the p-65 subunit of NF- κ B, thereby attenuating the pro-inflammatory pathways activated in microglia [6]. Additionally, purpurogallin alleviates ischemia-induced neuronal injury and microglial inflammation [7]. Therefore, purpurogallin plays a key role in neuronal protection.

Inflammation is a natural response to injury, infection or harmful stimuli, involving immune cells and molecules that help remove damage, initiate repairs, and protect against threats. Neuroinflammation is this process in the central nervous system, including immune cells like microglia and astrocytes [14]. However, chronic or dysregulated neuroinflammation contributes to neurological disorders, including neurodegenerative diseases, multiple sclerosis, brain injuries, infections, and neuropsychiatric disorders. Research aims to regulate neuroinflammation to balance its protective and harmful effects. Purpurogallin exhibits neuroprotective effects against cerebral ischemia/reperfusion (I/R) injury by reducing ER stress and neuroinflammation through inhibition of the Toll-like receptor 4 (TLR4)/NF- κ B pathway, suggesting its potential as a therapeutic candidate for treating cerebral I/R injury [11]. Purpurogallin mitigates ischemia-induced neuronal damage and microglial inflammation by promoting M2 polarization of microglia, reducing neuronal apoptosis, enhancing neurological functions, and suppressing the miR-124-3p/TNFR6/NF- κ B pathway [7].

Microglial activation is a significant process in the central nervous system, as microglia plays a key role in immune responses and neuroinflammation. When activated, microglia can release inflammatory molecules and contribute to neuronal damage, making the inhibition of their activation an attractive target for potential therapies in various neurological conditions [15]. In this work, purpurogallin has been proved to have neuroprotective or anti-inflammatory properties by preventing the overstimulation of microglia induced by SEV. This inhibition of microglial activation could potentially be beneficial in conditions where excessive microglial activation is detrimental to brain health, such as in neurodegenerative diseases or brain injuries [16]. Thus, purpurogallin can counteract the activation of microglial cells triggered by SEV, which could be an abbreviation for a specific stimulant or condition. Further research and clinical studies would be

needed to fully understand the therapeutic potential and safety of purpurogallin for these purposes.

Recently, several neuroprotective agents have been identified. Melatonin has shown efficacy as a postoperative anti-anxiety medication, while dexmedetomidine has demonstrated usefulness in alleviating postoperative pain [17]. Metformin exerts neuroprotective effects in neurodegenerative conditions associated with diabetes mellitus by inhibiting inflammation [18]. Isoviteixin improves cognitive dysfunction by inhibiting brain apoptosis and promoting autophagy targeting PGC-1 α /FNDC5 pathway [19]. Purpurogallin exhibits antioxidant, anticancer and anti-inflammatory effects. It protects human keratinocytes from severe damage and apoptosis induced by ultraviolet radiation and particulate matter 2.5 micrometers (PM_{2.5}), modulating pro-apoptotic and anti-apoptotic proteins *via* caspase and Mitogen-activated protein kinase (MAPK) signaling pathways [20]. One study on the neuroprotective effects of purpurogallin against cerebral ischemia/reperfusion injury revealed that purpurogallin treatment reduces endoplasmic reticulum (ER) stress and inflammation, improves cell viability, and lessens apoptosis [11]. Purpurogallin alleviates ischemia-induced neuronal apoptosis *via* promoting M2 polarization of microglia, enhancing neurological functions, and suppressing the miR-124-3p/TNFR6/NF- κ B pathway [7]. Purpurogallin prevents or reduces the programmed cell death (apoptosis) of neurons. This finding could have significant implications in the field of neuroprotection and neurodegenerative diseases, as preventing neuronal apoptosis is a critical factor in preserving brain function and preventing neurodegeneration [21]. However, it's essential to conduct further research and studies to understand the underlying mechanisms and to assess the potential therapeutic applications and safety of purpurogallin for neuronal protection.

