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Ellagic acid improved neurodegeneration caused by ischemic stroke through the regulation of glutamatergic and synaptic signaling

Pei Zhao[1] & Ting Wang[2]*

[1]Department of Neurology, Xi'an International Medical Center Hospital, Xi'an 710100, China

[2]Department of Rehabilitation Medicine, Xi'an Fengcheng Hospital, Xi'an 710021, China

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Ischemic stroke, caused by disrupted cerebral blood flow, leads to neurodegeneration through nutrient and oxygen deprivation. Despite ellagic acid's (EA) known antioxidant/anti-inflammatory roles in stroke, its direct modulation of synaptic NMDA/AMPA receptors remains unexplored. This study investigates EA's neuroprotection via glutamatergic signaling in ischemic stroke in a transient middle cerebral artery occlusion (MCAO) rat model. Wistar rats were divided into sham, MCAO, and EA-treated MCAO groups. Daily EA administration for 72 hours significantly improved neurobehavioral deficits and reduced cerebral edema in MCAO rats (P-value<0.001). EA increased anti-apoptotic Bcl-2 levels while decreasing pro-apoptotic CASP-3, protecting cortical neurons from ischemia-induced cell death. Furthermore, EA counteracted ischemia-induced declines in NR2a, NR2b, and GluR1 expression, while upregulated PSD95 expression (P-value<0.05). Oxidative stress (ROS) and neuroinflammatory cytokines (IL-6, IL-1β, TNF-α) were also reduced, though lipid peroxidation (MDA) remained unchanged. These findings demonstrate that EA mitigates ischemic neurodegeneration by enhancing synaptic survival signaling, suppressing apoptosis, and alleviating neuroinflammation and oxidative stress. The study highlights EA′s potential as a therapeutic agent for ischemic stroke, warranting further clinical validation.

Keywords: Synaptic plasticity, Neuroinflammation, MCAO model, Neuroprotection, Receptor

Stroke is acknowledged as a foremost cause of mortality and disability across the globe[1]. Among the multiple causes identified, ischemic stroke is particularly significant as it contributes to recurrent strokes, even after appropriate treatment measures have been implemented[2]. It is shown that the interaction of glutamate with N-methyl-D-aspartate (NMDA) receptors (NMDAR) activates these receptors[3]. This cascade initiates downstream signaling pathways resulting in cell death through mechanisms involving apoptotic factors[4]. Recent studies have categorised NMDARs into two distinct subtypes according to their subunit composition and cellular localization: NMDAR2a (NR2a) and NMDAR2b (NR2b)[5]. Postsynaptic density protein-95 (PSD95) serves as a scaffold protein that is vital for maintaining a complex network of protein interactions at the postsynaptic density of excitatory neurons, which is crucial for neuronal function and survival[6]. Moreover, ischemic stroke has been associated with heightened levels of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) at synaptic sites in the ipsilateral sensorimotor cortex[7]. NMDARs, along with AMPARs are considered the principal ionotropic glutamate receptors (GluRs) within the mammalian central nervous system (CNS) [7].

Ellagic acid (EA), a dietary polyphenol present in various fruits, vegetables, herbs, and nuts, can occur either in its pure form or as part of more complex molecular structures[8]. There is an indication that EA may act as a promising therapeutic agent for cerebral ischemia/reperfusion injury, potentially due to its interconnected anti-inflammatory properties[9]. While EA′s anti-inflammatory/antioxidant roles in stroke are documented, its direct modulation of synaptic NMDA/AMPA receptors remains unexplored. Hence, the present study hypothesises that EA mitigates ischemic neurodegeneration by selectively restoring synaptic NR2a-PSD95 signaling while downregulating extrasynaptic GluR1, a dual-target mechanism distinct from prior studies focused solely on EA's antioxidant/anti-inflammatory roles. This research aimed to develop an animal model for middle cerebral artery occlusion (MCAO) and to evaluate the effects of daily administration of EA on receptor-associated signaling pathways (NR2a, NR2b, PSD95, GluR1), markers of cell death (Bcl-2,

*Correspondence:
E-mail: ting_0712@outlook.com

CASP-3), neuroinflammatory factors (TNF-α, IL-6, IL-1β), and oxidative stress indicators (reactive oxygen species [ROS], malondialdehyde [MDA]).

Materials and Methods

Experimental animals

A total of 32 adult male Wistar rats, approximately eight weeks old and weighing between 200 and 250 grams, were used in this study. The animals were housed under standardised laboratory conditions, with unrestricted access to food and water. The environment was carefully controlled, maintaining a temperature of 22 ± 3 °C and relative humidity of 50 ± 5%. A 12-hour light/dark cycle was maintained to support normal physiological rhythms.

