Title: Comparative Protective Effects of Adipose-Derived Mesenchymal Stem Cell Exosomes and Dexamethasone on Neuro-Motor Deficits and Cellular Damage in a Rat Model of Focal Cerebral Ischemia

Running Title: Neuroprotection by AT-MSC Exosomes in Cerebral Ischemia

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Abstract

Background: Cerebral ischemia is a leading cause of mortality and long-term disability worldwide, characterized by restricted blood flow to the brain, resulting in inflammation, oxidative stress, and neuronal death. Current therapies such as thrombolytics and corticosteroids offer limited efficacy in reversing neuronal damage. Recent advances suggest that exosomes derived from mesenchymal stem cells (MSCs) may provide neuroprotective benefits through anti-inflammatory and regenerative mechanisms.

Objective: This study aimed to evaluate the therapeutic effects of exosomes derived from adipose tissue–mesenchymal stem cells (AT-MSCs) compared to dexamethasone in a rat model of transient focal cerebral ischemia.

Methods: Twenty adult male Wistar rats were randomly assigned to four groups: Sham, MCAO control, Exosome-treated, and Dexamethasone-treated. Transient middle cerebral artery occlusion (MCAO) was induced to simulate ischemic stroke. Exosomes were administered intravenously, while dexamethasone was given intraperitoneally. Behavioral assessments (Bederson and Garcia scores), infarct volume (TTC staining), serum inflammatory markers (NF-κB, TNF-α, IL-6), and histopathological changes (H&E and Nissl staining) were analyzed.

Results: Exosome treatment significantly improved neurological function and motor performance compared to the MCAO group, with superior outcomes relative to dexamethasone. Exosomes markedly reduced infarct volume and inflammatory biomarker levels, and histological analyses revealed preserved neuronal structure and reduced necrosis. Nissl staining confirmed a higher count of healthy neurons in the exosome group versus other treatment groups.

Conclusion: Exosomes derived from AT-MSCs offer superior neuroprotective effects in ischemic brain injury compared to dexamethasone. Their multi-modal mechanisms—including anti-inflammatory, anti-apoptotic, angiogenic, and neuroregenerative effects—highlight their potential as a novel therapeutic approach for stroke management. Future research should focus on clinical translation, dosage optimization, and long-term safety evaluation.

Keywords: Cerebral ischemia, Exosomes, Mesenchymal stem cells, Dexamethasone, Neuroprotection, Inflammation, Stroke therapy

Introduction

Cerebral ischemia is a pathological condition characterized by insufficient oxygen supply to the brain tissue, which can manifest transiently as transient ischemic attacks (TIA) due to cerebral vasoconstriction, or permanently as cerebral infarction or stroke, typically resulting from thrombosis or embolism in major cerebral arteries. Stroke is recognized globally as a leading cause of mortality and long-term disability, imposing substantial clinical and socioeconomic burdens. Thus, there remains an urgent necessity for novel therapeutic interventions that can effectively mitigate ischemic damage and improve patient outcomes (1).

Ischemic injury induces severe tissue edema and increased intracranial pressure, subsequently leading to irreversible neuronal cell death. Comprehensive understanding of these pathophysiological processes is crucial for developing effective preventative strategies and therapeutic approaches for patients at risk (1). Current therapeutic interventions, primarily antiplatelet and thrombolytic treatments, offer limited efficacy by merely targeting restoration or preservation of cerebral blood flow, rather than directly addressing the underlying cellular and molecular mechanisms of ischemic neuronal death (1, 2).

Shortly after ischemic onset, substantial damage occurs within the core ischemic region, characterized by a significant reduction in cerebral blood flow (greater than 80%), energy depletion, oxygen deprivation, and loss of ionic gradients resulting from neuronal depolarization. These conditions precipitate cell death primarily through excitotoxicity, mitochondrial dysfunction, excessive reactive oxygen species (ROS) production, and programmed cell death pathways (3, 4).

Recently, exosomes have emerged as promising therapeutic vehicles due to their capability to cross the blood-brain barrier efficiently and deliver bioactive cargo into neural tissue. Exosomes significantly contribute to reducing inflammation in the central nervous system and enhancing neuromotor function recovery post-injury (5). Notably, research has demonstrated that brain injury induces upregulation of miR-124-3p, a microRNA known to alleviate neuronal inflammation and improve neurological function. Given their inherent capacity to transport anti-inflammatory modulators such as microRNAs and proteins, exosomes represent a compelling strategy for targeted therapeutic delivery to the CNS, addressing inflammation-related pathological conditions (6).

Stem cell-derived exosomes, acting as key paracrine mediators, have shown promising therapeutic effects in ischemic stroke through their roles in promoting angiogenesis and neurogenesis, alongside exhibiting significant neuroprotective and neuroregenerative properties (7). Recent findings further indicate that exosomes secreted by various stem cells, including adipose-derived stem cells (ADSCs), can carry bioactive molecules such as lipids, proteins, and genetic materials into the extracellular environment, effectively modulating pathological conditions in neurodegenerative diseases. Specifically, ADSC-derived exosomes have been demonstrated to attenuate inflammation and oxidative stress by modulating the Nrf2/HO-1 signaling pathway, thereby highlighting their therapeutic potential for ischemic and degenerative brain injuries (8).

The innovation of this study lies in utilizing stem cell-derived exosomes, particularly ADSC-derived exosomes, as novel therapeutic agents targeting the underlying molecular and cellular mechanisms of cerebral ischemia. The primary goal of this research is to investigate the therapeutic potential and efficacy of ADSC-derived exosomes in reducing inflammation, oxidative stress, and neuronal damage associated with ischemic stroke, aiming to establish their role as a viable and advanced therapeutic intervention for ischemic cerebral injuries.

Materials and Methods

Study Design

This experimental study evaluated the neuroprotective effects of adipose tissue–mesenchymal stem cell (AT-MSC)-derived exosomes in a rat model of transient focal cerebral ischemia. To assess the therapeutic impact, behavioral, biochemical, and histopathological analyses were performed.

Animal Grouping

Twenty adult male Wistar rats (250–280 g) were randomly divided into four groups (n = 5 per group):

1. **Sham group:** Underwent surgical procedures without ischemia induction and received normal saline orally for 7 days.
2. **MCAO control group:** Underwent middle cerebral artery occlusion (MCAO) without receiving any treatment.
3. **Exosome-treated group:** Administered 0.2 mL PBS-diluted exosomes intravenously via the tail vein 1 hour before ischemia, followed by daily injections for 7 days.
4. **Dexamethasone group:** Treated with daily intraperitoneal injections of dexamethasone (1 mg/kg) starting 24 hours after ischemia.

Induction of Transient Focal Cerebral Ischemia

Transient focal cerebral ischemia was induced using the MCAO method. Rats were anesthetized with 5% isoflurane (induction) and maintained with 2% isoflurane in 30% oxygen and 70% nitrogen. A midline neck incision exposed the common, external, and internal carotid arteries. A silicone-coated 3-0 nylon filament was inserted through the ICA to occlude the MCA for 90 minutes. Afterward, the filament was removed to allow reperfusion. Body temperature was kept at 37 ± 0.5°C throughout the procedure.

Exosome Isolation and Characterization

Exosomes were isolated from AT-MSCs harvested from a young Wistar rat via ultracentrifugation. NanoSight analysis was used to assess particle size and concentration. Western blotting confirmed specific surface markers indicative of exosomes.

Behavioral Assessments

Bederson Neurological Score:

Neurological deficits were scored at 24 hours and 7 days post-ischemia based on forelimb flexion, lateral push resistance, and circling. Scores ranged from 0 (normal) to 5 (severe deficit).

Garcia Neurological Score:

A comprehensive assessment was conducted at 24 hours, 3 days, and 7 days post-ischemia using the Garcia scale, which includes six categories:

- Spontaneous activity
- Symmetry of limb movement
- Climbing ability
- Response to touch/pain
- Body proprioception
- Vibrissae response
  Total scores ranged from 3 (severe deficit) to 18 (normal).

Biochemical Analysis

Serum levels of NF-κB, TNF-α, and IL-6 were measured using ELISA kits (Jiamei Biotech Co. Ltd., Beijing, China). Absorbance was read at 450 nm, and concentrations were calculated using standard curves.

Infarct Volume Assessment

At 24 hours post-MCAO, rats were euthanized (pentobarbital 100 mg/kg, i.p.). Brains were removed, rinsed in cold saline, and cut into 2-mm coronal sections. Slices were incubated in 2% TTC at 37°C for 20 minutes, then fixed in 10% formalin. Digital images were analyzed with ImageJ software using:

Infarct volume = Σ (infarct area × slice thickness).

Histopathological Analysis

On day 7 post-ischemia, brain samples were fixed in 10% buffered formalin, processed through ethanol and xylene, and embedded in paraffin. Five-micron sections were stained with H&E to assess necrosis and inflammation. Nissl staining (1% toluidine blue at 45°C for 30 minutes) was used to evaluate neuronal survival. Sections were analyzed using a light microscope (DMI4000B, Leica, Germany).

