Original Article

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The effect of N-acetylcysteine on the expression of *miR-342-3p* and *Chi3l1* in the ischemic stroke model in rats

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Competing Interests

The authors state no conflict of interest.

Consent for Publication

Not applicable.

Data Availability Statement

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

Ethical Approval

The animal experiments adhered to the National Institute of Health Guide for the Care and Use of Laboratory Animals and received approval from the Research Ethics Committees of Shahid Beheshti University: (IR.SBU.REC.1402.027).

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Abstract

Background: Ischemic stroke is a leading global cause of death, and its severity is increased by inflammatory pathways. N-acetylcysteine (NAC), an FDA-approved anti-inflammatory agent, can significantly affect pro-inflammatory cytokines. On the other hand, microRNAs such as microRNA-342-3p (*miR-342-3p*), which likely play a role in reducing inflammation, could be a promising factor in the investigation of ischemic stroke. This research aims to investigate the impact of NAC on *miR-342-3p* expression and its downstream gene *Chi3l1* in animal models with middle cerebral artery occlusion (MCAO).

Methods: Rats were divided into four groups: MCAO, sham, NAC-only, and NAC+MCAO. The MCAO group underwent MCAO surgery without any treatment. In the NAC-only group, 150 mg/kg NAC was injected without MCAO surgery. Rats in the NAC+MCAO group were subjected to MCAO surgery, followed by 150 mg/kg NAC administered twice a day, 1 and 6 h after surgery. In all groups, neurological deficits, infarction volume, cerebral edema, blood-brain barrier permeability, and *miR-342-3p*/*Chi3l1* gene expression levels were evaluated.

Results: This study demonstrated notable differences in behavioral tests, blood-brain barrier permeability, and cerebral edema between the MCAO and NAC+MCAO groups. Infarct volume was significantly reduced in the NAC+MCAO group compared to the MCAO group. Also, *miR-342-3p* gene expression was down-regulated in the MCAO group, while its expression was up-regulated in the NAC+MCAO group. *Chi3l1* gene expression was reduced in the NAC+MCAO group. In addition, a significant correlation was observed between miR-342-3p and Chi3l1 expression levels.

Conclusion: This study supported the neuroprotective effect of NAC on reducing stroke damage in an animal model. Also, these findings provided evidence that the role of NAC in ischemia might be

related to modulating the pathway of inflammation (through alleviating the expression of *miR-342-3p* and *Chi3l1*).

Keyword: Ischemic Stroke; N-acetylcysteine; *MicroRNA-342-3p* gene; *Chi3l1* gene; Inflammation

1. Introduction

Stroke remains a common problem worldwide, and understanding of how the disease progresses and its impact on people's lives is crucial for developing more effective diagnostic tests and treatments [1]. Each year, about 13.5 million people have a stroke, and 5.5 million people die from stroke [2]. Extensive research has focused on unraveling the pathology of cerebrovascular disease, particularly ischemic strokes characterized by interrupted blood flow to the brain, often caused by arterial thrombosis or embolism. Ischemic brain damage involves a complex pathological and physiological process, including energy deficiency, increased calcium, oxidative stress, mitochondrial damage, excitotoxicity, and inflammatory responses, as well as apoptosis [3]. Inflammation initiates with the impairment of blood flow, the enhancement of intravascular leukocytes, and the release of pro-inflammatory agents from ischemic endothelium and cerebral tissue, thereby amplifying the risk of heightened damage. Although many aspects of post-ischemic inflammation persist for days or even weeks, dying neurons following ischemia release inflammatory elements in the adjacent brain parenchyma, and the inflammatory cascade promptly triggers more cerebral damage [4]. Inhibiting the inflammatory mediators has shown promise in reducing neuronal destruction and improving brain function [5]. In the field of inflammation, *miR-342-3p* acts as a critical player and regulates mechanisms within T cells due to its anti-inflammatory properties [6]. This microRNA holds promise for treating inflammation-related diseases and also plays a crucial role in maintaining the viability of macrophages by adjusting an anti-apoptotic gene network[6,7]. It has been suggested that *miR-342-3p* may play a role in the pathophysiological mechanisms of ischemia [6–8]. On the other hand, its downstream gene, *Chi3l1*, has a direct connection to inflammation [9]. *Chi3l1* is expressed in response to cytokines, such as interleukin 13 (IL-13) and interferon-γ (IFN-γ), which are involved in the inflammatory response [10]. This suggests that *Chi3l1* may also play a role in the inflammatory response in ischemic stroke. Investigating the

complex interaction between *miR-342-3p* and *Chi3l1* provides insights into their roles in regulating the inflammatory response.

N-acetylcysteine (NAC), a sulfur-containing compound with mucolytic properties, is known as a dietary supplement present in specific fruits and vegetables. NAC has been recognized for its potential, including boosting glutathione S-transferase activity, stabilizing protein structures, and functioning as an antioxidant and anti-inflammatory factor [11]. In particular, it has been demonstrated that NAC reduces pro-inflammatory biomarkers, such as Interleukin 6 (IL-6), providing a promising approach to alleviate inflammation-related symptoms associated with stroke [12].

Among the latest interventions in studies mitigating ischemic stroke injuries, microRNAs have attracted the researchers' attention [9,13]. According to the available evidence, there is no reported study investigating the effect of NAC on the level of *miR-342-3p* and its outcomes on injuries caused by ischemic stroke. Consequently, this study was designed to investigate the effect of NAC on one of the crucial pathophysiological pathways of cerebral vascular ischemia (inflammatory pathway) via the influence on the expression of *miR-342-3p* gene and its downstream gene, *Chi3l1*, in ischemic stroke. We hypothesized that NAC provides neuroprotection by upregulating *miR-342-3p* and suppressing *Chi3l1*, thereby attenuating inflammatory processes and reducing ischemic brain injury.

2. Materials and Methods

2.1. Animals

60 adult male *Wistar* rats (weighing between 250-350 g) were obtained from the Pasteur Research Production Complex of Iran. The rats were housed under standard temperature conditions (22 ±

2°C) and exposed to a 12-hour light/dark cycle. In this experiment, the minimum number of rats was utilized. All experimental procedures involving animals were conducted with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications, revised 2011). Also, every step of the experimental procedures was conducted according to the protocol approved by the Shahid Beheshti University Ethics Committee: (IR.SBU.REC.1402.027).

2.2. Experimental Groups

The *Wistar* rats were assigned to four principal categories: the MCAO group (n=20), the sham group (n=15), the N-acetylcysteine-only group (n=5), and the NAC+MCAO group (n=20). The sample size of the sham group was determined to ensure adequate statistical comparison for molecular analyses and to match the subgroup allocation used in the experimental groups. Each main group, except the NAC-only group, was separately evaluated for brain edema (n=5), blood-brain barrier permeability (n=5), and gene expression levels of *Chi3l1* and *miR-342-3p* using the real-time PCR (RT-PCR) technique (n=5). Infarct volume was measured in MCAO and NAC+ MCAO groups (Due to the complete use of brain tissue in each assay, rats were used independently in each of these subgroups). Also, neurological deficit scoring was evaluated in all groups except the NAC-only group. In the NAC-only group, *Chi3l1* and *miR-342-3p* genes levels were assayed (n=5). Rats in the MCAO group underwent middle cerebral artery occlusion (MCAO) through filament ischemic surgery without any treatment. Rats in the sham group were only subjected to surgical stress, and no filament was used to block the middle cerebral artery. Hence, infarction was not assayed in the sham group. NAC was dissolved in sterile normal saline and injected at a dose of 150 mg/kg twice per day with a 6 h interval time in the NAC-only group, and expression of *Chi3l1* and *miR-342-3p* genes was assayed in this group. The purpose of designing this group is solely to investigate the effect of NAC on gene expression under non-ischemic conditions, and there is no need for

evaluations such as brain edema, blood-brain barrier permeability, or infarct volume in this group. The NAC+ MCAO group underwent MCAO surgery and received NAC twice per day at a dose of 150 mg/kg, intraperitoneally (the first time was 1 h after induction of MCAO and the second time was 6 h after reperfusion) [14] (Table 1) (Figure 1). In this study, the mortality rate was about %10. Total 66 rats were purchased and six rats died following MCAO surgery due to surgical complications or severe ischemic injury.