Study design

The study was designed with a randomised allocation of the rats into four separate experimental groups: 1. Sham operation group (n = 8), 2. Ellagic acid Sham group (EA+Sham, n = 8), 3. Middle Cerebral Artery Occlusion (MCAO) group (n = 8), and 4. Ellagic acid-treated MCAO group (EA+MCAO, n = 8). The Sham operation group served as the control for baseline biomarker levels and surgical procedural effects. EA (Sigma-Aldrich, ≥95% purity) was administrated at 50 mg/kg daily as previous dose-response studies showed peak efficacy at 50 mg/kg in MCAO models and brain bioavailability data confirming blood-brain barrier penetration at this dose[10,11], though human-equivalent dosing requires further pharmacokinetic profiling. Following the administration of chloral hydrate anesthesia (350 mg/kg, intraperitoneally) and the maintenance of a stable body temperature at 37°C, MCAO procedures followed established methods and were validated by prior studies[12,13]. A midline incision was performed to expose the right common carotid artery, along with the external carotid artery and internal carotid arteries. A 4/0 nylon filament featuring a heated, rounded tip was then inserted through the external carotid artery and advanced approximately 20 mm to occlude the origin of the middle cerebral artery. The sham-operated group underwent the same procedures, excluding the filament insertion. After a 90-minute occlusion period, the 4/0 filament was carefully removed to allow for reperfusion over a duration of 24 hours. Seventy-two hours following the occlusion, the animals were anesthetized, and brain tissue samples were collected.

Neurobehavioral examination and brain water content

Before the trial began, the rats underwent thorough monitoring and training for a duration of three days. Behavioural evaluations were conducted at 24, 48, and 72 hours following surgery[14]. The assessment of sensorimotor function employed a modified 28-point neuro-scoring system, which includes 11 distinct tests that together can yield a maximum score of 28 points. The assessment comprises multiple components, each scored within defined parameters to evaluate neurological and motor function: Circumnavigation: Assesses spatial navigation ability (maximum 4 points). Motility: Gauges coordination and movement efficiency (maximum 3 points). Overall Condition: Evaluates general health and responsiveness (maximum 3 points). Righting Reflex: A single point is awarded if the subject promptly corrects its posture when positioned supine. Paw Placement: Measures adaptive limb positioning on a flat surface (maximum 4 points). Horizontal Bar Pull-Up: Tests upper-body strength and endurance (maximum 3 points). Sloped Surface Navigation: Examines balance and agility on an incline (maximum 3 points)and Grip Strength: Quantifies forelimb force using a standardised apparatus (maximum 2 points).

The scoring scale ranges from 0 to 28, where a score of 0 indicates severe impairment and a score of 28 reflects no neurological injury. A previous study was conducted to analyse the water content in the brain, as the current research adhered to the methodology outlined[15].

Real-time quantitative PCR

Total RNA was extracted from experimental samples with the PureLink RNA Mini Kit (Thermo Fisher Scientific, USA; Cat. 12183018A), adhering to the manufacturer′s recommended protocol. RNA purity and concentration were determined using a NanoDrop spectrophotometer (Biotek, USA). For cDNA synthesis, the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, USA; Cat. 4368814) was employed to convert RNA into complementary DNA. Gene expression analysis was conducted via quantitative PCR (qPCR) on the StepOne Real-time PCR System (Applied Biosystems, USA) using Maxima SYBR Green qPCR Master Mix (Thermo Fisher Scientific, USA; Cat. K0253). To standardise expression data, GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was selected as the housekeeping gene. Relative quantification of target genes was performed using the

$2^{-\Delta\Delta Ct}$ method, with results expressed as fold changes relative to controls. Primer sequences designed for this study are comprehensively listed in Table 1.

Enzyme-Linked Immunosorbent Assay (ELISA)

Following the conclusion of the therapeutic protocols, the animals were euthanised in a humane manner, and their tissues were harvested for subsequent analysis. Specifically, cerebral cortex tissue samples weighing approximately 100 milligrams were homogenised in RIPA lysis buffer fortified with protease inhibitors to ensure protein integrity during processing. The study focused on assessing NMDA receptor signaling markers (NR2a, NR2b, PSD95, GLUR1), apoptosis markers (CASP-3, and Bcl-2), and inflammatory cytokines (IL-1β, IL-6, and TNF-α) using enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer guidelines.

Oxidative stress markers

The levels of oxidative stress in homogenised cortical tissues from rats were quantitatively measured by evaluating the concentrations of key biomarkers, including reactive oxygen species (ROS) and malondialdehyde (MDA). This analysis was conducted using ELISA kits as previously validated[16-18], which were meticulously employed throughout the procedure, adhering strictly to the manufacturer's guidelines and protocols.

Statistical analysis

Data are presented in the format of mean ± standard deviation. To assess statistical significance, one-way ANOVA was employed, accompanied by Tukey's post-hoc test. Statistical analyses were conducted using IBM SPSS Statistics version 24.0 (IBM Corp., Chicago, IL, USA), and graphical visualisations were created with GraphPad Prism version 8 (San Diego, CA, USA). Statistical significance was defined using a threshold of $P < 0.05$.