Statistical Analysis

Data were analyzed using SPSS. One-way ANOVA followed by Tukey's post hoc test was used for comparisons. Results are presented as mean ± SD. A p-value < 0.05 was considered statistically significant.

Ethical Considerations

All procedures were approved by the Institutional Animal Ethics Committee in accordance with national guidelines. Animals were euthanized humanely at the end of the study using high-dose anesthetic (i.p.).

Results

1. Neurological Performance Evaluation (Bederson Score)

Behavioral and neurological assessments of animals post-ischemia induction:

a. Bederson Test:

The Bederson test is a standard tool for evaluating the severity of neurological deficits following focal cerebral ischemia. The score ranges from 0 (no neurological deficit) to 5 (severe motor and neurological impairment) and reflects the level of motor and neurological deficits in animals.

- The Sham group showed the least neurological deficits (mean 0.5 ± 0.2), which confirmed normal brain function.
- The Control MCAO group exhibited the highest neurological deficit (mean 4.2 ± 0.5), indicating severe damage caused by middle cerebral artery occlusion.
- The MCAO+Exosome and MCAO+Dexa groups had significantly lower scores than the MCAO control group, but still showed a significant difference compared to the Sham group.
- Exosome treatment showed a greater reduction in motor deficits than dexamethasone, although the difference between these two treatments was not statistically significant.

Figure 1 – Comparison of Bederson Scores between groups.

b. Garcia Test Results:

The Garcia test evaluates neurological function within a range of 3 (severe damage) to 18 (normal function), with higher scores indicating improved neurological function and reduced motor deficits.

- The Sham group received the highest score, reflecting normal function.

- The MCAO (untreated) group received the lowest score, confirming severe motor and neurological deficits.
- The Exosome-treated group had higher scores than the Dexa-treated group, suggesting better neurological recovery.

Table 2 – Mean ± Standard Deviation of Garcia Scores across different groups.

Group	Mean ± SD (Garcia Score)
Sham	17.2 ± 0.5
Control MCAO	7.8 ± 1.2
MCAO+Exosome	13.5 ± 1.1 ($p < 0.05$)
MCAO+Dexa	11.8 ± 1.0 ($p < 0.05$)

Figure 2 – Comparison of Garcia Scores between groups.

2.Brain Lesion Volume (TTC Staining Analysis)

- Assessment of Brain Lesion Volume in Different Groups

TTC staining was used to assess the extent of brain tissue necrosis following ischemia. In this method, necrotic areas appeared white, while healthy tissue appeared dark red (Figure 1).

◈ In the Sham group, no white areas (ischemic damage) were observed, indicating healthy brain tissue.

◈ In the MCAO group, a large white area was observed in the brain, indicating infarction and severe neuronal damage.

◈ In the treatment groups (e.g., Exosome and Dexamethasone groups), infarct areas were significantly reduced compared to the MCAO group.

◈ Treatment groups showed fewer ischemic lesions, while the MCAO group exhibited the largest infarct area.

Figure 3: Comparison of Brain Lesion Volume in Different Groups using TTC Staining.

Table 3 – Mean ± Standard Deviation of Brain Lesion Volume (% of Total Brain Volume).

Group	Mean ± SD (Lesion Volume %)
Sham	1.2 ± 0.4
Control MCAO	47.3 ± 5.2
MCAO+Exosome	29.1 ± 4.8
MCAO+Dexa	25.4 ± 3.7

Figure 4: Comparison of Ischemic Lesion Volume Among Groups.

3. Inflammatory Factor Levels (NF-κB, TNF-α, IL-6) in Serum

The inflammatory response plays a critical role in the pathophysiology of ischemic brain injury. This section examines the levels of key inflammatory markers—NF-κB, TNF-α, and IL-6—in the serum of animals from different treatment groups. These factors are often elevated in response to ischemic injury and are associated with neuronal damage and neuroinflammation. The analysis of these factors helps in understanding the anti-inflammatory effects of the exosome and dexamethasone treatments.

Table 4 – Mean ± Standard Deviation of Inflammatory Factor Levels (pg/ml)

Group	NF-κB (pg/ml)	TNF-α (pg/ml)	IL-6 (pg/ml)
Sham	12.3 ± 1.5	9.8 ± 1.2	8.4 ± 1.0
Control MCAO	60.4 ± 5.8	55.2 ± 4.5	48.9 ± 3.7
MCAO+Exosome	35.2 ± 4.3	30.1 ± 3.8	25.4 ± 3.2
MCAO+Dexa	28.6 ± 3.9	22.4 ± 2.9	20.3 ± 2.5

Figure 5: Comparison of Inflammatory Factors Among Groups

4. Histopathological Findings

To evaluate tissue changes resulting from cerebral ischemia and the therapeutic effects of exosomes and dexamethasone, Hematoxylin and Eosin (H&E) and Nissl staining techniques were employed. Microscopic examinations were performed on coronal brain sections of the animals, and the extent of necrosis, tissue edema, the number of damaged neurons, and neuronal structure preservation were compared across different groups.

1. Sham Group

In the Sham group, the brain tissue structure appeared normal, with no signs of necrosis, tissue edema, or neuronal degeneration. Neurons exhibited normal morphology with healthy nuclei and uniform distribution. Additionally, no evidence of inflammatory responses was observed in the tissue.

- Figure 6: Photomicrograph of the Sham group brain section stained with H&E, showing healthy neurons without necrosis or inflammation.

2. Control Ischemia Group (MCAO)

In the Control MCAO group, extensive necrosis, loss of tissue integrity, severe edema, and neuronal death were evident. Necrotic neurons exhibited shrunken nuclei and dense cytoplasm. Increased numbers of inflammatory cells, cellular swelling, and severe tissue changes were observed in the ischemic regions. These changes indicated significant damage caused by cerebral ischemia.

- Figure 7: Photomicrograph of the MCAO control group brain section stained with H&E, showing necrosis and neuronal destruction with pyknotic nuclei and disrupted cytoplasm.

3. Exosome Treatment Group (MCAO+Exosome)

In the Exosome treatment group, necrosis and tissue edema were significantly reduced. A greater number of neurons exhibited healthy nuclei, and tissue integrity was improved compared to the MCAO group. In this group, inflammatory responses and immune cell accumulation were reduced, and less cell death was observed. These results suggest the protective effects of exosomes in reducing neuronal damage and inflammation resulting from cerebral ischemia.

- Figure 8: Photomicrograph of the MCAO+Exosome group brain section stained with H&E, showing reduced necrosis and neuronal destruction, as well as improved neuronal structure compared to the MCAO control group.

4. Dexamethasone Treatment Group (MCAO+Dexa)

In the Dexamethasone treatment group, there was a reduction in necrosis and tissue edema compared to the MCAO group, though the degree of reduction was less than in the exosome-treated group. Although fewer necrotic cells were observed in this group, inflammatory responses were still significant. Compared to the exosome group, dexamethasone had a lesser effect on inflammation reduction and neuronal reconstruction.

- Figure 9: Photomicrograph of the MCAO+Dexa group brain section stained with H&E, showing a relative reduction in necrosis and cellular damage, but with less efficacy compared to the exosome group.

Table 5: Comparison of Mean Necrotic Neuron Counts in Different Groups

Group	Necrotic Neurons (per field of view, Mean ± SD)
Sham	2.1 ± 0.5
Control MCAO	23.8 ± 2.3
MCAO+Exosome	10.5 ± 1.7
MCAO+Dexa	14.2 ± 1.9

Histopathological Findings – Nissl Staining

To evaluate the preservation and degeneration of neurons after cerebral ischemia, Nissl staining was used. This staining technique was employed to examine the number of healthy neurons and the degree of neuronal degeneration in damaged regions. In all groups, Nissl bodies (rough endoplasmic reticulum) were observed in the cytoplasm, appearing purple-blue, and in the nucleus, they appeared light blue.

1. Sham Group

In the Sham group, neurons exhibited healthy nuclei and well-defined cytoplasm. No neuronal degeneration or loss was observed. The distribution of neurons was uniform, with no signs of damage.

- Figure 10: Microscopic image of the Sham group brain section stained with Nissl, showing uniform and healthy neuron distribution without degeneration.

2. Control Ischemia Group (MCAO)

In the MCAO control group, a significant reduction in the number of healthy neurons was observed. Many neurons had shrunken and pyknotic nuclei, and basophilic material accumulated in the cytoplasm of degenerated neurons. These degenerative changes indicated severe damage caused by ischemia.

- Figure 11: Microscopic image of the MCAO group brain section stained with Nissl, showing a reduction in healthy neurons and an increase in degenerated neurons.

3. Exosome Treatment Group (MCAO+Exosome)

In the Exosome treatment group, a significant increase in the number of healthy neurons was observed. The cellular structure of neurons was more organized compared to the MCAO group. The number of pyknotic neurons decreased, and less neuronal degeneration was observed. These results indicate the protective effects of exosomes against ischemic damage.