Table 1. Evaluation of ischemic damages in various experimental groups. MCAO: Middle cerebral artery occlusion; NAC: N-acetylcysteine

Assessment	Sham (n=15)	MCAO (n=20)	NAC-only (n=5)	NAC+MCAO (n=20)
NDS	+	+	-	+
Brain Edema (n=5)	+	+	-	+
Infarction (n=5)	-	+	-	+
BBB integrity (n=5)	+	+	-	+
miR-342-3p / *Chi3l1* Genes Expression (n=5)	+	+	+	+

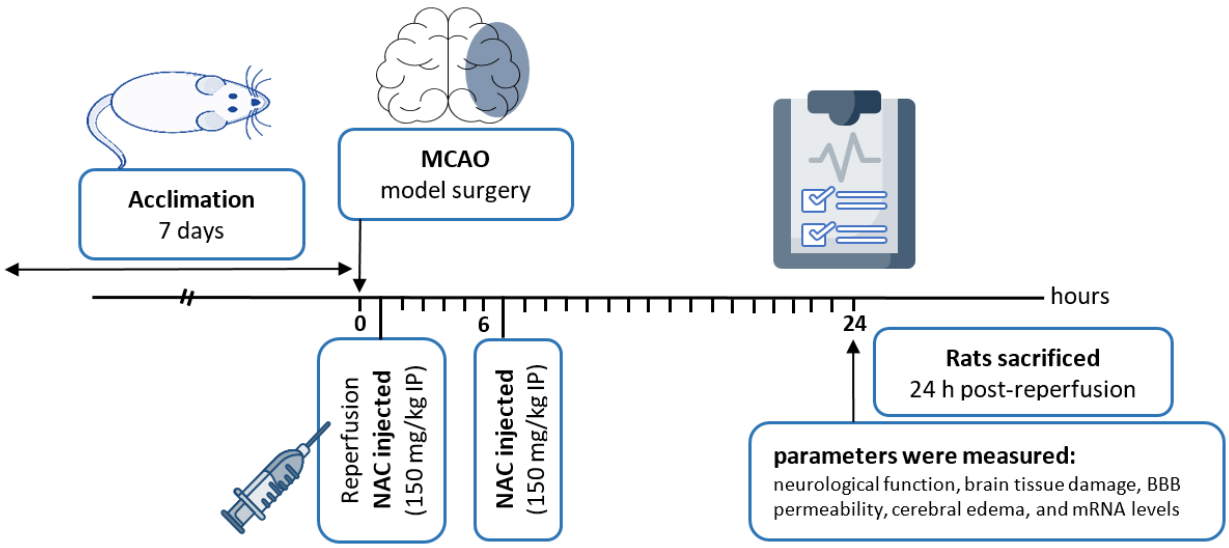

Fig. 1 Schematic timeline of the experimental protocol. Rats underwent right middle cerebral artery occlusion (MCAO) for 60 minutes followed by reperfusion. NAC was administered at 1 hour after MCAO induction and at 6 hours after reperfusion. Neurological deficits were evaluated 24 hours after reperfusion. Animals were euthanized 24 hours after surgery, and brain tissues were collected. MCAO, middle cerebral artery occlusion; NAC, N-acetylcysteine; IP, intraperitoneal.

2.3. Cerebral Ischemia Modeling

Ischemia was induced in rats by surgically blocking the middle cerebral artery using the MCAO method [15]. Anesthesia was induced by intraperitoneal administration of ketamine (80 mg/kg) and xylazine (10 mg/kg). Throughout the surgical procedure, additional maintenance doses were administered as required to maintain a stable anesthetic plane. Subsequently, the intraluminal filament approach was used in this procedure to block the MCA of the right hemisphere, as described by Longa [15]. The right side of the rat's neck was incised, and the carotid arteries were exposed after tissue dissection. The right external carotid artery and right common carotid artery

were ligated with a suture. A rounded, coated 3–0 nylon suture filament (with poly-L-lysine (Sigma, USA)) was created. An incision was made in the right common carotid, and the filament was inserted into the internal carotid artery up to the point where the filament met resistance and could not be advanced further (20 mm). In this way, the filament completely blocked the blood flow to the right MCA. Reperfusion was initiated by withdrawing the filament after 60 minutes of ischemia. During the operation, the rectal temperature was monitored. All assessments were conducted 24 h post-reperfusion.

2.4. Behavioral Assessment of Neurological Deficits

Neurological deficits were assessed 24 hours after MCAO surgery. The behavioral assessment consisted of movement tests, sensory tests, balance assessments, absence of reflexes, and abnormal movements. The neurological functions were scored on a scale from 0 to 18, with 0-6 indicating normal function, 7-12 indicating mild damage, and 13-18 indicating severe damage. A score of 18 means severe damage to the nervous system, which controls the animal's behavior [16] (Supplementary Table).

2.5. Assessment of Infarct Volume

Twenty-four hours post-reperfusion, the rats' brains were removed under deep anesthesia and preserved in cold saline for 5 min to allow sufficient tissue firmness for sectioning. The brains were then immediately processed without freezing for TTC staining. Following sectioning of the rats' brains from the frontal to the temporal side, 2 mm thick coronal segments were obtained. A 2% solution of 2.3.5-triphenyl tetrazolium chloride (TTC) dye (Merck-Germany) was prepared for staining. After 15 min, normal tissues appeared red due to the presence of succinate dehydrogenase, while damaged and ischemic tissues remained white due to the lack of this enzyme, indicating the occurrence of a stroke. Subsequently, the sections of the rat brain were

photographed with a digital camera. Finally, the areas of the rat brain, including the cortex, piriform cortex-amygdala (Pri-Amy), and striatum, were calculated using Image J software (version 1.50). These areas were then multiplied by 2 mm, and all the values obtained from all the sections were added together and calculated according to the following formula [17,18].

Corrected volume of the affected area = the volume of the left hemisphere – (volume of the right hemisphere - volume of the affected area)

2.6. Assessment of the Cerebral Edema

To assess cerebral edema, one of the common methods is to measure the wet and dry weights of the rat brain. After separating the Pri-Amy, striatum, and cortex of the left and right hemispheres, their weights were measured and defined as the wet weight (WW). Then, the brain pieces were placed in an incubator at 120°C (PID-C168 Zist Fanavar, Iran) for 24 hours. The weight of tissues was measured again to obtain the dry weight. In the end, the brain water content was calculated by the formula ([(WW-DW)/WW]×100) [19].

2.7. Assessment of the Permeability of the Blood-brain Barrier (BBB)

To evaluate the permeability of the blood-brain barrier, the extravasation of Evans Blue was quantified. In this method, 30 minutes after entering the filament into the common carotid artery and stopping the blood supply, 2% Evans Blue solution (Sigma, USA) was injected at a dose of 4 ml/kg. 24 hours post-reperfusion, 250 ml of saline was transcardially injected into the left ventricle to remove Evans Blue from the blood vessels. Subsequently, the brain was extracted from the cranium, and each area of the left and right hemispheres was separated and then weighed before being transferred to individual microtubes. Four times the weight of each area, phosphate buffer (PBS) was added to the microtubes and the samples were homogenized for 10 min. Then, 60%

trichloroacetic acid was added to precipitate proteins. They were mixed for three minutes and kept at 4 °C for thirty minutes. Subsequently, the specimens underwent centrifugation for 30 min at 1000 revolutions per minute (rpm). Finally, the optical absorbance of the supernatant was measured at 610 nm with an ELISA reader (BioTek 800 TS) to determine the dye concentration using a standard curve [20].

2.8. Assessment of Expression of *miR-342-3p* and *Chi3l1* Genes

Following the separation of brain regions, RNA extraction from brain tissue was conducted according to the manufacturer's instructions (Pars Tos, Iran, cat. A101231). Subsequently, the extracted RNA was reverse-transcribed into cDNA using a cDNA synthesis kit (Pars Tos, Iran, cat. A101161). PCR was carried out using SYBR green master mix (Pars Tos, Iran, cat. C101021) in duplicate with the MIC_BMS (no rox) machine. The TM Primer Quest tool was used to design the primers (Table 2). To analyze the data, the $2^{-\Delta\Delta CT}$ formula was used for normalization. *β-actin* for *Chi3l1* and *Snord87* for *miR-342-3p* were used as housekeeping genes, respectively.

Table 2. The primers of *miR 342-3P*, *Chi3l1*, and *housekeeping (snord89* and *β-actin*) genes.

Gene	Sequence of Primers
Mir-342-3P	*Stem loop: 5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACGGGT-3′*
	Forward: 5′-CCACTCTCACACAGAAATCGC -3′
	Reverse 5′-CAGTGCAGGGTCCGAGGTA-3′
Snord89	*Forward: 5′-GCTGGCACAATGATGACTTATGTT-3′*
	Reverse: 5′-CAGTGCAGGGTCCGAGGTA-3′
Chi3l1	*Forward: 5′-GGACCTATGGCTTTGATGGA-3′*
	Reverse 5′-TGAGCAGGAGTTTCTCTGTG-3′
β-actin	*Forward: 5′-CAACTGGGACGATATGGAGAAG-3′*
	Reverse: 5′-CAGAGGCATACAGGGACAAC-3′

2.9. Statistical Analysis

The Kruskal-Wallis non-parametric test analyzed the neurological deficits score (NDS) data, Dunn's post hoc. Also, analysis of cerebral edema, BBB, *Chi3l1,* and *mir342-3p* genes data was done by a one-way ANOVA test. Infarct volume was quantified using ImageJ software (version 1.50) and analyzed by an unpaired t-test. Data are presented as mean ± SEM. $P<0.05$ was considered a significant criterion for each group.