Results

Neurobehavioral and brain water content

Daily neurological assessment was performed for 72 hours to determine the effects of EA on MCAO-caused neurobehavioral changes (Fig. 1). The findings of the present study showed that MCAO animals had severe neurological deficits accompanied by significantly increased levels of cerebral edema (P-value<0.001). However, in animals subjected to

Table 1 — The sequences of the studied primers

Primers	Forward primer sequence (5′–3′)	Reverse primer sequence(5′–3′)
NR2a	GGGGTTCTGCATCGACATCC	GACAGCAAAGAAGGCCCACAC
NR2b	CGATGGCGTCTGGAATGG	CTGGCAAGAAAGATGACCGC
PSD95	TCTGTGCGAGAGGTAGCAGA	AAGCACTCCGTGAACTCCTG
GluR1	CTTCACTGCAGGATAAATGGTGG	CAAAGATGTACGGCATATTCC
Bcl-2	TGTGGATGACTGACTACCTGAACC	CAGCCAGGAGAAATCAAACAGAGG
CASP-3	GTGGAACTGACGATGATATGGC	CGCAAAGTGACTGGATGAACC
GAPDH	GCAAGGATACTGAGAGCAAGAG	GGATGGAATTGTGAGGGAGATG

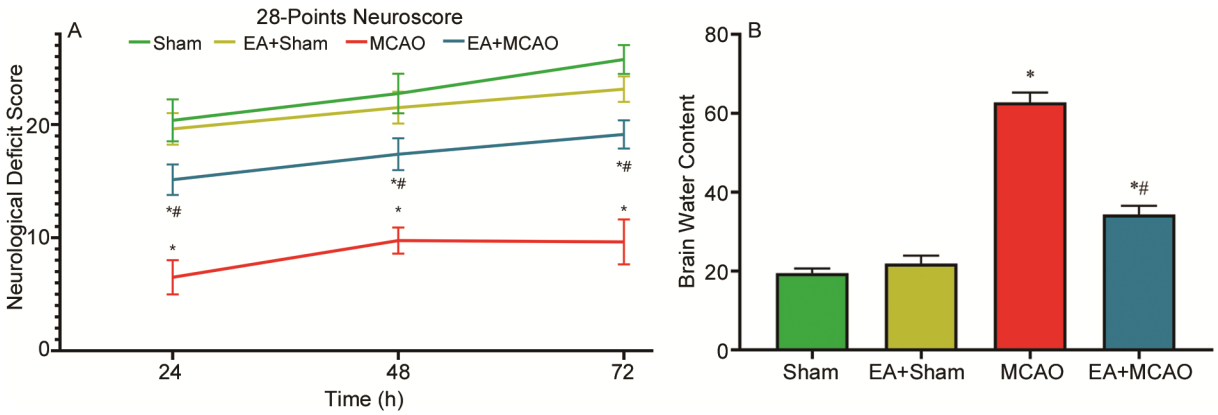

Fig. 1 — Neurodegeneration assessment and neuroprotective role of EA. Neurobehavioral performance (A) and brain water content (B) are illustrated. [One-way ANOVA accompanied by Tukey's post-hoc test was employed to assess statistical significance. *: significant difference with Sham group; *#: significant difference with both Sham and MCAO groups; P-value<0.05 was considered significant]

MCAO that were administered EA daily, impairments associated with neurological deficits as well as edema were significantly restored (*P*-value<0.001). Although Sham and EA+Sham groups did not show significant differences in terms of the neurological indices examined (*P*-value>0.05), EA+MCAO animals represented significant differences from sham animals in terms of impaired neurobehavior and cerebral edema (*P*-value<0.001).

Apoptosis was suppressed upon EA administration

The evaluation of the apoptotic status in the cortex was performed by measuring the levels of Bcl-2 and CASP-3 gene expression and proteins. The results of the current investigation indicated that no differences occurred between Sham and EA+Sham groups regarding gene expression and protein levels of Bcl-2 and CASP-3 genes between groups Sham and EA+Sham (*P*-value>0.05). Conversely, MCAO reduced *Bcl-2* expression by 54.13% and protein by 60.05% vs. Sham (*P*-value<0.001), while elevating *CASP-3* expression by 10.04-fold and protein levels by 2.25-fold (*P*<0.001). EA treatment reversed these changes (Bcl-2 increased by 57.52% while CASP-3 decreased by 48.56%; *P*<0.01 vs. MCAO) (Fig. 2).

EA restored NMDA and AMPA receptor signaling

Previous studies have shown that synaptic glutamate activity predominantly stimulates NMDA receptors, which contain NR2a and NR2b subunits and regulate several downstream pathways. *NR2a* gene expression did not differ between Sham and EA+Sham. However, EA+Sham animals exhibited reduced *NR2b* expression (26.81%, *P*=0.037) compared to Sham. Moreover, the findings indicated that there was no significant difference between Sham and EA+Sham groups in terms of protein levels of NR2a and NR2b (*P*-value>0.05). However, in MCAO animals, the levels of NR2a and NR2b were significantly reduced by 54.19% and 53.99%, respectively (*P*-value<0.001). Administration of EA to MCAO rats resulted in a significant 46.69% increase in *NR2a* gene expression compared to the MCAO group (*P*-value=0.025), although *NR2b* gene expression did not show a significant difference between EA+MCAO and MCAO (*P*-value=0.166). Notably, administration of EA to rats with MCAO caused a significant increase of 1.40-fold (*P*-value=0.0235) and 1.51-fold (*P*-value=0.001) in the protein levels of NR2a and NR2b, respectively, compared to the MCAO group. Although the levels of