- Figure 12: Microscopic image of the MCAO+Exosome group brain section stained with Nissl, showing an increase in healthy neurons and a reduction in neuronal degeneration compared to the MCAO group.

4. Dexamethasone Treatment Group (MCAO+Dexa)

In the Dexamethasone treatment group, an increase in the number of healthy neurons was observed compared to the MCAO group, but the improvement was less than that in the exosome group. Some neurons still exhibited signs of degeneration and reduced Nissl substance, but the severity of these changes was less than in the MCAO group.

- Figure 13: Microscopic image of the MCAO+Dexa group brain section stained with Nissl, showing a relative improvement in neuronal structure, but with less efficacy compared to the exosome group.

Table 6: Statistical Analysis of Nissl Staining (Number of Healthy Neurons per Field of View)

Group	Healthy Neurons (Mean ± SD)
Sham	65.2 ± 4.1
Control MCAO	20.3 ± 3.2
MCAO+Exosome	45.7 ± 4.5
MCAO+Dexa	38.9 ± 3.8

Discussion

1. Comparison of Results with Previous Studies

1.1. Neurological Function and Motor Recovery

Neurological deficits are a common consequence of stroke, primarily due to neuronal death, inflammation, and damage to neural circuits. In the present study, a significant reduction in Bederson scores was observed in both the exosome- and dexamethasone-treated groups compared to the MCAO control group, indicating improved neurological outcomes.

Xin et al. (2013) demonstrated that mesenchymal stem cell-derived exosomes could enhance neurological function and reduce motor impairment in a stroke animal model [9]. Similarly, Doeppner et al. (2015) reported that exosomes reduce neuronal necrosis and promote motor recovery by transferring neuroprotective microRNAs [10].

1.2. Reduction of Infarct Volume and Pathological Findings

TTC staining revealed that infarct size and necrotic damage were significantly reduced in the exosome-treated groups. Jiang et al. (2022) found that stem cell-derived exosomes could mitigate necrosis and promote neuronal survival and regeneration in ischemic brains through the delivery of microRNAs and growth factors [11].

Wang et al. (2022) also reported that exosomes suppress the expression of inflammatory mediators such as IL-1β, TNF-α, and NF-κB, leading to reduced tissue necrosis and smaller infarct volumes [12].

Our results support the notion that exosomes exert superior neuroprotective effects compared to conventional treatments like dexamethasone, largely through modulation of inflammatory signaling and stimulation of cellular repair mechanisms.

Exosomes have also been shown to inhibit apoptosis and increase the expression of neurotrophic factors like BDNF and NGF, which are critical for neuronal regeneration and reducing secondary damage after ischemia [13].

Furthermore, recent evidence suggests that exosome therapy improves mitochondrial function and decreases oxidative stress via the reduction of reactive oxygen species (ROS), thereby enhancing neuronal survival and recovery [14].

Another mechanism includes the modulation of microglial activity. Exosomes can attenuate chronic inflammation by suppressing pro-inflammatory responses in microglia, creating a more favorable environment for brain tissue regeneration [15].

In contrast to dexamethasone, which mainly acts through anti-inflammatory pathways, exosome treatment not only reduces inflammation but also promotes neuroregeneration. While further research is needed to elucidate precise mechanisms, current evidence highlights the high therapeutic potential of exosomes in ischemic brain injury.

3. Mechanisms of Therapeutic Effects

3.1. Potential Mechanisms of Exosome Action

Stem cell-derived exosomes exert their therapeutic effects through a variety of mechanisms due to their cargo of neuroprotective microRNAs, growth factors, and anti-inflammatory proteins, including:

- Delivery of anti-inflammatory microRNAs such as miR-124 and miR-21, which suppress inflammatory pathways and enhance neuronal viability.
- Inhibition of NF-κB and IL-6 signaling pathways, reducing inflammation and preventing necrosis.
- Upregulation of neurotrophic factors like BDNF and NGF, facilitating neuronal repair.
- Promotion of angiogenesis and increased blood supply in the ischemic region via VEGF expression.
- Inhibition of apoptosis, leading to improved cell survival [16].

3.2. Mechanisms of Dexamethasone

Dexamethasone is a potent corticosteroid with well-known anti-inflammatory properties. Its actions include:

- Inhibition of NF-κB and MAPK pathways, leading to reduced production of pro-inflammatory cytokines.
- Reduced infiltration of immune cells into the ischemic region and suppression of cerebral edema.
- Decreased chronic inflammation and necrosis [17].

4. Study Limitations

1. Lack of long-term assessment of neuronal regeneration and cognitive function.
2. Absence of detailed investigation into molecular signaling pathways involved in inflammation and neurorepair.
3. Use of a single animal model (rat), which may limit the generalizability of the results.

5. Recommendations for Future Research

1. Evaluate the effects of these therapies in other animal models and over extended time periods.
2. Use advanced techniques like RNA sequencing to analyze molecular mechanisms and identify key signaling pathways.
3. Compare exosome therapy with other regenerative approaches, such as direct mesenchymal stem cell transplantation.
4. Investigate combinatory strategies involving exosomes and other neuroprotective agents to enhance therapeutic efficacy.

Conclusion

The findings of this study revealed that both treatments—stem cell-derived exosomes and dexamethasone—were effective in reducing ischemic damage, inflammation, and neurological deficits. However, exosomes demonstrated superior efficacy due to their multifaceted mechanisms, including neuroprotection, angiogenesis, and anti-inflammatory effects.

Exosomes can promote neural tissue repair by delivering bioactive factors such as BDNF, NGF, and VEGF. They mitigate neuroinflammation and oxidative stress by downregulating inflammatory gene expression (e.g., IL-1β, TNF-α) and inhibiting NF-κB activation, leading to decreased cell death and enhanced brain repair.

Compared to dexamethasone, exosomes act through more complex signaling pathways, exerting broader effects on neurons, endothelial cells, and immune cells. While dexamethasone mainly suppresses inflammation, exosomes stimulate neurogenesis, synaptic plasticity, and vascular remodeling. This gives them a therapeutic edge in treating stroke.

Animal studies have confirmed their efficacy in reducing brain lesions and improving cognitive and motor functions. For example, recent studies demonstrated that exosome injection leads to decreased infarct volume, enhanced behavioral performance, and upregulation of protective factors in the brain [18].

Future research should focus on optimizing exosome production, purification, and delivery methods. Key challenges remain in determining the optimal dosage and administration route, as well as assessing long-term safety in stroke patients.

Overall, this study highlights the therapeutic promise of exosome-based strategies in stroke treatment. By delivering biological agents that reduce inflammation and promote neuroregeneration, exosomes represent a novel and potentially transformative approach for managing neurological disorders.

Authors' Contribution

Authors' Contribution:
Conceptualization, Ali Reza Eftekhari Moghadam, Mahdi Heydari;
Methodology, Mahdi Heydari, Bahman Jalali Kondori, Mehdi Raei, Ali Reza Eftekhari Moghadam;
Investigation, Mahdi Heydari, Bahman Jalali Kondori, Mehdi Raei;
Data Curation, Mahdi Heydari, Bahman Jalali Kondori;
Writing – Original Draft Preparation, Mahdi Heydari, Mehdi Raei;
Writing – Review & Editing, Ali Reza Eftekhari Moghadam, Bahman Jalali Kondori;
Visualization, Mahdi Heydari;
Supervision, Ali Reza Eftekhari Moghadam;
Project Administration, Ali Reza Eftekhari Moghadam;
Funding Acquisition, Ali Reza Eftekhari Moghadam.

Ethical Considerations

Ethical Considerations:

All animal procedures were approved by the Institutional Animal Ethics Committee of Baqiyatallah University of Medical Sciences, Tehran, Iran, and performed in accordance with the national guidelines for animal research. All efforts were made to minimize animal suffering and the number of animals used.