3. Results

3.1. The Effect of N-acetylcysteine (NAC) on Behavioral Deficits

24 hours after reperfusion, the effect of NAC on neurological deficits was evaluated. The results showed that the MCAO group exhibited a significant increase in the total neurological score in comparison with the sham group ($P=0.0002$). Also, a significant increase in each behavioral test (raise the tail, motor function, sensory function, and reflex activity) was observed between the MCAO and sham groups ($P<0.05$, $P<0.001$, $P<0.001$, $P<0.01$, respectively). In addition, the total score of all behavioral evaluations in the NAC+MCAO group decreased when compared with the MCAO group ($P=0.029$) (Figure 2A). A separate analysis of each behavioral test also indicated that NAC in ischemia-subjected rats attenuated neurological deficits compared with the MCAO group ($P<0.05$, $P<0.05$, $P<0.05$, $P<0.01$, respectively) (Figure 2B-E).

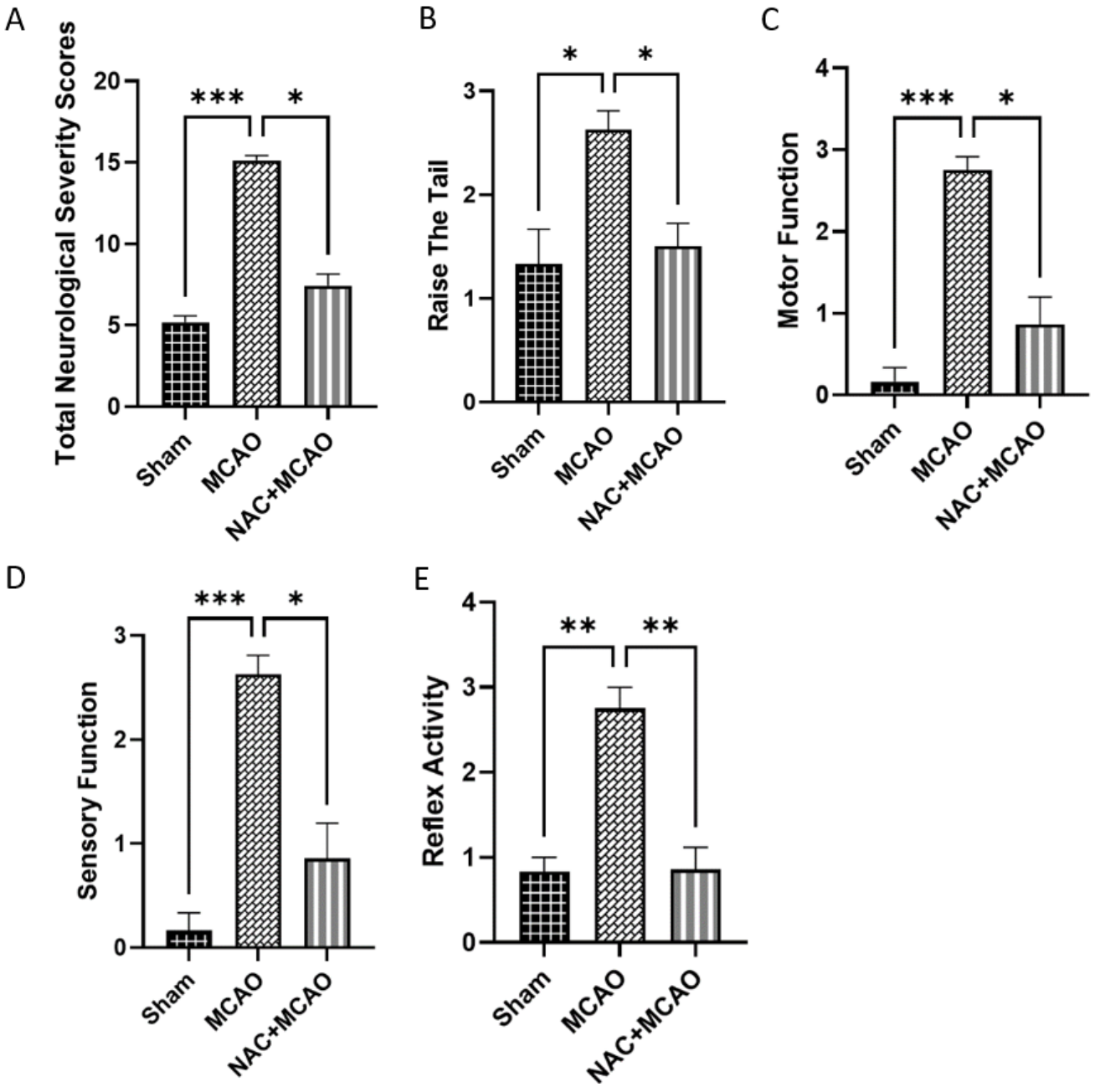

Fig. 2 The effect of N-acetylcysteine (NAC) on neurological deficits in the sham, MCAO, and NAC + MCAO groups 24 h after MCAO surgery. The graphs show the total neurological score (A), rising rat by the tail (B), motor tests (C), sensory tests (D), and reflex activity (E). Kruskal-Wallis non-parametric test; Each column represents Mean ± SEM (n=5). **P*<0.05, ***P*<0.01, and ****P*<0.001. Sham, surgery without MCAO; MCAO (control), ischemia without treatment; MCAO+NAC, ischemia with NAC treatment.

3.2. The Effect of N-acetylcysteine (NAC) on Infarction Volume

The volume of tissue damage in the brain was evaluated 24 hours after reperfusion. The brain sections were stained and the damage volume of the cortex, piriform cortex-amygdala (Pri-Amy), and striatum was calculated using ImageJ software for each group. The achieved results indicated that the total infarction volume in the NAC+MCAO group (120.5±10.17) was markedly diminished in comparison with the MCAO group (205.9±16.4) (*P*=0.002) (Figure 3A). Moreover, the NAC+MCAO group (49.62±10.13) exhibited a significant decrease in the infarcted volume of the cortex area when compared to the MCAO group (101.8±18.02) (*P*=0.03) (Figure 3B). A marked reduction was also observed within the Pri-Amy in the NAC+MCAO group (13.56±2.545) compared to the MCAO group (36.68±4.244) (*P*=0.001) (Figure 3C). Furthermore, the NAC+MCAO group (3.58±1.11) manifested a considerable decrease in the striatum area compared to the MCAO group (13.31±2.48) (*P*=0.007) (Figure 3D). The macroscopic images reflected that white and red areas of brain sections indicate ischemia and non-ischemic tissues, respectively (Figure 3E).