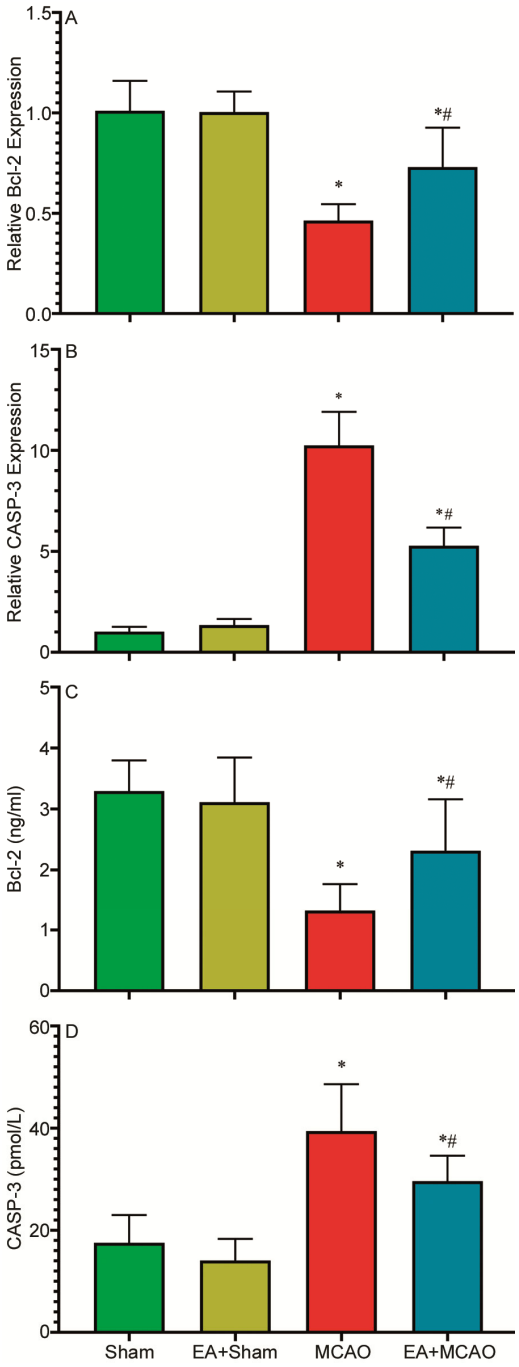

Fig. 2 — EA suppressed apoptotic damage in the cortex of rats with MCAO. The expression of *Bcl-2* (A) and *CASP-3* (B) genes, along with Bcl-2 (C) and CASP-3 (D) proteins, was evaluated. As the figure illustrates, Bcl-2 mRNA and protein levels were significantly reduced in the MCAO group, whereas CASP-3 mRNA and protein levels were significantly increased. Treatment of MCAO animals with EA caused a significant alleviation in MCAO-caused alterations in apoptotic markers. [One-way ANOVA accompanied by Tukey's post-hoc test was employed to assess statistical significance. *: significant difference with Sham group; *#: significant difference with both Sham and MCAO groups; *P*-value<0.05 was considered significant]

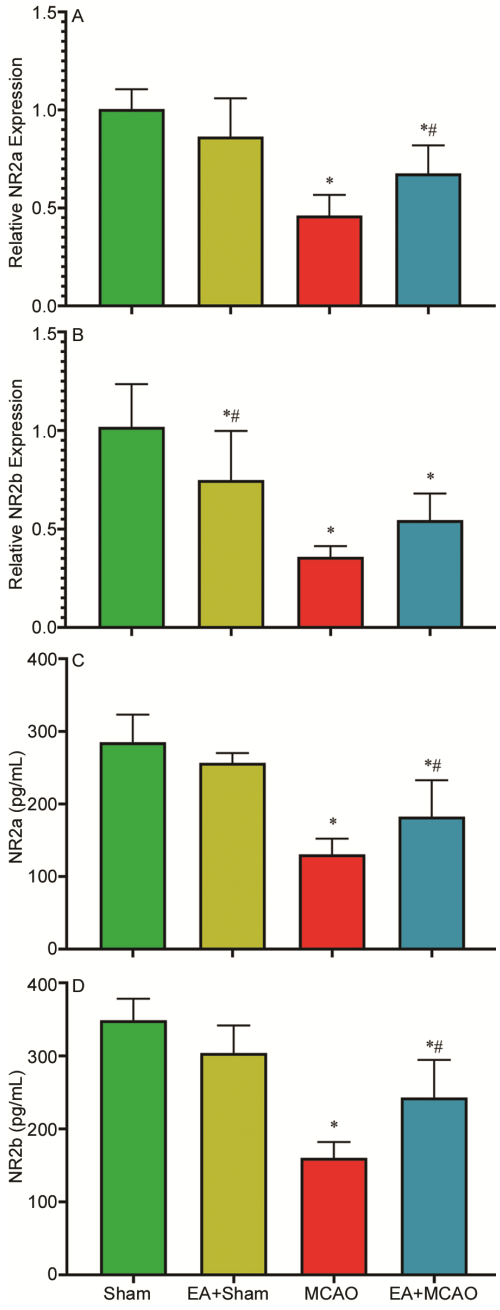

Fig. 3 — EA upregulated NMDAR subunits in MCAO animals. The gene expression and protein levels of NR2a (A & C) and NR2b (B & D) are depicted. As the figure illustrates, NR2a and NR2b mRNA and protein levels were significantly reduced in the MCAO group. Treatment of MCAO animals with EA caused a significant alleviation in MCAO-caused alterations in these receptors. [One-way ANOVA accompanied by Tukey's post-hoc test was employed to assess statistical significance. *: significant difference with Sham group; *#: significant difference with both Sham and MCAO groups; *P*-value<0.05 was considered significant]

NR2a and NR2b in EA+MCAO animals were significantly lower than in the Sham group (*P*-value<0.05, Fig. 3).