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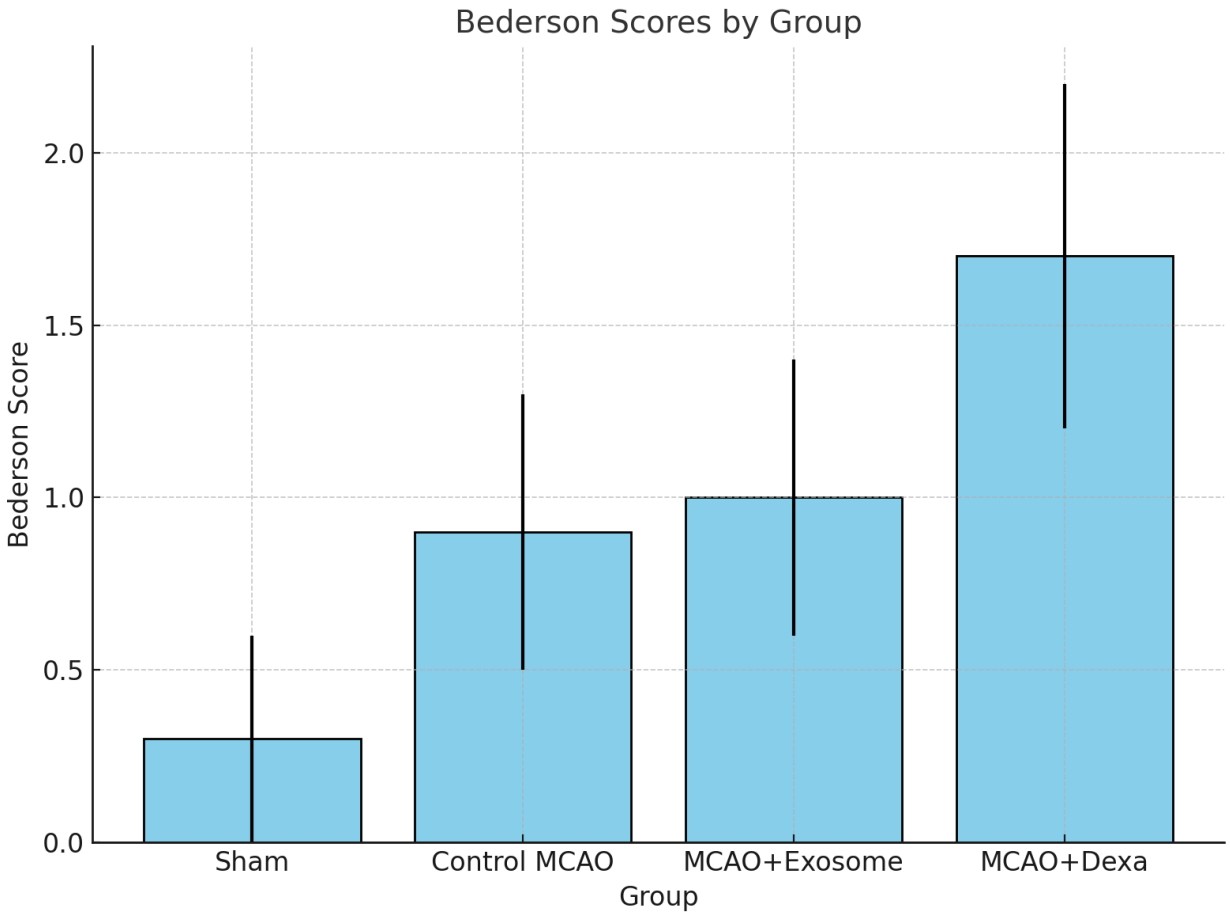

Figure 1 – Comparison of Bederson Scores between groups**.**

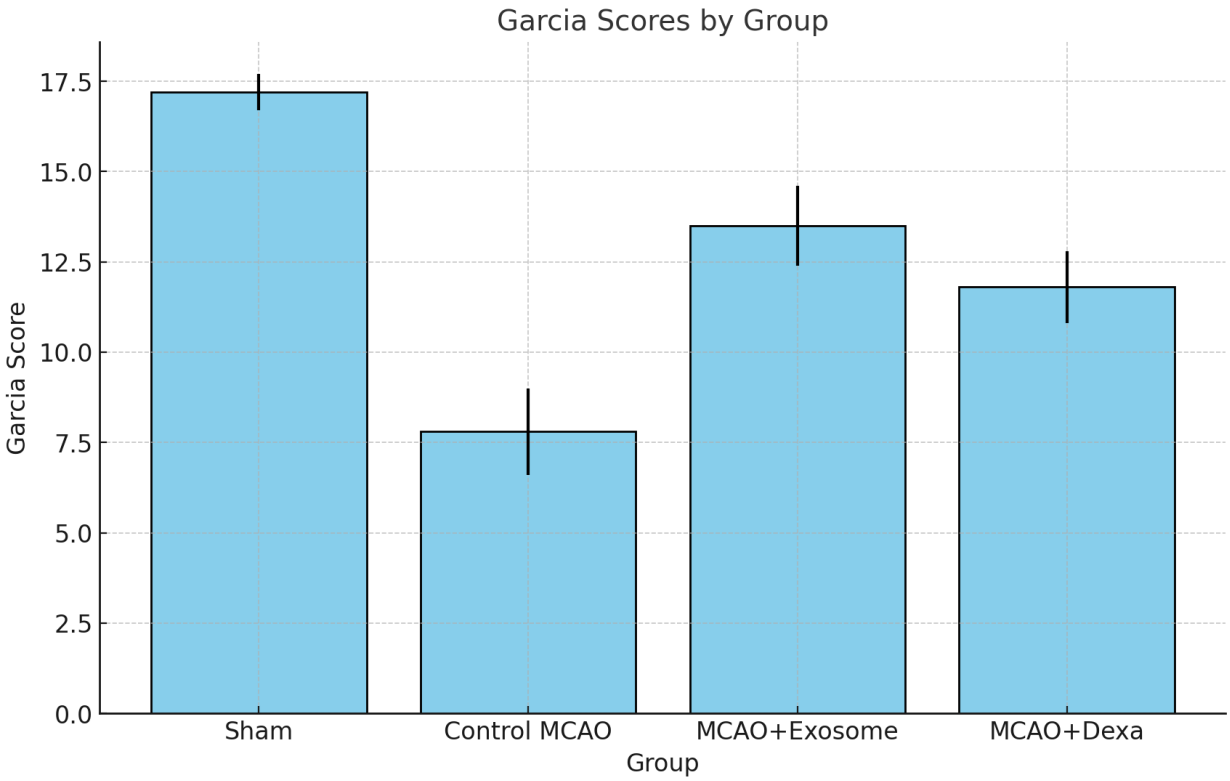

Figure 2 – Comparison of Garcia Scores between groups.

Figure 3: Comparison of Brain Lesion Volume in Different Groups using TTC Staining.

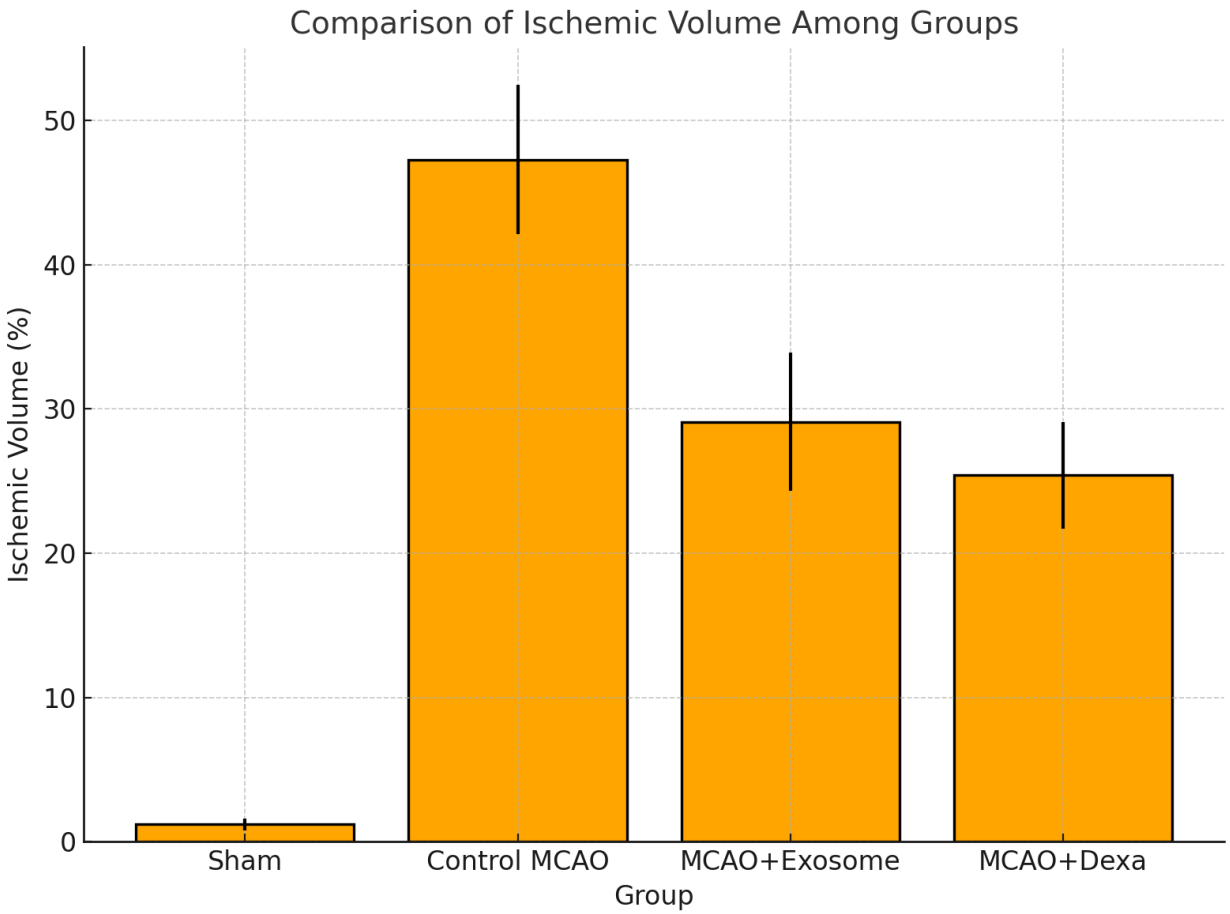

Figure 4: Comparison of Ischemic Lesion Volume Among Groups.