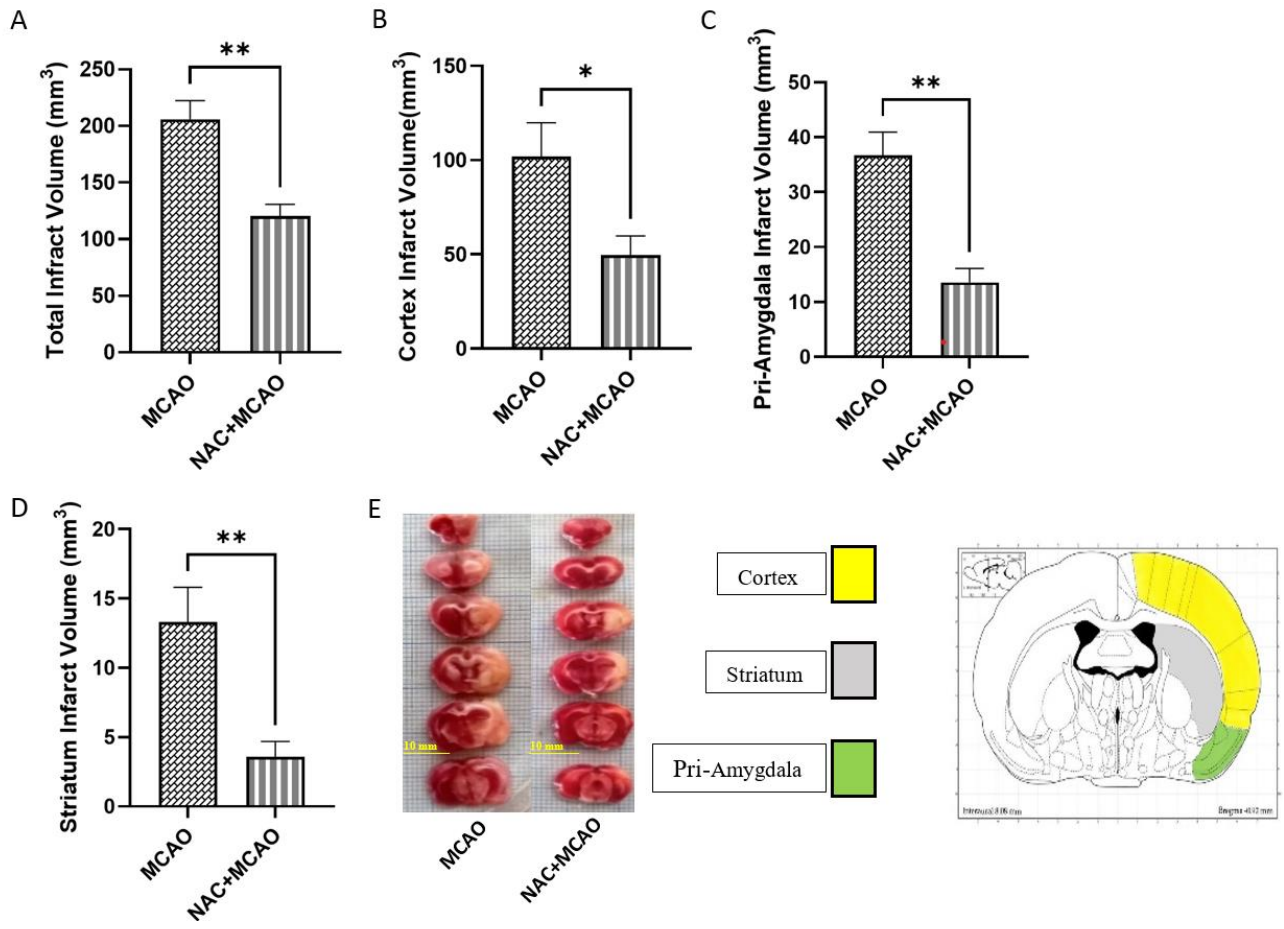

Fig. 3 The effect of N-acetylcysteine (NAC) on infarct volume 24 h after MCAO surgery. The graphs show the total infarct volume (A), infarct volume in cortex (B), infarct volume in piriform cortex-amygdala (Pri-Amygdala) (C), infarct volume in striatum (D). Also, staining of brain tissue slides was characterized by red color in non-ischemic and white in infarcted areas (E). Unpaired t-test; Each column represents Mean ± SEM (n=5). *P<0.05 and **P<0.01. MCAO (control), ischemia without treatment; MCAO+NAC, ischemia with NAC treatment.

3.3. The Effect of N-acetylcysteine (NAC) on the Cerebral Water Content

24 hours after the induction of ischemia, the weights of the cortex, striatum, and Pri-Amy areas of the left and right hemispheres were evaluated. In three regions of the left hemisphere, no

significant difference was observed between the experimental groups. Analyzing the data and comparing the outcomes of the groups indicated that the cerebral water content (edema) in the cortex, Pri-Amy, and striatum areas of the right hemisphere in the MCAO group was significantly enhanced in comparison to the sham group (P=0.0002, P=0.001, and P=0.0002; respectively). Furthermore, the results of the cortical area in the right hemisphere revealed a remarkable decrease in edema of the NAC+MCAO group (74.13±2.07) in comparison with the MCAO group (79.90±5.66) (P=0.002) (Figure 4A). In addition, there was a reduction in the Pri-Amy area of the NAC+ MCAO group (74.10±1.50) compared to the MCAO group (79.45±3.65) and this difference was significant (P=0.01) (Figure 4B). Also, the cerebral edema of the striatal area in the right hemisphere of the NAC+ MCAO group (74.23±2.62) was reduced when compared with the MCAO group (78.40±5) (P=0.008) (Figure 4C).

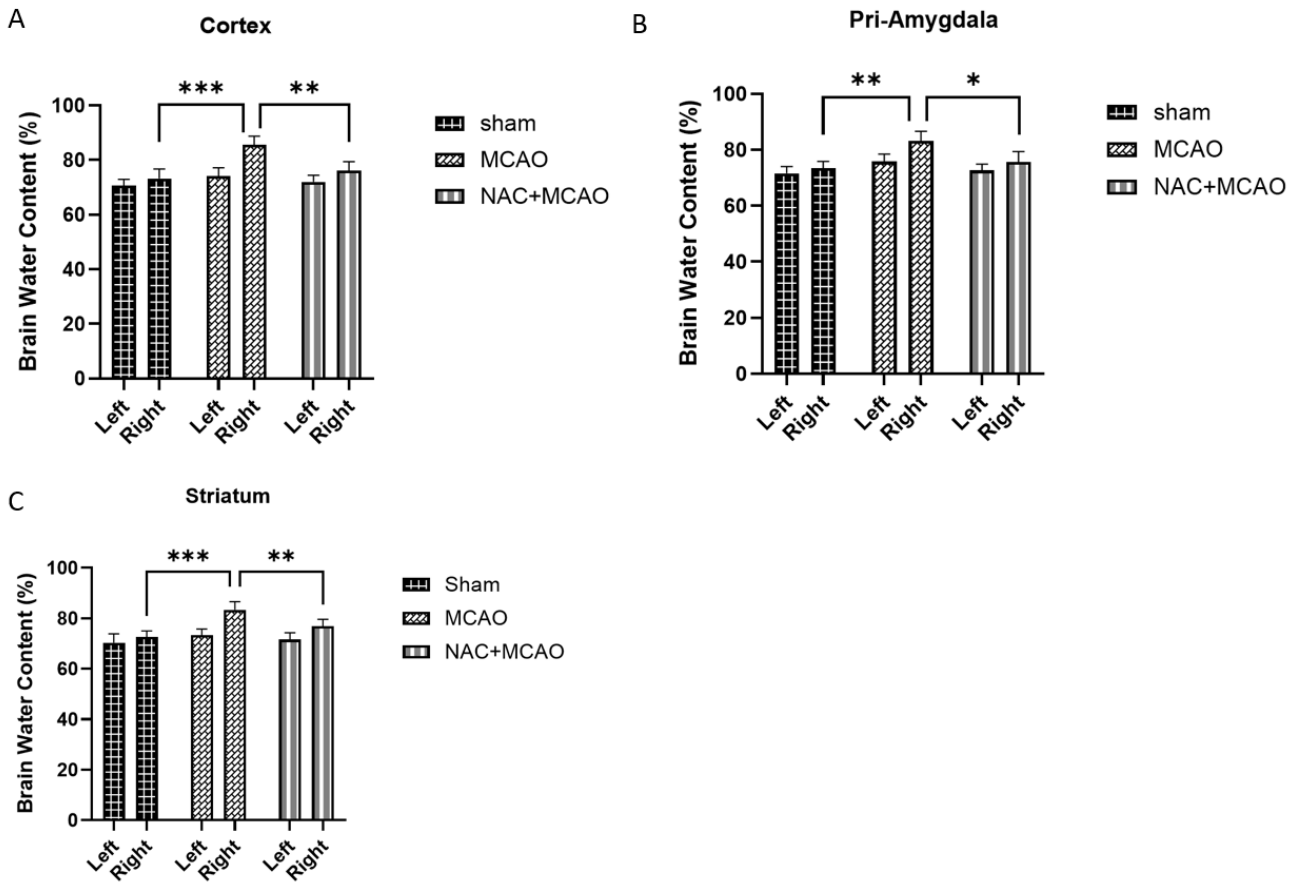

Fig. 4 The effect of N-acetylcysteine (NAC) on cerebral edema 24 h after MCAO surgery in cortex (A), piriform cortex-amygdala (Pri-Amygdala) (B), and striatum (C) areas of rat brain. One-way ANOVA; Each column represents Mean ± SEM (n=5). *P<0.05, **P<0.01, and ***P<0.001. Sham, surgery without MCAO; MCAO (control), ischemia without treatment; MCAO+NAC, ischemia with NAC treatment.

3.4. The Effect of N-acetylcysteine (NAC) on Blood-brain Barrier Permeability

The detection of Evans Blue amount in brain tissue reflects permeability of the blood-brain barrier, leading to the release of Evans Blue from the blood vessels into cerebral tissue. In three regions of

the left hemisphere, no significant difference was observed between the groups. The statistical comparison of experimental groups indicated that the Evans Blue amount in the cortex, Pri-Amy, and striatum areas of the right hemisphere in the MCAO group was significantly increased in comparison to the sham group (P=0.0006, P=0.003, P=0.001, and P=0.0002; respectively). In addition, the results indicated that the permeability of the BBB in the cortex of the right hemisphere in the NAC+ MCAO group (0.106±0.024) was attenuated compared to the MCAO group (0.162±0.055) (P=0.01) (Figure 5A). Similarly, Evans Blue amount in the Pri-Amy region of the NAC+MCAO group (0.186±0.048) showed a significant reduction compared to the MCAO group (0.349±0.162) (P=0.01) (Figure 5B). A remarkable decrease in the striatum of the NAC+ MCAO group (0.174±0.052) was also observed compared with the MCAO group (0.279±0.117) (P=0.03) (Figure 5C).