NF- κ B resides in an inactive state in the cytoplasm. When I- κ B undergoes phosphorylation or ubiquitination, NF- κ B is released into the nucleus to regulate inflammatory responses, neural differentiation, proliferation, and cognitive functions [9]. As is reported that purpurogallin demonstrated significant inhibition of lipopolysaccharide (LPS)-induced pro-inflammatory mediators and cytokines in microglia cells by downregulating nitric oxide (NO), Prostaglandin E₂ (PGE₂), Inducible nitric oxide synthase (iNOS), Cyclooxygenase-2 (COX-2), IL-1 β and TNF- α production, suppressing NF- κ B signaling, and attenuating phosphoinositide 3-kinases (PI3K)/protein kinase B (Akt) and MAPK pathways, indicating its neuroprotective potential [6, 22]. In this work, purpurogallin was found to repress the phosphorylation of p-65 and I κ B α , illustrating its role in inhibiting NF- κ B pathway, which is consistent with previous reports.

5. Conclusions

Purpurogallin effectively countered SEV-induced cognitive impairment, neuroinflammation, hippocampal injury, microglial activation and neuronal apoptosis through inhibiting NF- κ B pathway. Purpurogallin showed potential to be a drug candidate for postoperative cognitive dysfunction.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article. The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

YY and ZHL—designed the study, completed the experiment and supervised the data collection; JQZ—analyzed the data, interpreted the data; JZW, BHW and FJW—prepare the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Animal experiments were approved by the Committee of Experimental Animals of Changchun University of Chinese Medicine (Approval No. 2023211). All experiments were performed according to the guidelines and rules of Institutional Animal Care and Use Committee.

ACKNOWLEDGMENT

Not applicable.