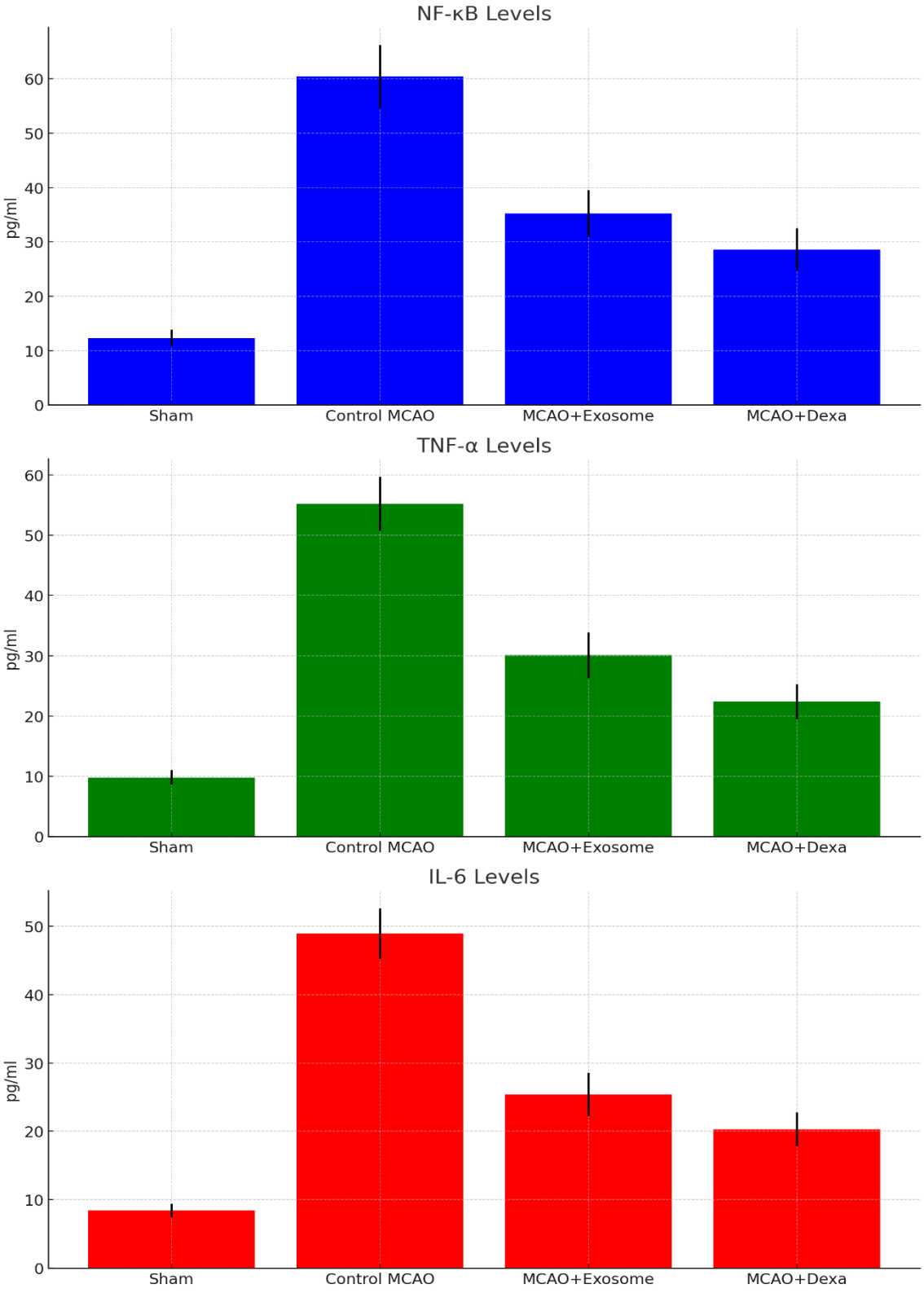

Figure 5: Comparison of Inflammatory Factors Among Groups

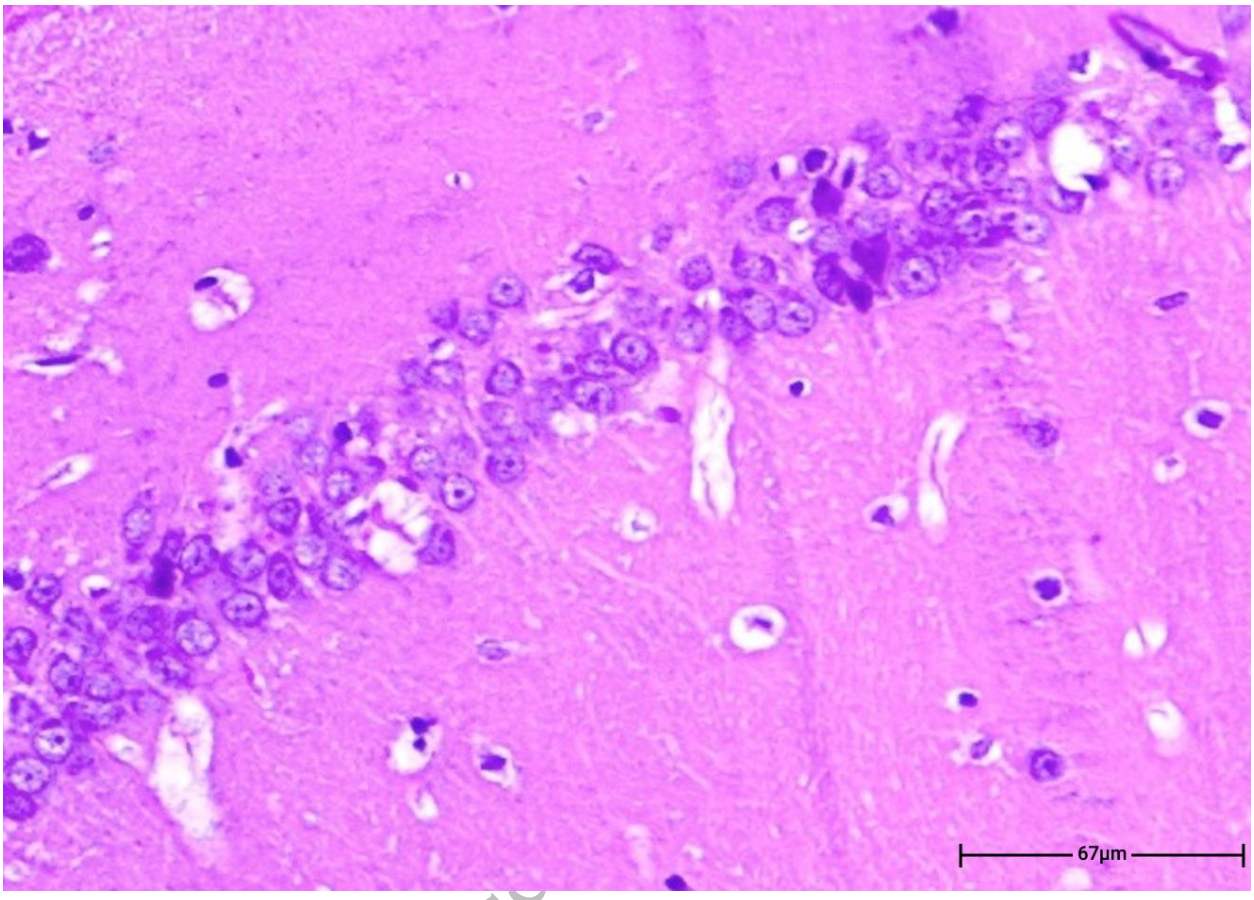

Figure 6: Photomicrograph of the Sham group brain section stained with H&E, showing healthy neurons without necrosis or inflammation.