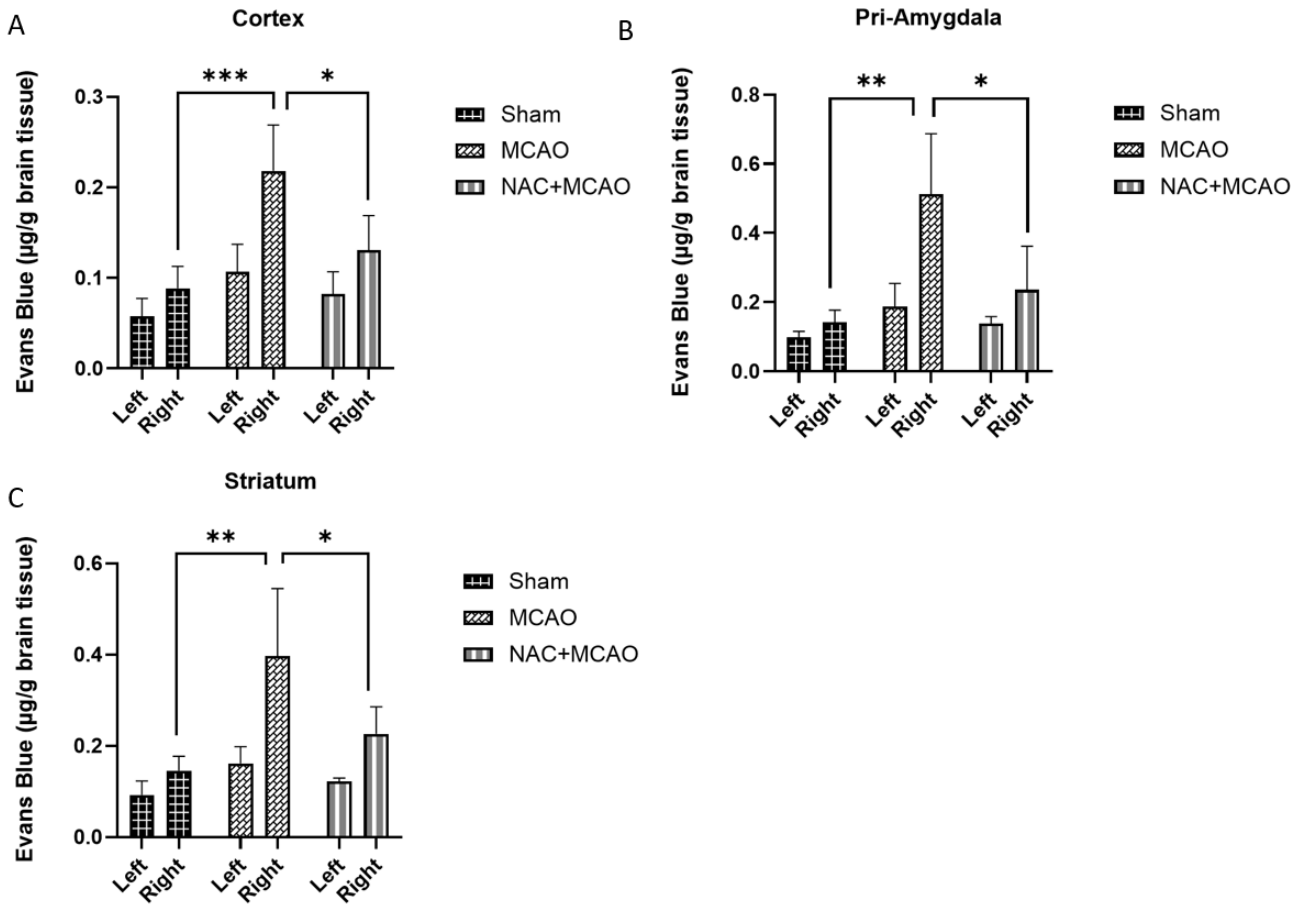

Fig. 5 The effect of N-acetylcysteine (NAC) on the permeability of the blood-brain barrier in the MCAO, sham, and NAC+ MCAO groups 24 h after MCAO surgery in cortex (A), piriform cortex-amygdala (Pri-Amygdala) (B), and striatum (C) areas of rat brain. One-way ANOVA; Each column represents Mean ± SEM (n=5). *P<0.05, **P<0.01, and ***P<0.001. Sham, surgery without MCAO; MCAO (control), ischemia without treatment; MCAO+NAC, ischemia with NAC treatment.

3.5. The Effect of N-acetylcysteine (NAC) on the Expression of *miR-342-3p* Gene

The expression of the *miR-342-3p* was individually assessed in the cortex, Pri-Amy, and striatum regions. The results indicated that *miR-342-3p* level gene did not change following injection of NAC

in NAC-only group rats. As illustrated in Figure 6, ischemia in three areas of rat brain in the MCAO group markedly led to a reduction in *miR-342-3p* level compared to the sham group (*P*<0.001). Moreover, the administration of NAC after induction of ischemia (0.91±0.079) resulted in a significant elevation in the *miR-342-3p* level of the cortex region compared to the MCAO group (0.37±0.051) (*P*=0.0004*)* (Figure 6A). Furthermore, the *miR-342-3p* level in the Pri-Amy region of the NAC+MCAO group (0.83±0.11) was enhanced compared with the MCAO group (0.41±0.041) (*P*=0.006) (Figure 6B). Also, the NAC+ MCAO group (0.82±0.075) displayed an elevation in the level of *miR-342-3p* gene in the striatum compared to the MCAO group (0.26±0.067) (*P*<0.001) (Figure 6C).

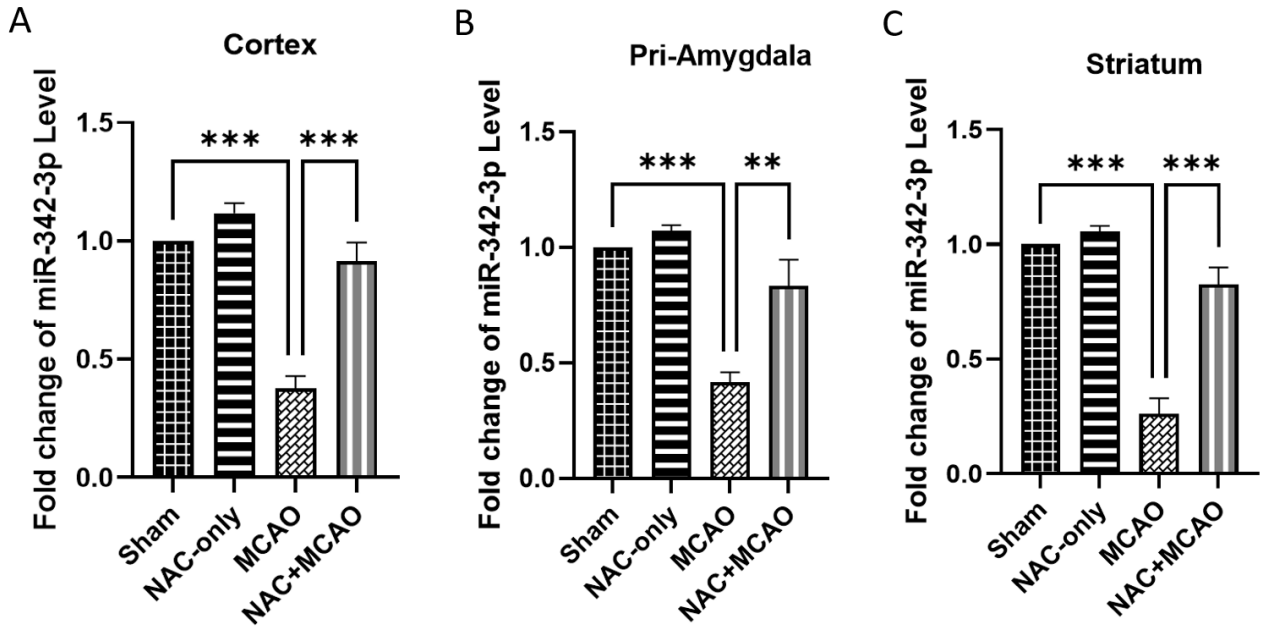

Fig. 6 The effect of N-acetylcysteine (NAC) on the expression of *miR-342-3P* level in cortex (A), piriform cortex-amygdala (Pri-Amygdala) (B), and striatum (C) areas of rat brain. One-way ANOVA test; Each column represents Mean ± SEM (n=5). ***P*<0.01 and ****P*<0.001. Sham, surgery without MCAO; MCAO (control), ischemia without treatment; NAC-only, NAC administration without MCAO; MCAO+NAC, ischemia with NAC treatment.