FUNDING

This research received no external funding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- [1] Yang Y, Liu Y, Zhu J, Song S, Huang Y, Zhang W, *et al.* Neuroinflammation-mediated mitochondrial dysregulation involved in postoperative cognitive dysfunction. *Free Radical Biology and Medicine*. 2022; 178: 134–146.
- [2] Zhao G, Jing J. *HOMER1A* restores sevoflurane-induced cognitive dysfunction by regulating microglia's activation through activating the AMPK/TXNIP axis. *Signa Vitae*. 2023; 19: 202–212.
- [3] Wang P, Wang P, Yin M, Wang S. Sevoflurane ameliorates LPS-induced inflammatory injury of HK-2 cells through Sirtuin1/NF- κ B pathway. *Allergologia et Immunopathologia*. 2022; 50: 115–123.
- [4] Zhang Q, Li Y, Yu J, Yin C, Guo J, Zhao J, *et al.* TLR3 deletion inhibits programmed necrosis of brain cells in neonatal mice with sevoflurane-induced cognitive dysfunction. *Aging*. 2022; 14: 4714–4727.
- [5] Kim K, Kim TH, Ihn HJ, Kim JE, Choi JY, Shin HI, *et al.* Inhibitory effect of purpurogallin on osteoclast differentiation *in vitro* through the downregulation of c-Fos and NFATc1. *International Journal of Molecular Sciences*. 2018; 19: 601.
- [6] Park HY, Kim TH, Kim CG, Kim GY, Kim CM, Kim ND, *et al.* Purpurogallin exerts anti-inflammatory effects in lipopolysaccharide-stimulated BV2 microglial cells through the inactivation of the NF- κ B and MAPK signaling pathways. *International Journal of Molecular Medicine*. 2013; 32: 1171–1178.
- [7] Cheng Z, Li X, Ye X, Yu R, Deng Y. Purpurogallin reverses neuronal apoptosis and enhances “M2” polarization of microglia under ischemia *via* mediating the miR-124-3p/TRAF6/NF- κ B Axis. *Neurochemical Research*. 2023; 48: 375–392.
- [8] Liu L, Huan L, Zhang Y, Wei W, Chen Z, Xu D, *et al.* Ubiquitin-specific protease 8 inhibits lipopolysaccharide-triggered pyroptosis of human bronchial epithelial cells by regulating PI3K/AKT and NF- κ B pathways. *Allergologia et Immunopathologia*. 2022; 50: 96–103.
- [9] Zhao W, Song S, Chu W, Li Y, Chen S, Ji Y, *et al.* Disruption of hippocampal P2RX2/CaMKII/NF- κ B signaling contributes to learning and memory impairment in C57BL/6 mice induced by surgery plus anesthesia in neonatal period. *Biomedicine & Pharmacotherapy*. 2022; 149: 112897.
- [10] Sun M, Dong Y, Li M, Zhang Y, Liang F, Zhang J, *et al.* Dexmedetomidine and clonidine attenuate sevoflurane-induced tau phosphorylation and cognitive impairment in young mice *via* α -2 adrenergic receptor. *Anesthesia and Analgesia*. 2021; 132: 878–889.
- [11] Li X, Cheng Z, Chen X, Yang D, Li H, Deng Y. Purpurogallin improves neurological functions of cerebral ischemia and reperfusion mice by inhibiting endoplasmic reticulum stress and neuroinflammation. *International Immunopharmacology*. 2022; 111: 109057.
- [12] Jaszczyk A, Stankiewicz AM, Juszczak GR. Dissection of mouse hippocampus with its dorsal, intermediate and ventral subdivisions combined with molecular validation. *Brain Sciences*. 2022; 12: 799.
- [13] Huang J, Dai W, Xiao D, Xiong Q, Liu C, Hu J, *et al.* Acetylation-dependent SAGA complex dimerization promotes nucleosome acetylation and gene transcription. *Nature Structural & Molecular Biology*. 2022; 29: 261–273.
- [14] Liu X, Nasab EM, Athari SS. Anti-inflammatory effect of N-(trifluoromethylphenyl)-2-cyano-3-hydroxy-crotonic acid amide and gluconic acid on allergic rhinitis and asthma controlling. *Allergologia et Immunopathologia*. 2022; 50: 71–75.
- [15] Chen J, Zheng ZV, Lu G, Chan WY, Zhang Y, Wong GKC. Microglia activation, classification and microglia-mediated neuroinflammatory modulators in subarachnoid hemorrhage. *Neural Regeneration Research*. 2022; 17: 1404–1411.
- [16] Lu J, Liu Z, He W, Shao S. Knockdown of TNFAIP1 mitigates sevoflurane-induced cognitive dysfunction by activating CREB/Nrf2 pathway. *Tropical Journal of Pharmaceutical Research*. 2022; 21: 1419–1424.
- [17] Jouybar R, Kazemifar S, Asmari N, Karami A, Khademi S. Comparison of the effect of melatonin, dexmedetomidine, and gabapentin on reduction of postoperative pain and anxiety following laminectomy: a randomized clinical trial. *BMC Anesthesiology*. 2022; 22: 318.
- [18] Karami F, Jamaati H, Coleman-Fuller N, Zeini MS, Hayes AW, Gholami M, *et al.* Is metformin neuroprotective against diabetes mellitus-induced neurodegeneration? An updated graphical review of molecular basis. *Pharmacological Reports*. 2023; 75: 511–543.
- [19] Guo Y, Chen Q, Wu B, Sun L. Isovitexin restores sevoflurane-induced cognitive dysfunction by mediating autophagy through activation of the PGC-1 α /FNDC5 signaling pathway. *Acta Neurobiologiae Experimentalis*. 2022; 82: 373–379.
- [20] Zhen AX, Piao MJ, Hyun YJ, Kang KA, Ryu YS, Cho SJ, *et al.* Purpurogallin protects keratinocytes from damage and apoptosis induced by ultraviolet b radiation and particulate matter 2.5. *Biomolecules & Therapeutics*. 2019; 27: 395–403.
- [21] Islam F, Islam MM, Khan Meem AF, Nafady MH, Islam MR, Akter A, *et al.* Multifaceted role of polyphenols in the treatment and management of neurodegenerative diseases. *Chemosphere*. 2022; 307: 136020.
- [22] Xie X, Zu X, Liu F, Wang T, Wang X, Chen H, *et al.* Purpurogallin is a novel mitogen-activated protein kinase kinase 1/2 inhibitor that suppresses esophageal squamous cell carcinoma growth *in vitro* and *in vivo*. *Molecular Carcinogenesis*. 2019; 58: 1248–1259.

How to cite this article: Yu Yao, Zhihong Li, Jingqi Zhang, Jinzhao Wang, Baohua Wang, Fengjuan Wang. Purpurogallin ameliorates sevoflurane-induced cognitive impairment and hippocampal neuroinflammation in neonatal mice. *Signa Vitae*. 2024; 20(3): 89-96. doi: 10.22514/sv.2024.032.