These results reinforce recent observations indicating that MCAO diminishes the expression of NR2a, thereby impacting the critical signaling pathways necessary for neuronal survival (Fig. 4). The activation of calpain leads to the degradation and decreased levels of full-length NR2a in the context of MCAO, a process that could potentially be counteracted by an increased interaction between PSD95 and NR2a. Hence, the present research investigated the influence of EA on PSD95 expression. The present data demonstrated that the *PSD95* gene expression in the MCAO group was notably increased by 4.54-fold compared to Sham-operated controls (*P*-value<0.001). Also, the results regarding PSD95 protein levels align with those of NR2a (2.06-fold increase compared to the Sham-operated group, *P*-value<0.001), as the overexpression of PSD95 in MCAO can be interpreted as enhancing the NR2a-mediated survival signaling pathway. After the occurrence of brain ischemia, the accumulation of glutamate leads to the activation of AMPA receptors. Consistent with previous studies, current findings indicated a significantly reduced expression of the AMPA receptor (GluR1, *P*-value<0.001) following cerebral ischemia when compared to the Sham-operated control group. Importantly, administration of EA to animals with MCAO resulted in significant recovery of *PSD95* and *GluR1* gene expression (*P*-value<0.001 and *P*-value=0.044, respectively) and protein (*P*-value=0.006 and *P*-value=0.003, respectively) levels, although PSD95 levels in the EA+MCAO group showed a significant difference from the Sham group (*P*-value=0.0134).

EA modulated inflammation

Available ELISA kits were purchased to measure the levels of inflammatory markers (Fig. 5). The results revealed that EA administration to Sham-operated rats caused a significant decrease of 47.03% (*P*-value<0.001) and 41.35% (*P*-value=0.001) in the IL-6 and TNF-α levels compared to the Sham group, while there was no significant difference between the two groups in terms of IL-16 levels (*P*-value=0.0994). In contrast, in the MCAO group, the levels of IL-6, TNF-α, and IL-1β markers increased significantly by 1.84-fold, 1.53-fold, and 1.88-fold, respectively, compared to Sham controls (*P*-value<0.001). Notably, EA reduced MCAO-elevated IL-6, TNF-α, and IL-1β by 35–50% (*P*<0.05).

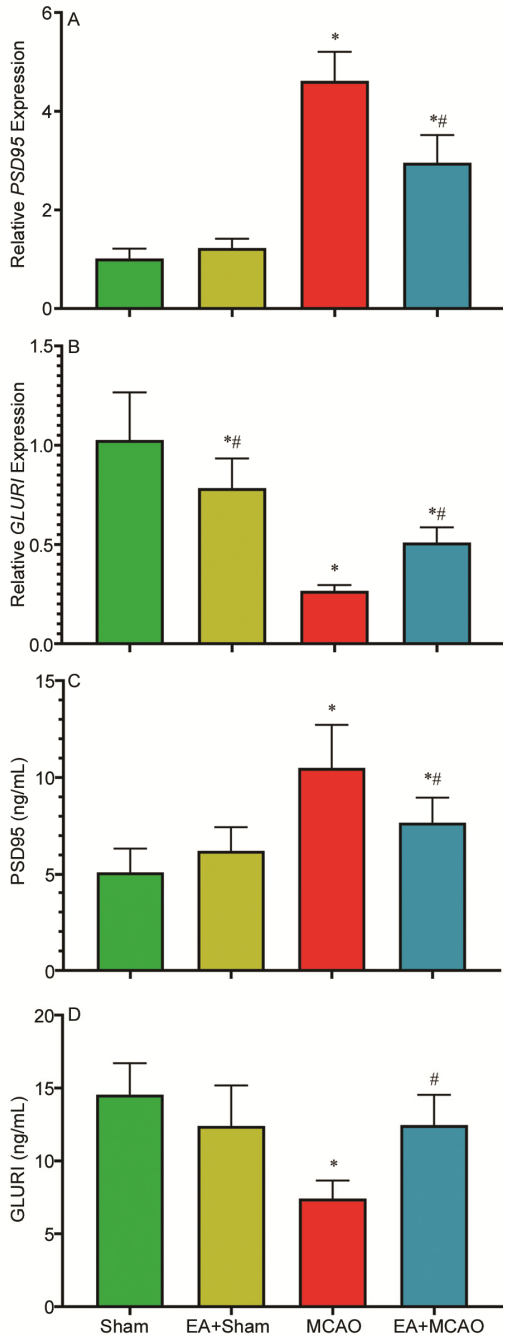

Fig. 4 — Downregulation of PSD95 and upregulation of GluR1 upon EA administration. Graphs A & B illustrate the gene expression of *PSD95* and *GluR1*, respectively. The protein levels are depicted in C & D. As the figure illustrates, PSD95 mRNA and protein levels were significantly increased in the MCAO group, whereas GLUR1 mRNA and protein levels were significantly decreased. Treatment of MCAO animals with EA caused a significant alleviation in MCAO-caused alterations in these markers. [One-way ANOVA accompanied by Tukey's post-hoc test was employed to assess statistical significance. *: significant difference with Sham group; *#: significant difference with both Sham and MCAO groups; *P*-value<0.05 was considered significant]