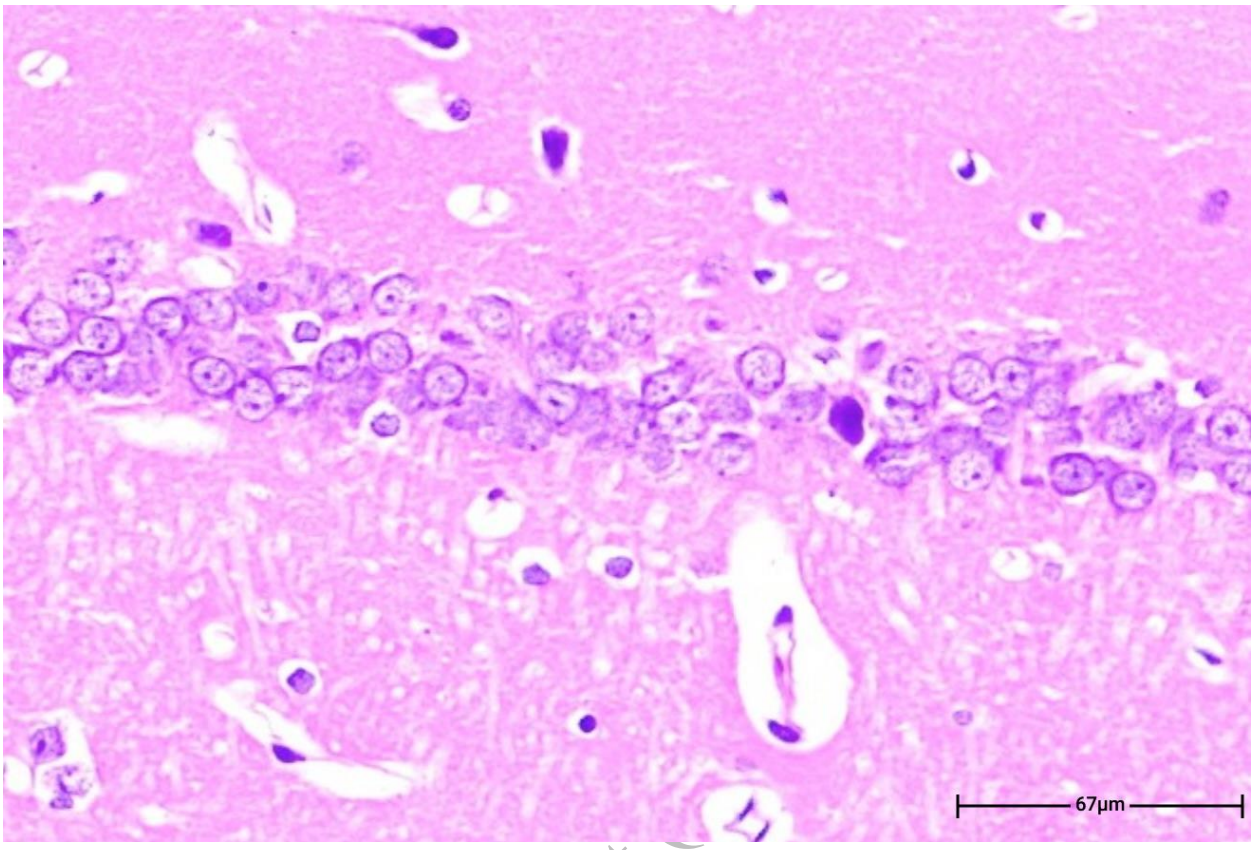

Figure 7: Photomicrograph of the MCAO control group brain section stained with H&E, showing necrosis and neuronal destruction with pyknotic nuclei and disrupted cytoplasm.

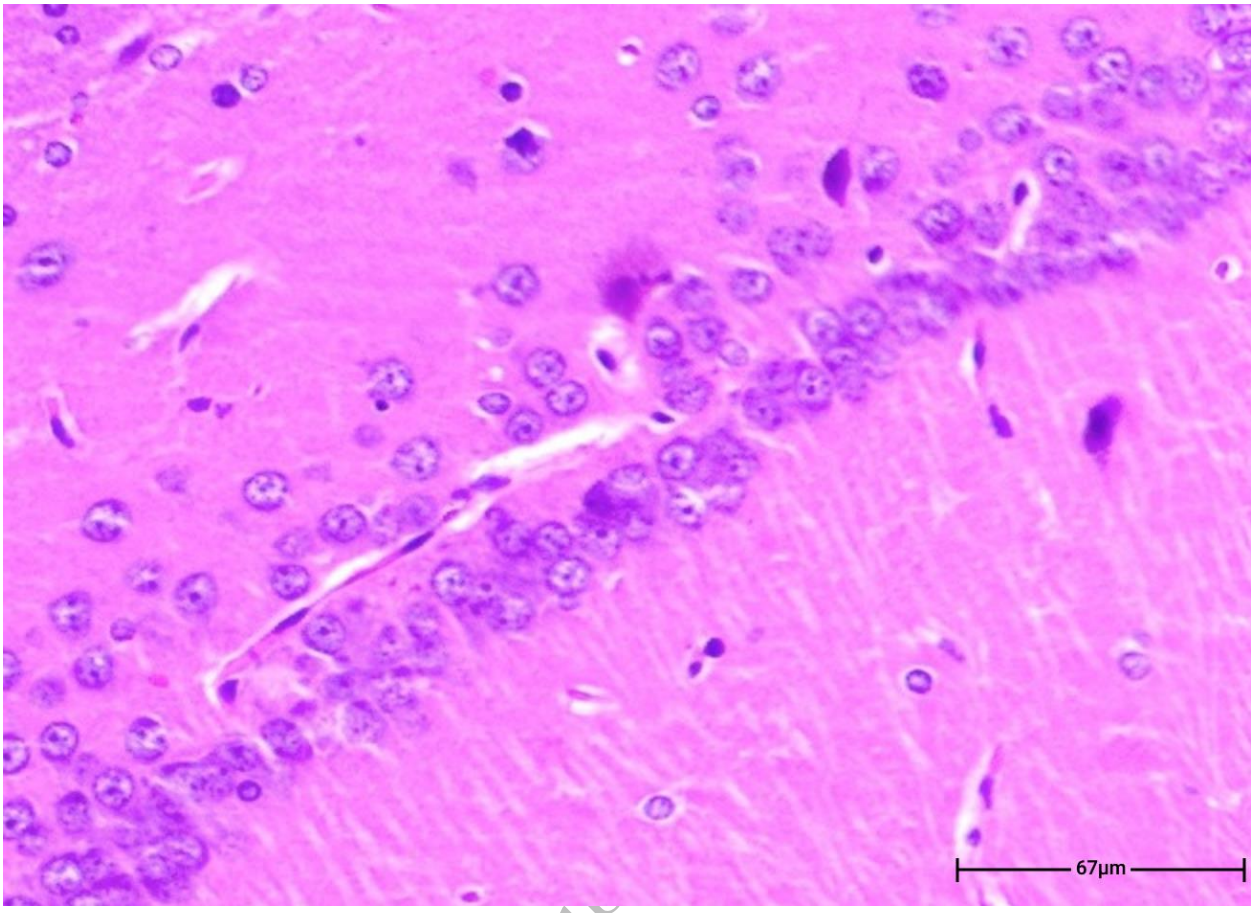

Figure 8: Photomicrograph of the MCAO+Exosome group brain section stained with H&E, showing reduced necrosis and neuronal destruction, as well as improved neuronal structure compared to the MCAO control group.