3.6. The Effect of N-acetylcysteine (NAC) on the Expression of *Chi3l1* Gene

The data analysis was independently conducted for the cortex, Pri-Amy, and striatum areas between the experimental groups. The findings of the present study indicated a significant increase in the expression of the *Chi3l1* gene in the cortex, Pri-Amy, and striatum areas of the MCAO group in comparison with the sham group (*P*=0.0003, *P*=0.0006, and *P*=0.001; respectively). In addition, *Chi3l1* gene expression in the cortical region of the NAC+MCAO group (0.64±0.043) was down-regulated compared to the MCAO group (3.91±0.53) (*P*=0.0001) (Figure 7A). Furthermore, administration of NAC in ischemic rats (the NAC+MCAO group) (0.45±0.054) attenuated the expression of the *Chi3l1* gene in the Pri-Amy area in comparison with the MCAO group (3.21±0.45) (*P*=0.0001) (Figure 7B). In the striatal area, the down-regulation of the *Chi3l1* gene was also observed in the NAC+MCAO group (0.60±0.095) when compared to the MCAO group (2.71±0.38) (*P*=0.0008) (Figure 7C).

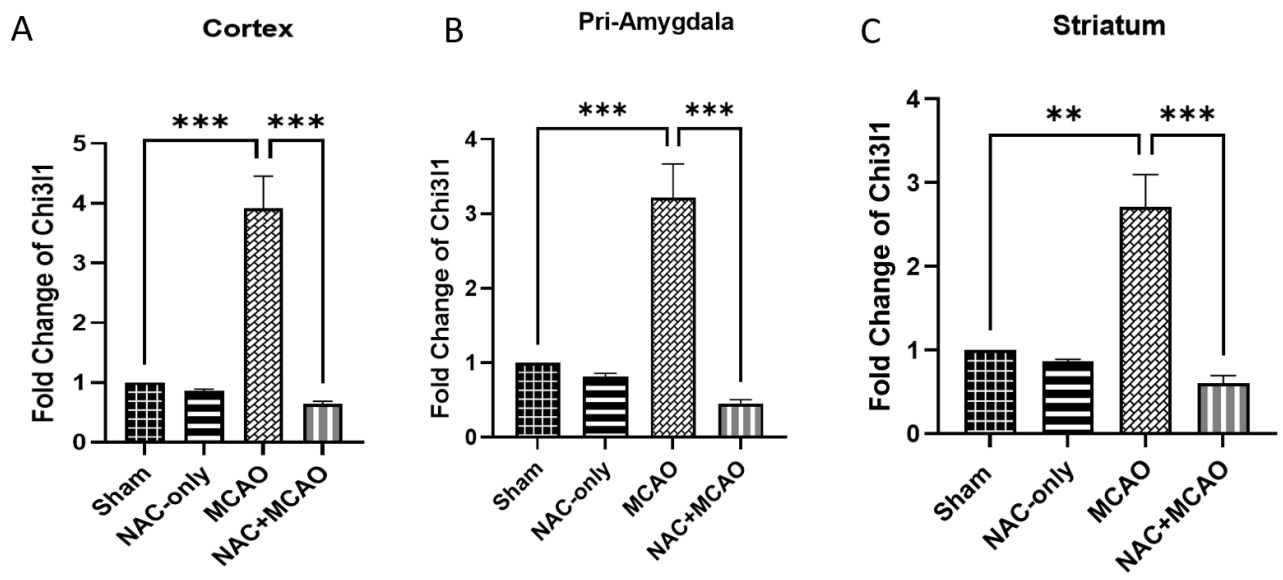

Fig. 7 The effect of N-acetylcysteine (NAC) on *Chi3l1* gene level in cortex (A), piriform cortex-amygdala (Pri-Amygdala) (B), and striatum (C) areas of rat brain. One-way ANOVA test; Each column represents Mean ± SEM (n=5). ***P*<0.01 and ****P*<0.001. Sham, surgery without MCAO; MCAO (control), ischemia without treatment; NAC-only, NAC administration without MCAO; MCAO+NAC, ischemia with NAC treatment.

3.7. Region-specific correlation between *miR-342-3p* and *Chi3l1*

A strong negative correlation between *miR-342-3p* and *Chi3l1* expression was observed across three brain regions. Specifically, in the cortex, pri-amygdala, and striatum, the correlation coefficients were r=–0.907 (*P*<0.0001), r = –0.815 (*P*=0.0013), and r= –0.825 (*P*=0.001), respectively (Figure 8A-C). These results indicate a consistent inverse association between *miR-342-3p* and *Chi3l1* across all examined regions, suggesting a robust regulatory relationship.

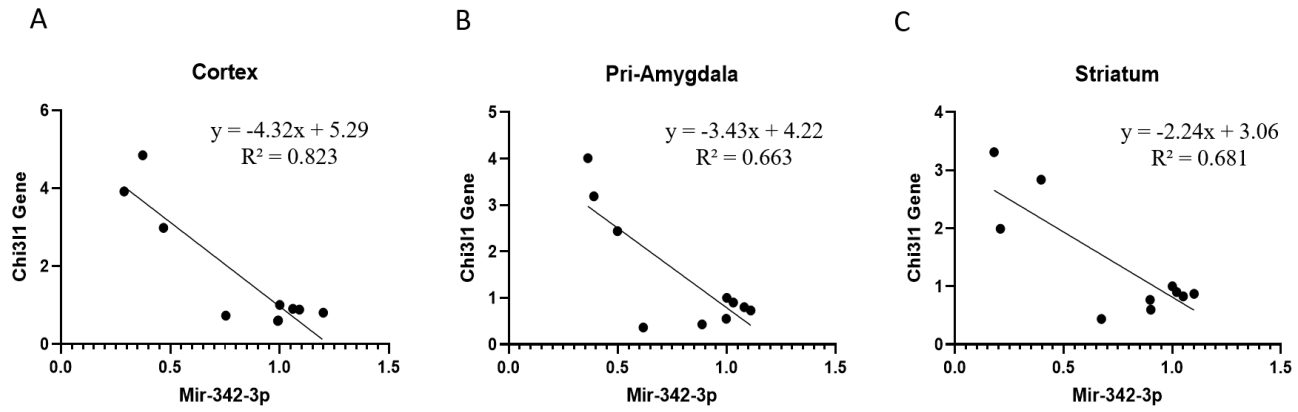

Fig. 8 Correlation between *miR-342-3p* and *Chi3l1* levels in the cortex (A), piriform cortex-amygdala (Pri-Amygdala) (B), and striatum (C) regions of the rat brain. A significant correlation was observed between the NAC-induced increase in *miR-342-3p* and the corresponding decrease in *Chi3l1* expression ($P<0.0001$, $P<0.01$, and $P<0.001$, respectively).

4. Discussion

The results of this study indicated that administering NAC following ischemia induction in rats alleviated ischemic damage, including neurological deficits, infarction volume, cerebral edema, and BBB breakdown.

In this investigation, NAC alleviated various types of behavioral deficits caused by stroke. Previous evidence also indicated that reperfusion led to an increase in the expression of tumor necrosis factor (TNF-α) and inducible nitric oxide synthase (iNOS), while the administration of NAC alleviated these cytokines, resulting in a 50% reduction in neurological deficits [21]. NAC not only directly neutralizes free radicals by providing sulfhydryl groups but also enhances antioxidant activity in the cells by restoring intracellular GSH (glutathione) [11]. Hence, NAC probably improved neurological outcomes due to its anti-inflammatory and antioxidant properties. Also, a decrease in infarction

was observed following the administration of NAC. Similarly, it was reported that the combination of NAC and normobaric hyperoxia (NBO) treatment in a rat model of cerebral ischemia effectively reduced tissue infarction [22]. Infarcted tissue is known as a result of neuronal death. It was demonstrated that the application of NAC can notably restrict the damage of ischemic stroke by regulating glutamate expression [22–24]. Consistently, NAC inhibited caspase activation, reduced apoptosis, and enhanced angiogenesis [25]. In line with these observations, recent evidence has demonstrated that NAC markedly attenuates neuronal apoptosis following cerebral ischemia–reperfusion by modulating intrinsic mitochondrial apoptotic pathways. Specifically, NAC has been shown to reduce cytochrome c release and caspase-3 activation, while restoring the NGF–Akt/Bad survival signaling axis in hippocampal neurons, ultimately limiting ischemia-induced neuronal loss[26]. Therefore, it can be stated that NAC may reduce the infarction due to its anti-apoptotic and anti-excitotoxic properties. Moreover, the result of this study showed that NAC significantly attenuated the cerebral water content and BBB permeability [23]. In agreement with the present result, it was reported that NAC affected blood-brain barrier permeability through ROS-induced changes [27,28]. Previous reports revealed that NAC regulated cytokines by inhibiting nuclear factor kappa B (NF-κB), subsequently resulting in a decrease of TNF-α, interleukin 1 (IL-1), iNOS, and ED1 levels [14,29]. Supporting this mechanism, NAC has also been reported to suppress oxidative stress–induced apoptosis and inflammation in ischemia-related tissue injury through inhibition of the TLR4/NF-κB/NLRP3 signaling axis. By attenuating NF-κB activation and downstream inflammasome components such as NLRP3, NAC effectively reduces caspase-dependent apoptosis and tissue injury, highlighting its dual antioxidant and anti-inflammatory actions[30]. Other anti-inflammatory effects of NAC have also been reported, including the reduction of pro-inflammatory cytokines such as IL-6, malondialdehyde (MDA), soluble intercellular adhesion molecule-1 (sICAM-1), nitric oxide (NO), and neuron-specific enolase [12]. Inflammation and oxidative stress signaling pathways are

considered the main contributors to BBB breakdown and increased cerebral water content. Therefore, it is hypothesized that NAC mitigates the ischemic damage mentioned above through its anti-inflammatory and antioxidant properties.