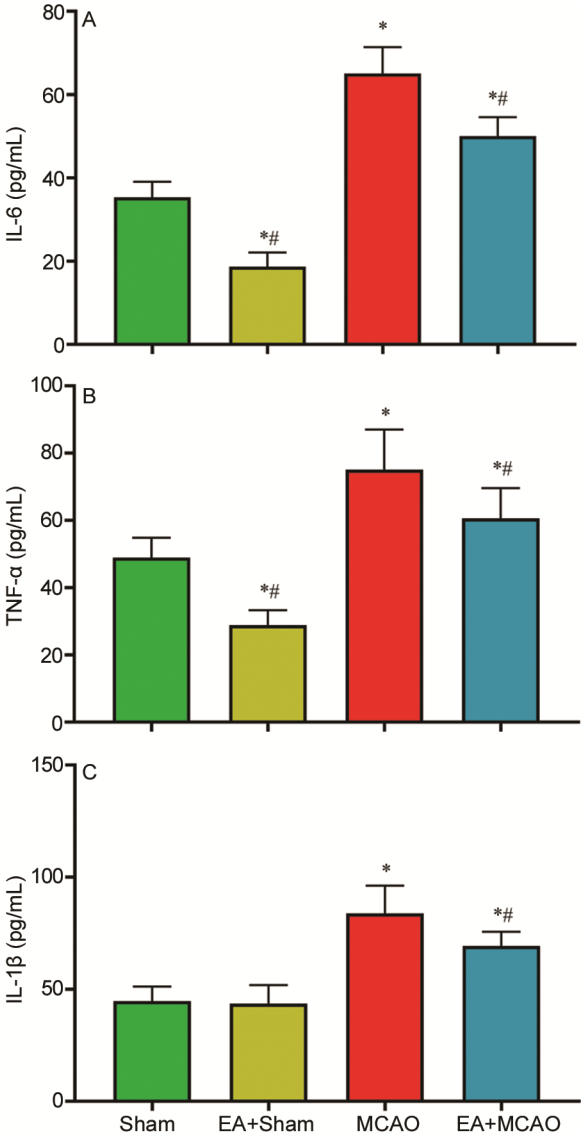

Fig. 5 — Neuroinflammatory cytokines and effects of EA. The levels of IL-6 (A), TNF-α (B), and IL-1β (C) were measured. [One-way ANOVA accompanied by Tukey's post-hoc test was employed to assess statistical significance. *: significant difference with Sham group; *#: significant difference with both Sham and MCAO groups; *P*-value<0.05 was considered significant]

EA alleviated oxidative stress in rats with MCAO

The level of ROS, along with lipid peroxidation, determined by measuring MDA, was assessed to reveal the oxidative stress status (Fig. 6). The findings demonstrated that the induction of MCAO was accompanied by a significant increase of 2.89-fold (*P*-value<0.001) and 1.52-fold (*P*-value=0.004) in the levels of ROS and MDA, respectively, compared to Sham-operated controls. Moreover, the administration of EA to the Sham animals slightly reduced both

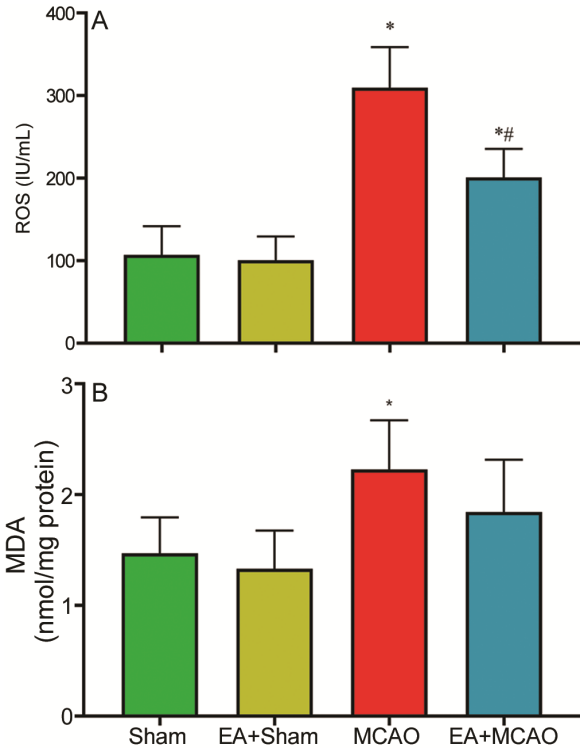

Fig. 6 — The induction of oxidative stress in MCAO animals and the effects of EA. The levels of ROS (A) and MDA (B) were assessed. [One-way ANOVA accompanied by Tukey's post-hoc test was employed to assess statistical significance. *: significant difference with Sham group; *#: significant difference with both Sham and MCAO groups; *P* value<0.05 was considered significant]

ROS (*P*-value=0.986) and MDA (*P*-value=0.899). Importantly, EA reduced ROS by 35.14% (*P*<0.001), but not MDA (*P*-value=0.243).