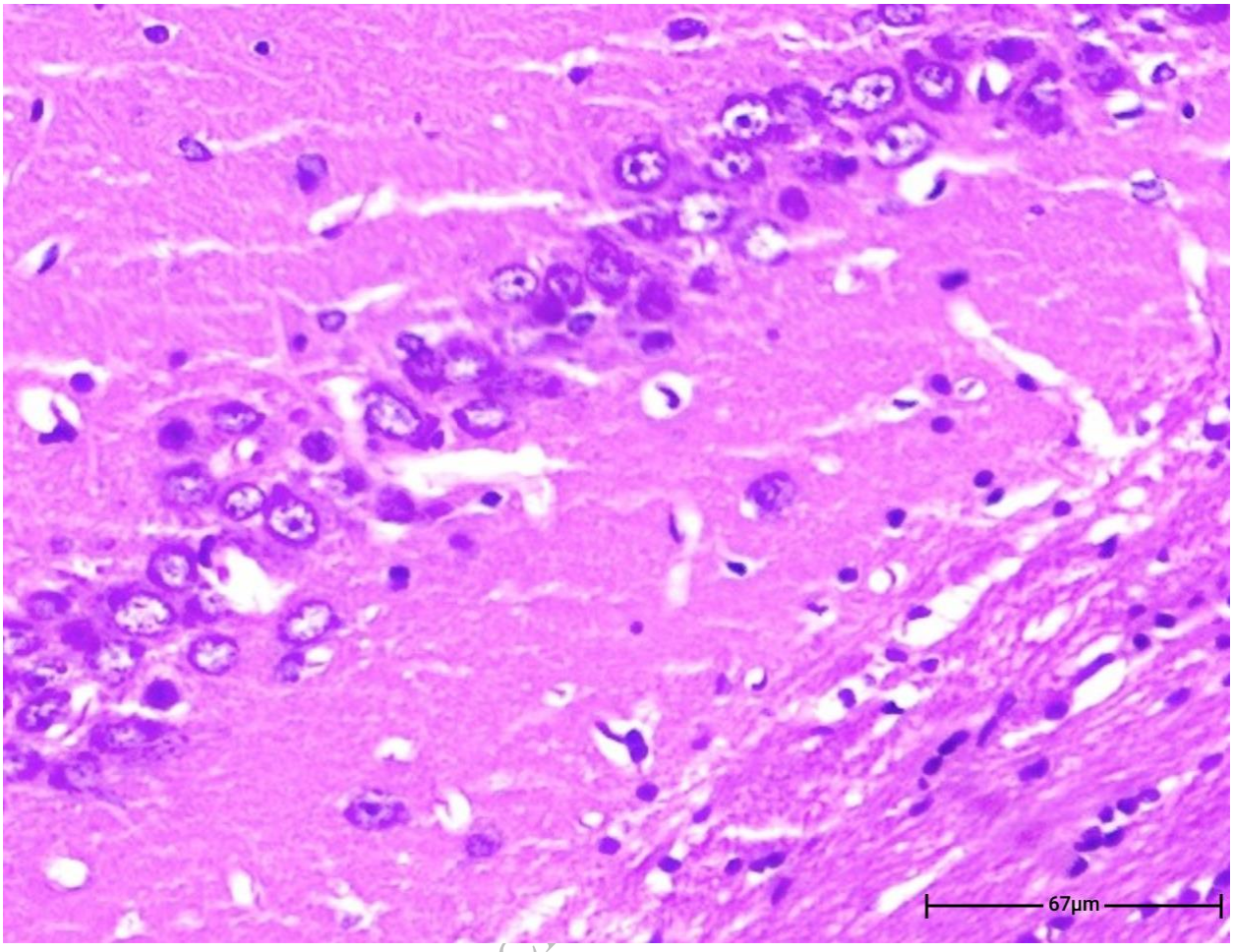

Figure 9: Photomicrograph of the MCAO+Dexa group brain section stained with H&E, showing a relative reduction in necrosis and cellular damage, but with less efficacy compared to the exosome group.

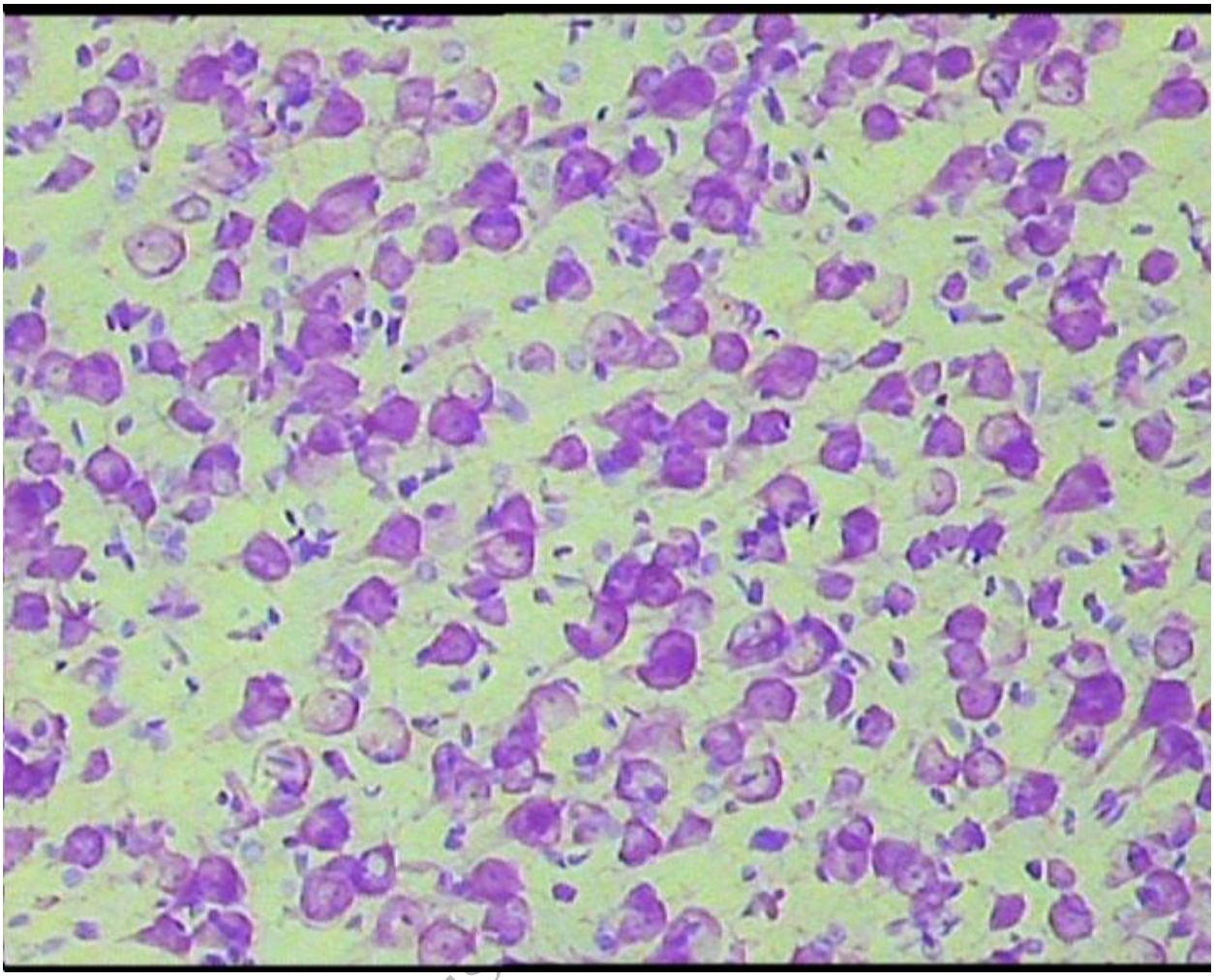

Figure 10: Microscopic image of the Sham group brain section stained with Nissl, showing uniform and healthy neuron distribution without degeneration.

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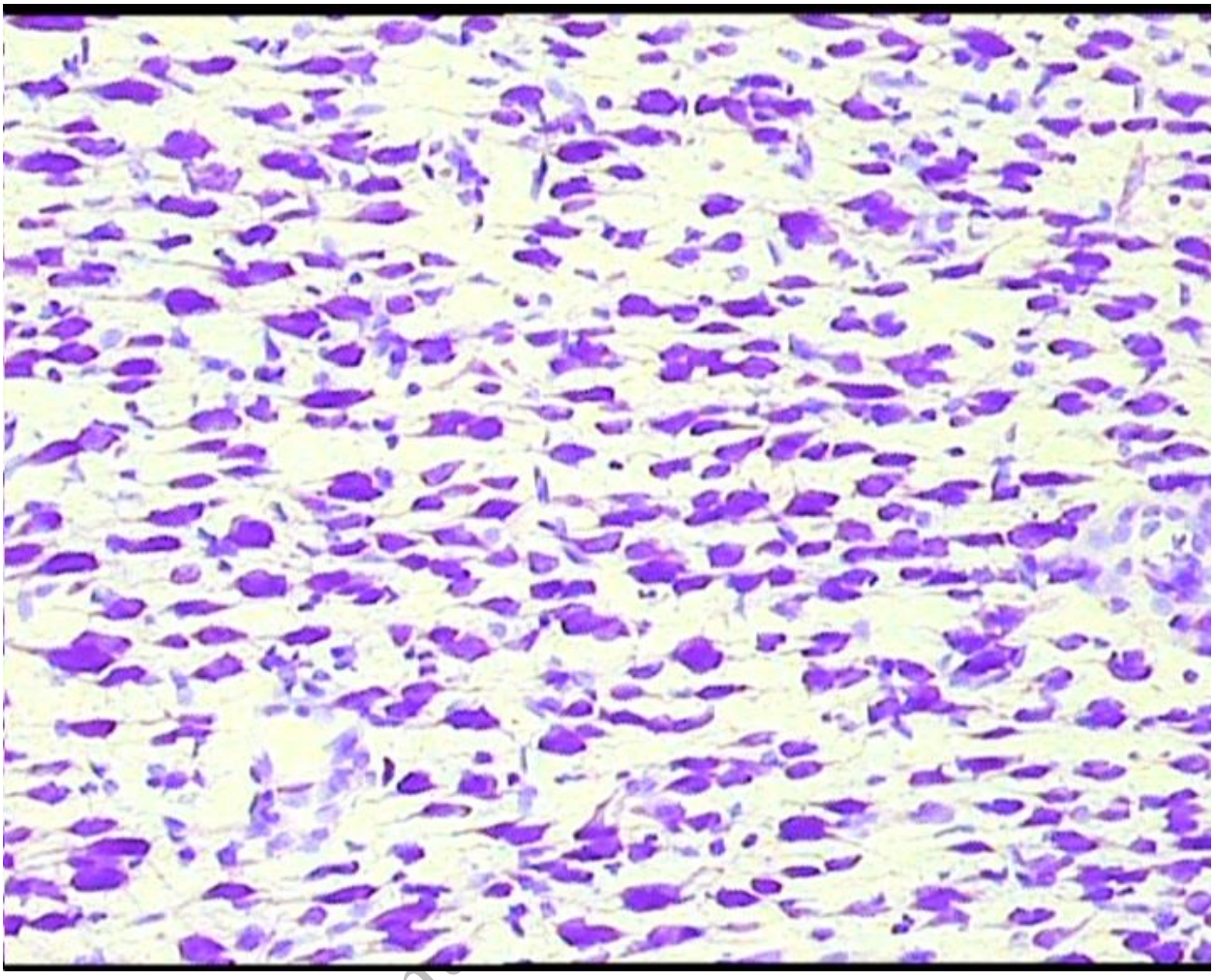

- **Figure 11**: Microscopic image of the MCAO group brain section stained with Nissl, showing a reduction in healthy neurons and an increase in degenerated neurons.