The results of this study confirmed the expression of the *miR-342-3p* gene and its downstream *Chi3l1* gene 24 hours after ischemia induction. NAC did not affect *miR-342-3p* or *Chi3l1* levels under non-ischemic conditions, but its regulatory effect was evident in the context of ischemic stroke. In line with these findings, previous studies have demonstrated that NAC exerts neuroprotective effects in cerebral ischemia–reperfusion models by reducing infarct size, suppressing inflammatory responses, and reversing ischemia-induced alterations in specific microRNAs and inflammatory signaling pathways[31]. Moreover, a strong negative correlation between *miR-342-3p* and *Chi3l1* expression was observed across three brain regions. This suggests that *miR-342-3p* may play a key role in controlling *Chi3l1* expression in regions affected by ischemic injury. The correlations were particularly pronounced in the cortex and pri-amygdala, areas known for prominent inflammatory and glial responses, and were also robust in the striatum, highlighting the broad relevance of the *miR-342-3p–Chi3l1* interaction across anatomically distinct, ischemia-vulnerable brain regions.

While these data support a strong associative link, they do not establish causality. Future mechanistic studies using *miR-342-3p* gain- or loss-of-function approaches, together with protein-level validation of *Chi3l1*, will be necessary to confirm a direct regulatory effect. Nevertheless, the consistent and significant inverse correlations observed across three brain regions highlight the potential importance of *miR-342-3p–Chi3l1* interactions in the context of ischemic brain injury. In line with these correlations, NAC alleviated the decrease in *miR-342-3p* and the increase in *Chi3l1* expression. It is plausible that the observed regulation of *miR-342-3p* by NAC is mediated through its antioxidant actions. NAC restores GSH and lowers ROS, suppressing NF-κB–driven oxidative

stress and inflammation, while preventing pathological tissue remodeling and supporting neuroprotection in ischemic stroke [32,33]. Since these transcription factors are critically involved in inflammation and oxidative stress responses, their inhibition could relieve the suppression of anti-inflammatory microRNAs such as *miR-342-3p*. In parallel, the reduction of pro-inflammatory mediators like TNF-α and IL-1β by NAC may further contribute to the downregulation of *Chi3l1* [33,34]. Therefore, it can be speculated that NAC influences upstream redox-sensitive signaling pathways to modulate the *miR-342-3p*/*Chi3l1* axis, ultimately leading to reduced ischemic brain injury.

These results suggest that the protective effects of NAC are likely exerted through interactions with pathways related to ischemia/inflammation, rather than through baseline changes in the expression of these genes. This study is the first attempt to indicate the effect of NAC on the expression levels of the mentioned genes. A previous study reported the inverse relationship between *miR-342-3p* and *Chi3l1*. The finding showed that the absence of the *Chi3l1* gene in arterial endothelium might prevent the progression of arterial atherosclerosis. Moreover, *miR-342-3p* attenuated atherosclerosis by reducing *Chi3l1* levels in arterial endothelium [9]. It is obvious that atherosclerosis is the main cause of stroke.

It can be hypothesized that inflammation, as a key pathophysiological pathway, plays a potential role in triggering subsequent brain damage following ischemia. Changes in the expression of certain genes and microRNAs involved in inflammation may serve as important markers for reducing ischemic injury. Conflicting reports exist regarding the role of *miR-342-3p* in inflammatory pathways. It has been reported that *miR-342-3p* is involved in inflammation-related disorders, particularly cardiovascular diseases, leading to a reduction in inflammatory cytokines such as IL-6, IL-7, IL-8, and TNF-α. Conversely, *miR-342-3p* can activate MAP3K1, IKBKγ, and PDGFB, indirectly suppressing inflammation [35]. Furthermore, the *Chi3l1* gene plays a crucial role in oxidative damage,

apoptosis regulation, and inflammation modulation. Excessive *Chi3l1* expression is observed in various inflammatory conditions, including diabetes, asthma, cirrhosis, sepsis, rheumatoid arthritis, preeclampsia, and coronary artery disease [36]. Elevated TNF-α and IL-1β also enhance *Chi3l1* expression [37]. In neurological diseases such as multiple sclerosis, increased *Chi3l1* expression is observed in regions of active demyelination [38]. Given the role of *miR-342-3p* in reducing inflammation and targeting *Chi3l1*, the effect of NAC on these factors could lead to further decrease of inflammation and consequently neuroprotection. NAC may have been able to control inflammation process in ischemic conditions by interfering the expression and activity levels of the *miR-342-3p* and *Chi3l1*. However, to present this hypothesis accurately, it is necessary to investigate whether the observed changes in the activity of these factors are directly related to anti-inflammatory or anti-oxidant effects of NAC. Importantly, these apparently conflicting reports are likely explained by the fact that the role of *miR-342-3p* is highly context-dependent, varying with the cell type involved (e.g., neurons, microglia, or endothelial cells) and the disease model under investigation (ischemia, atherosclerosis, or autoimmune disorders). This variability highlights the importance of studying *miR-342-3p* specifically within the ischemic stroke context.

5. Conclusion

In general, two main findings were achieved in this research: 1) the neuroprotective effect of NAC on reducing stroke damage in an animal model, and 2) the alleviating effect of NAC on the expression of *miR-342-3p* and a related gene called *Chi3l1* in cerebral ischemic conditions. As was mentioned above, this hypothesis that NAC may attenuate the ischemic injuries through the modulation of *miR-342-3p* and *Chi3l1* factors and the reduction of inflammation is strengthened. There is hope that further research into the pathways associated with the mentioned factors (*miR-*

342-3p and *Chi3l1*), including the inflammatory pathway, as well as investigating the upstream and downstream factors of these pathways, will enable these factors to be used as biomarkers for both diagnosis and monitoring the treatment process of stroke.

References

1. Thayabaranathan T, Kim J, Cadilhac DA, et al. Global stroke statistics 2022. *Int J Stroke*. 2022;17(9):946-956. doi:10.1177/17474930221123175

2. Kuriakose D, Xiao Z. Pathophysiology and Treatment of Stroke: Present Status and Future Perspectives. *Int J Mol Sci*. 2020;21(20):7609. doi:10.3390/ijms21207609

3. Zheng T, Shi Y, Zhang J, et al. MiR-130a exerts neuroprotective effects against ischemic stroke through PTEN/PI3K/AKT pathway. *Biomedicine & Pharmacotherapy*. 2019;117:109117. doi:10.1016/j.biopha.2019.109117

4. Anrather J, Iadecola C. Inflammation and Stroke: An Overview. *Neurotherapeutics*. 2016;13(4):661-670. doi:10.1007/s13311-016-0483-x

5. Li G, Morris-Blanco KC, Lopez MS, et al. Impact of microRNAs on ischemic stroke: From pre- to post-disease. *Progress in Neurobiology*. 2018;163-164:59-78. doi:10.1016/j.pneurobio.2017.08.002

6. Czimmerer Z, Varga T, Kiss M, et al. The IL-4/STAT6 signaling axis establishes a conserved microRNA signature in human and mouse macrophages regulating cell survival via miR-342-3p. *Genome Med*. 2016;8:63. doi:10.1186/s13073-016-0315-y

7. Fu B, Lin X, Tan S, et al. MiR-342 controls Mycobacterium tuberculosis susceptibility by modulating inflammation and cell death. *EMBO Rep*. 2021;22(9):e52252. doi:10.15252/embr.202052252

8. Mohammadinasr M, Montazersaheb S, Molavi O, et al. Multiplex Analysis of Cerebrospinal Fluid and Serum Exosomes MicroRNAs of Untreated Relapsing Remitting Multiple Sclerosis (RRMS) and Proposing Noninvasive Diagnostic Biomarkers. *NeuroMolecular Medicine*. 2023;25(3):402-414. doi:10.1007/s12017-023-08744-3