Discussion

This study sought to determine whether EA could alleviate neurodegeneration and apoptosis caused by MCAO in the cortical region through the modulation of the glutamatergic pathway. The findings revealed that EA significantly strengthens the interaction between NR2a and PSD95, thereby facilitating cellular survival. Furthermore, the research underscored the synaptic-protective effects of EA, in addition to its anti-inflammatory and antioxidant properties. In the context of ischemic stroke, the impacted areas can be categorised into two distinct zones: the ischemic core, which is marked by irreversible necrosis, and the penumbra, where apoptosis occurs[13,19]. MCAO leads to a relatively uniform infarct within 3 to 12 hours following occlusion, predominantly affecting the neocortex[13,19]. This study provides the first evidence that EA directly modulates synaptic glutamate receptor partitioning in ischemic stroke.

Excitotoxicity has emerged as the predominant and extensively studied pathogenic mechanism associated with ischemic stroke. This phenomenon is characterised by the excessive release of glutamate from injured neurons, which leads to the overactivation of NMDARs. Such overactivation results in calcium overload within neurons, triggering a cascade of pro-apoptotic events, including the generation of ROS, activation of calpain, inflammatory responses, and mitochondrial impairment, ultimately culminating in either apoptosis or necrosis[12,20]. Research indicates that various receptor subtypes elicit distinct functional responses to ischemic challenges. Notably, the synaptic NR2a subunit is crucial for neuroprotective mechanisms and neuronal survival, whereas the extrasynaptic NR2b subunit is implicated in promoting excitotoxicity and apoptosis in the context of ischemic injury[21]. Given the opposing roles of NMDAR subunits, an optimal therapeutic approach would involve inhibiting NR2b-mediated pro-death pathways while preserving NR2a function. Nonetheless, the widespread distribution of both subunits complicates the application of this strategy in human subjects[15]. Therefore, targeting downstream mechanisms associated with cell death may offer a promising avenue for mitigating ischemic damage[15]. The current findings corroborate previous studies[22,23], demonstrating that EA treatment enhances the NR2a protein levels, indicating that EA may exert its neuroprotective effects, at least in part, by modulating the expression of NMDAR subunits. EA administration correlated with upregulated synaptic NR2a and PSD95 expression, which may stabilise postsynaptic density and potentially enhance pro-survival ERK/CREB/AKT pathways[24-26]. Concurrently, EA downregulated GluR1, suggesting a reduction in AMPA receptor-mediated Ca^{2+} influx and excitotoxicity[27,28]. This dual modulation aligns with strategies to preserve synaptic NMDA function while mitigating excitotoxic damage[5]. Indeed, EA's upregulation of synaptic NR2a and PSD95 may counteract ischemia-induced calpain-mediated NR2a degradation, while PSD95 recruitment could inhibit nNOS-driven excitotoxicity. Conversely, suppressed GluR1 expression likely limits AMPA receptor-mediated Ca^{2+} overload and ROS production, consistent with studies showing 40–60% infarct reduction after GluR1 inhibition[29,30]. The present findings also indicated the restoration of Bcl-2 and

CASP-3 levels upon EA administration, which were significantly downregulated and upregulated, respectively, by MCAO. Bcl-2 is considered the most important inhibitor of apoptosis, whereas CASP-3 is a serine protease that promotes the apoptosis process[31]. Consequently, the findings indicate the anti-apoptotic activity of EA in the cortex of animals with MCAO. Maintaining cell viability is one of the most important properties of phytochemicals such as EA, which has led to its recommendation in various neuropathological conditions[8,32,33].

Glutamate not only activates NMDAR but also stimulates AMPAR, a ligand-gated cation channel permeable to sodium ions[34]. The phosphorylation of specific subunits endows AMPAR with varied functionalities. The excitotoxic effects of glutamate are primarily mediated by the AMPAR subunits GluR1 to GluR4[35]. Notably, GluR1 is mainly found in the hippocampus, and numerous studies indicate that its phosphorylation is essential for the channel's properties, synaptic presence, plasticity, memory formation, and spatial learning[36,37]. The GluR1 subunit enhances cognitive processes upon synaptic integration, primarily through phosphorylation at two key sites: Ser845, mediated by protein kinase A (PKA), and Ser831, regulated by calcium/calmodulin-dependent protein kinase II (CaMKII)[30]. Consistent with earlier research, the present findings revealed that EA treatment can influence the expression of GluR1[22,29]. EA′s upregulation of synaptic NR2a and PSD95 enhances pro-survival ERK/CREB pathways, while GluR1 downregulation limits Ca^{2+}-mediated excitotoxicity. This dual modulation aligns with recent strategies to spare synaptic NMDA function while inhibiting excitotoxicity[5]. EA suppresses GluR1 expression, limiting AMPA receptor-mediated Ca^{2+} influx and subsequent ROS overproduction. This aligns with evidence that GluR1 inhibition reduces infarct volume by 40–60% in stroke models[27,28]. Nevertheless, further studies are encouraged to clarify the impact of EA on downstream pathways of GluR1 and the probable contribution to the amelioration of ischemic damage.