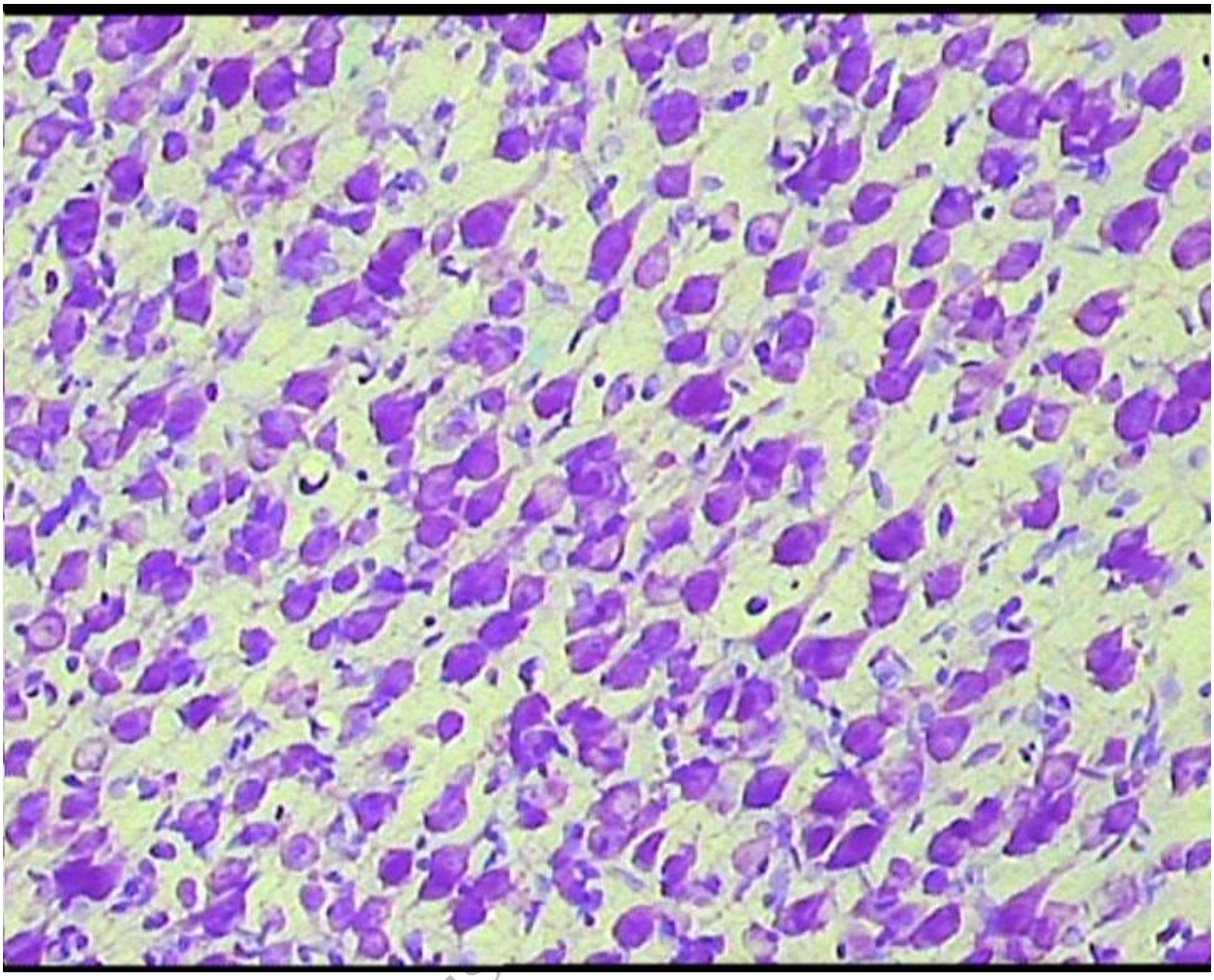

Figure 12: Microscopic image of the MCAO+Exosome group brain section stained with Nissl, showing an increase in healthy neurons and a reduction in neuronal degeneration compared to the MCAO group.

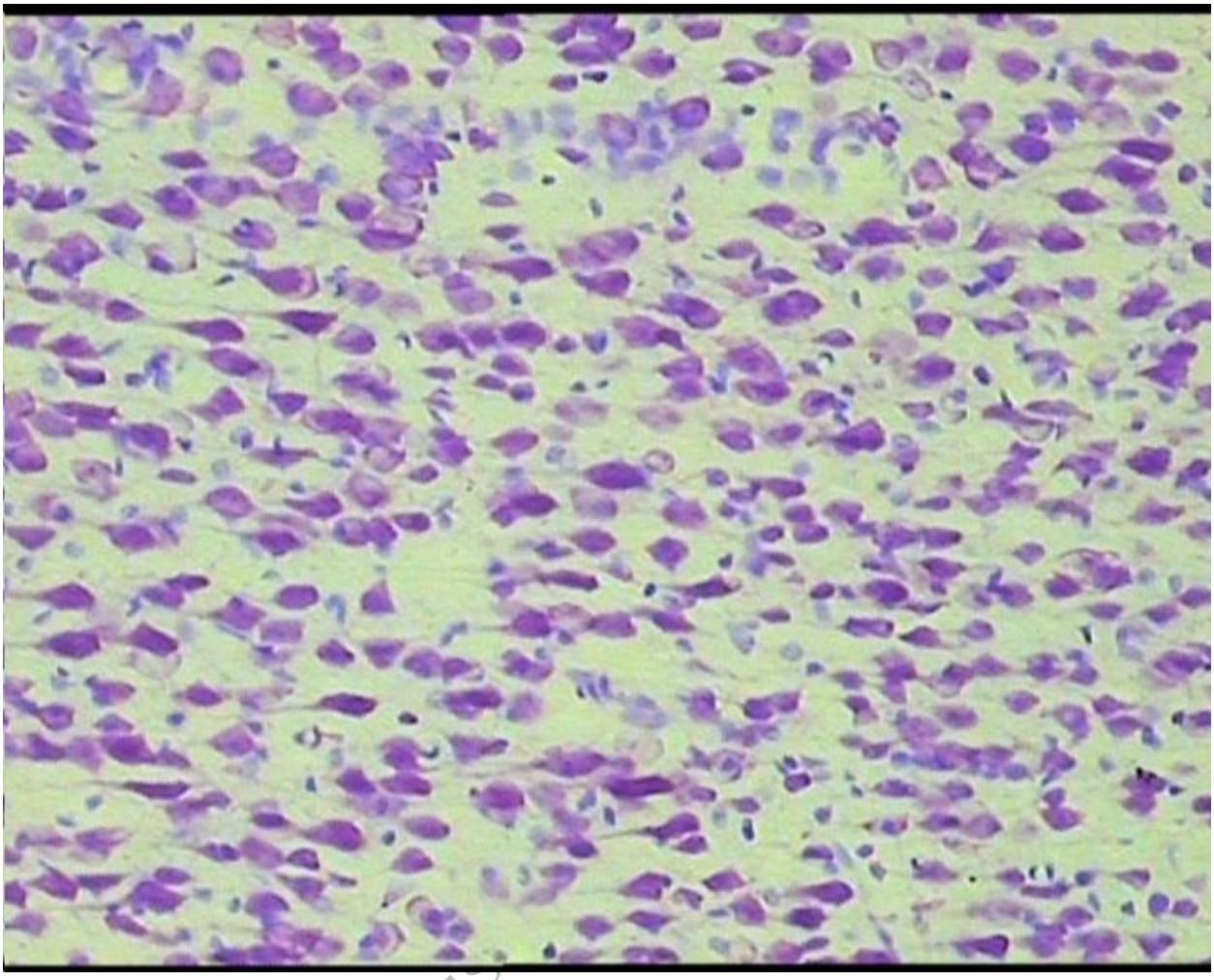

Figure 13: Microscopic image of the MCAO+Dexa group brain section stained with Nissl, showing a relative improvement in neuronal structure, but with less efficacy compared to the exosome group