9. Ray SL, Coulson DJ, Yeoh MLY, et al. The Role of miR-342 in Vascular Health. Study in Subclinical Cardiovascular Disease in Mononuclear Cells, Plasma, Inflammatory Cytokines and PANX2. *Int J Mol Sci*. 2020;21(19):7217. doi:10.3390/ijms21197217

10. Kim DH, Choi JM. Chitinase 3-like-1, a novel regulator of Th1/CTL responses, as a therapeutic target for increasing anti-tumor immunity. *BMB Rep*. 2018;51(5):207-208. doi:10.5483/BMBRep.2018.51.5.094

11. Bavarsad Shahripour R, Harrigan MR, Alexandrov AV. N-acetylcysteine (NAC) in neurological disorders: mechanisms of action and therapeutic opportunities. *Brain Behav*. 2014;4(2):108-122. doi:10.1002/brb3.208

12. Sabetghadam M, Mazdeh M, Abolfathi P, Mohammadi Y, Mehrpooya M. Evidence for a Beneficial Effect of Oral N-acetylcysteine on Functional Outcomes and Inflammatory Biomarkers in Patients with Acute Ischemic Stroke. *Neuropsychiatr Dis Treat*. 2020;16:1265-1278. doi:10.2147/NDT.S241497

13. Khoshnam SE, Winlow W, Farzaneh M. The Interplay of MicroRNAs in the Inflammatory Mechanisms Following Ischemic Stroke. *Journal of Neuropathology & Experimental Neurology*. 2017;76(7):548-561. doi:10.1093/jnen/nlx036

14. Khan M, Sekhon B, Jatana M, et al. Administration of N-acetylcysteine after focal cerebral ischemia protects brain and reduces inflammation in a rat model of experimental stroke. *Journal of Neuroscience Research*. 2004;76(4):519-527. doi:10.1002/jnr.20087

15. Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke*. 1989;20(1):84-91. doi:10.1161/01.str.20.1.84

16. Chen J, Li Y, Wang L, et al. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke*. 2001;32(4):1005-1011. doi:10.1161/01.str.32.4.1005

17. Benedek A, Móricz K, Jurányi Z, et al. Use of TTC staining for the evaluation of tissue injury in the early phases of reperfusion after focal cerebral ischemia in rats. *Brain Research*. 2006;1116(1):159-165. doi:10.1016/j.brainres.2006.07.123

18. Vahidi S, Bigdeli MR, Shahsavarani H, Ahmadloo S, Roghani M. Neuroprotective Therapeutic Potential of microRNA-149-5p against Murine Ischemic Stroke. *Molecular Neurobiology*. Published online April 4, 2024:1-18. doi:10.1007/s12035-024-04159-8

19. Mardookhi J, Bigdeli MR, Khaksar S. The effect of pre-treatment with olive oil on TNFR1/NF-κB inflammatory pathway in rat ischemic stroke model. *Physiology and Pharmacology*. Published online November 10, 2016. Accessed September 7, 2025. http://ppj.phypha.ir/article-1-1203-en.html

20. Fernández-López D, Faustino J, Daneman R, et al. Blood–Brain Barrier Permeability Is Increased After Acute Adult Stroke But Not Neonatal Stroke in the Rat. *J Neurosci*. 2012;32(28):9588-9600. doi:10.1523/JNEUROSCI.5977-11.2012

21. Sekhon B, Sekhon C, Khan M, Patel SJ, Singh I, Singh AK. *N*-Acetyl cysteine protects against injury in a rat model of focal cerebral ischemia. *Brain Research*. 2003;971(1):1-8. doi:10.1016/S0006-8993(03)02244-3

22. Liu Y, Liu WC, Sun Y, et al. Normobaric Hyperoxia Extends Neuro- and Vaso-Protection of N-Acetylcysteine in Transient Focal Ischemia. *Molecular Neurobiology*. 2016;54(5):3418-3427. doi:10.1007/s12035-016-9932-0

23. Chen G, Shi J, Hu Z, Hang C. Inhibitory Effect on Cerebral Inflammatory Response following Traumatic Brain Injury in Rats: A Potential Neuroprotective Mechanism of N-Acetylcysteine. *Mediators Inflamm*. 2008;2008:716458. doi:10.1155/2008/716458

24. Krzyżanowska W, Pomierny B, Bystrowska B, et al. Ceftriaxone- and N-acetylcysteine-induced brain tolerance to ischemia: Influence on glutamate levels in focal cerebral ischemia. *PLoS One*. 2017;12(10):e0186243. doi:10.1371/journal.pone.0186243

25. Ali M, Tabassum H, Alam MM, Parvez S. N-acetyl-L-cysteine ameliorates mitochondrial dysfunction in ischemia/reperfusion injury via attenuating Drp-1 mediated mitochondrial autophagy. *Life Sciences*. 2022;293:120338. doi:10.1016/j.lfs.2022.120338

26. Saniei H, Naderi R. The effect of N-acetylcysteine on apoptosis and NGF-Akt/Bad pathway in the hippocampus tissue of cerebral ischemia-reperfusion in male rats. *Metabolic Brain Disease*. 2025;40(5):217. doi:10.1007/s11011-025-01641-7

27. Abdullahi W, Tripathi D, Ronaldson PT. Blood-brain barrier dysfunction in ischemic stroke: targeting tight junctions and transporters for vascular protection. *Am J Physiol Cell Physiol*. 2018;315(3):C343-C356. doi:10.1152/ajpcell.00095.2018

28. Amouri A, Waker C, Huang L, Smith C, Mayes D. N-Acetylcysteine Modulation of Mitochondrial Respiration and Blood Brain Barrier Permeability Is Time Dependent and Cell Type Specific. *Molecular Biology*. 2018;3(6):266-276. doi:10.15406/ijmboa.2018.03.00086

29. Yamamoto Y, Gaynor RB. Therapeutic potential of inhibition of the NF-κB pathway in the treatment of inflammation and cancer. *J Clin Invest*. 2001;107(2):135-142.

30. Amini H, Shirpoor A, Naderi R. Nandrolone decanoate induces heart injury via oxidative damage and mitochondrial apoptotic pathway by regulation of TLR4/NF-κB/NLRP3 axis in male rats: The rescue effect of *N*-acetylcysteine. *Steroids*. 2025;214:109563. doi:10.1016/j.steroids.2025.109563

31. Saniei H, Shirpoor A, Naderi R. Cerebral Ischemia-Reperfusion Induced Neuronal Damage, Inflammation, miR-374a-5p, MAPK6, NLRP3, and Smad6 Alterations: Rescue Effect of N-acetylcysteine. *Pharmaceutical Sciences*. 2024;30(4):502-511. doi:10.34172/PS.2024.28

32. Sztolsztener K, Bzdęga W, Hodun K, Chabowski A. N-Acetylcysteine Decreases Myocardial Content of Inflammatory Mediators Preventing the Development of Inflammation State and Oxidative Stress in Rats Subjected to a High-Fat Diet. *Int J Inflam*. 2023;2023:5480199. doi:10.1155/2023/5480199

33. Cui Y, Zhu Q, Hao H, Flaker GC, Liu Z. N-Acetylcysteine and Atherosclerosis: Promises and Challenges. *Antioxidants*. 2023;12(12):2073. doi:10.3390/antiox12122073

34. Liu D, Hu X, Ding X, Li M, Ding L. Inflammatory Effects and Regulatory Mechanisms of Chitinase-3-like-1 in Multiple Human Body Systems: A Comprehensive Review. *International Journal of Molecular Sciences*. 2024;25(24):13437. doi:10.3390/ijms252413437

35. Jung YY, Kim KC, Park MH, et al. Atherosclerosis is exacerbated by chitinase-3-like-1 in amyloid precursor protein transgenic mice. *Theranostics*. 2018;8(3):749-766. doi:10.7150/thno.20183

36. Zhao T, Su Z, Li Y, Zhang X, You Q. Chitinase-3 like-protein-1 function and its role in diseases. *Signal Transduct Target Ther*. 2020;5:201. doi:10.1038/s41392-020-00303-7

37. Chen X, Jiao J, He X, et al. CHI3L1 regulation of inflammation and the effects on osteogenesis in a Staphylococcus aureus-induced murine model of osteomyelitis. *The FEBS Journal*. 2017;284(11):1738-1747. doi:10.1111/febs.14082

38. Talaat F, Abdelatty S, Ragaie C, Dahshan A. Chitinase-3-like 1-protein in CSF: a novel biomarker for progression in patients with multiple sclerosis. *Neurological Sciences*. 2023;44(9):3243-3252. doi:10.1007/s10072-023-06764-2