Ischemic stroke induces neuroinflammation, which occurs initially depending on activated resident non-neural cells in the brain[38]. Microglia and astrocytes, two types of non-neuronal cells, actively contribute to neuroinflammation during ischemic stroke by releasing pro-inflammatory proteins, which in turn activate stress kinases such as JNK and P38, leading to mitochondrial apoptosis. Additionally, inflammatory cytokines and mediators, including TNF-α, IL-6, and IL-1β are released in response to ischemic damage, resulting in further collateral injury[39]. Increasing evidence suggests that modifying neuroinflammatory responses may mitigate ischemic damage[40,41]. The current findings demonstrated that MCAO was followed by a significant increment in the levels of neuroinflammatory mediators, whereas EA daily administration significantly downregulated the levels of inflammatory markers. This aligns with previous studies that highlight the significant anti-inflammatory properties of EA in various ischemic stroke models[32,42,43]. Inflammatory responses in MCAO models are often accompanied by overproduction of ROS, which exacerbates ischemic damage[44]. EA′s suppression of TNF-α/IL-1β indirectly preserves NR2a function, as neuroinflammation disrupts NMDA receptor trafficking[45]. The interaction of overproduced ROS with membrane lipids leads to peroxidation of these macromolecules, which may be followed by cellular damage and ultimately cell death[46], hence, antioxidants are suggested as therapeutic options for ischemic stroke[47]. The present findings showed that MCAO progressively increased the levels of ROS and MDA. Antioxidant activity is one of the main properties of EA, which maintains cellular oxidative homeostasis via scavenging free radicals[10], thus preventing ischemic damage mediated by oxidative stress. The current study demonstrated that daily treatment of EA was accompanied by a significant reduction in the levels of ROS, although it did not lead to a decrease in lipid peroxidation. Lipid peroxidation is usually a delayed process, and restoring the elevated levels of MDA requires a longer treatment period[48,49]. Thereby, the lack of MDA reduction despite ROS decrease may reflect delayed lipid peroxidation kinetics[50] as well as EA's preferential scavenging of water-soluble ROS (e.g., •OH) over lipid peroxyl radicals[51,52].

This study provides the first evidence that EA directly modulates synaptic glutamate receptor partitioning in ischemic stroke. Unlike previous reports on EA's antioxidant effects[8,32], the current findings demonstrate its unique ability to enhance NR2a-PSD95 coupling as pro-survival signaling, suppress GluR1 as excitotoxicity reduction; and concurrently attenuate neuroinflammation/oxidative stress. This tripartite mechanism represents a

significant advance over single-pathway targeting in prior EA/stroke studies. Moreover, while Chen *et al*.[42] reported EA's IGF-1-mediated neuroprotection, our work identifies glutamatergic synaptic reorganization as a novel pathway. Similarly, unlike Shah *et al*.[15] who studied quercetin's effects on NMDA receptors, we establish EA's specific efficacy in rescuing NR2a-PSD95 complexes altered by ischemia.

This study exclusively used male rats, ignoring sex-specific responses influenced by estrogen's neuroprotective role in stroke recovery[53]. Future work must evaluate EA in females and address pharmacokinetics (e.g., brain bioavailability via LC-MS), dose optimisation, and combinatorial therapies with thrombolytics (e.g., tPA). Translational potential also requires validation in higher-order species and clinical cohorts. The absence of histological validation (e.g., Nissl staining for neuronal loss, IHC for NR2a/PSD95 colocalization) along with electrophysiological assessments of synaptic plasticity is a limitation that precludes definitive conclusions about functional outcomes of EA-mediated receptor modulation. Future studies will incorporate these to visualise EA's neuroprotective effects. Although the present findings indicated the promising potential of EA in improving ischemic stroke in an animal model through the modulation of NMDAR and AMPAR, as well as inhibition of neuroinflammation and reduction of oxidative stress, it is necessary to conduct further animal models and especially clinical trials, to confirm the therapeutic effects of EA. Future studies should assess: (i) EA pharmacokinetics (e.g., brain bioavailability via LC-MS); (ii) combinatorial therapies with thrombolytics (e.g., tPA); and (iii) sex-specific responses, given estrogen′s role in stroke recovery.

Conclusion

This study demonstrates that ellagic acid (EA) administration mitigates neurodegeneration in a transient MCAO rat model by selectively restoring synaptic NR2a-PSD95 signaling and downregulating extrasynaptic GluR1 expression, thereby attenuating excitotoxicity, neuronal apoptosis, neuroinflammation, and oxidative stress. These findings establish EA′s dual-target mechanism, distinct from its previously documented antioxidant/anti-inflammatory roles, in preserving glutamatergic synaptic integrity and neuronal survival post-ischemia, directly addressing the objective of investigating EA′s receptor-mediated neuroprotection. While EA exhibits compelling therapeutic potential for ischemic stroke, further preclinical optimisation and clinical validation are imperative to ensure its translational applicability.

Ethical statement

The experimental procedures and animal care and handling were approved by the Animal Ethics and Welfare Committee (AEWC) of the Tianjin XINRUI Procedures for Laboratory Animal Center (XinRui-DWLL-2025009) and the research has complied with all relevant national regulations. Moreover, all efforts were made to minimise the animals' suffering based on the ARRIVE guidelines.

Conflict of interest

The authors declare that they have no conflicts of interest.

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