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Neuroprotective effects of ascorbic acid and edaravone in experimental cerebral ischemia-reperfusion injury

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ABSTRACT

Aims: Oxidative stress-mediated ischemia-reperfusion (I/R) injury plays a critical role in secondary neuronal damage following acute ischemic stroke. This study aimed to evaluate the neuroprotective effects of edaravone and ascorbic acid administered at different doses in an experimental cerebral ischemia-reperfusion injury model.

Methods: Forty female Sprague-Dawley rats were randomly assigned using a computer-generated randomization method into five groups (n=8/group): control, low-dose edaravone (1 mg/kg), high-dose edaravone (5 mg/kg), low-dose ascorbic acid (100 mg/kg), and high-dose ascorbic acid (500 mg/kg). Experimental cerebral ischemia-reperfusion injury was induced by transient unilateral carotid artery occlusion. Treatments were administered intraperitoneally 60 minutes after ischemia induction and immediately before reperfusion. Serum malondialdehyde (MDA) and nitric oxide (NO) levels and histopathological neuronal injury were evaluated.

Results: High-dose ascorbic acid treatment was associated with significantly lower MDA, NO, and neuronal injury values compared with the control group. Serum MDA levels were significantly reduced in both low-dose and high-dose ascorbic acid groups ($p<0.05$ and $p<0.01$, respectively), whereas NO and histopathological neuronal injury values were significantly lower only in the high-dose ascorbic acid group ($p<0.05$ and $p<0.01$, respectively). Edaravone-treated groups did not demonstrate statistically significant biochemical improvement under the present experimental conditions, although mild histopathological improvement was observed.

Conclusion: In conclusion, high-dose ascorbic acid treatment was associated with reduced oxidative stress markers and decreased histopathological neuronal injury in this experimental cerebral ischemia-reperfusion model. Among the evaluated treatment protocols, high-dose ascorbic acid was associated with comparatively more favorable biochemical and histopathological findings under the present experimental conditions. However, further studies including functional neurological assessment and molecular inflammatory analyses are required to clarify the underlying mechanisms and potential translational relevance of antioxidant-based interventions in ischemic stroke.

Keywords: Ischemic stroke, cerebral ischemia-reperfusion injury, oxidative stress, neuroprotection, ascorbic acid, edaravone

INTRODUCTION

Acute ischemic stroke (AIS) remains one of the leading causes of mortality and long-term neurological disability worldwide despite substantial advances in diagnostic and therapeutic strategies.[1,3,4] Early reperfusion therapies, including thrombolytic treatment and mechanical thrombectomy, have significantly improved clinical outcomes by restoring cerebral blood flow and salvaging ischemic penumbral tissue.[3,4] However, restoration of perfusion may paradoxically induce secondary neuronal injury, known as ischemia-reperfusion (I/R) injury, which substantially contributes to infarct progression and neurological deterioration.[5,6]

The pathophysiology of cerebral I/R injury is highly complex and involves multiple interconnected molecular and cellular mechanisms, including excitotoxicity, mitochondrial dysfunction, oxidative stress, endothelial injury, inflammatory cascade activation, and apoptosis.[6,7,15-17] Among these mechanisms, oxidative stress plays a central role in the progression of neuronal damage after cerebral ischemia. During ischemia and subsequent reperfusion, excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) leads to disruption of cellular homeostasis, lipid peroxidation, protein oxidation, DNA damage, and ultimately neuronal cell death.[5-10]

Nitric oxide (NO) has dual physiological and pathological roles in cerebral ischemia. Although physiological NO

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production contributes to vascular regulation and cerebral perfusion, excessive NO generation during ischemia, particularly through neuronal NO synthase activation, promotes the formation of peroxynitrite and other cytotoxic metabolites that aggravate neuronal injury.[8,9] Peroxynitrite-mediated oxidative damage induces lipid membrane degradation and DNA oxidation, resulting in irreversible cellular injury.[9,10] Malondialdehyde (MDA), one of the major end-products of lipid peroxidation, is widely accepted as a reliable biomarker of oxidative stress and free radical-mediated tissue injury.[11-14] Therefore, evaluation of NO and MDA levels may provide important information regarding the severity of oxidative neuronal damage following cerebral ischemia-reperfusion.

Because oxidative stress is a major therapeutic target in ischemic stroke, antioxidant agents have attracted increasing attention as potential neuroprotective strategies.[7,18] Edaravone is a potent free radical scavenger with low molecular weight and lipophilic properties that enable efficient penetration through the blood-brain barrier.[18-24] Experimental studies have demonstrated that edaravone reduces oxidative stress, suppresses lipid peroxidation, preserves endothelial integrity, and attenuates inflammatory responses following cerebral ischemia.[20-24] Moreover, previous animal studies and systematic reviews have reported favorable functional and histopathological outcomes associated with edaravone treatment in focal cerebral ischemia models.[2,20,24]

Ascorbic acid (vitamin C) is another important antioxidant molecule that exerts neuroprotective effects through scavenging reactive oxygen radicals and preventing oxidative cellular injury.[25-27] As a water-soluble antioxidant, ascorbic acid contributes to the maintenance of intracellular redox balance and inhibits lipid peroxidation within the aqueous phase.[25,27] Previous experimental studies have shown that ascorbic acid administration may reduce infarct volume and neuronal injury following focal cerebral ischemia.[26,32] Nevertheless, comparative data regarding the comparative neuroprotective efficacy of edaravone and ascorbic acid in cerebral ischemia-reperfusion injury remain limited.

Therefore, the present experimental study aimed to investigate and compare the neuroprotective effects of different doses of edaravone and ascorbic acid in a rat model of acute cerebral ischemia-reperfusion injury using biochemical oxidative stress markers and histopathological neuronal injury parameters. We hypothesized that antioxidant treatment, particularly at higher doses, would attenuate oxidative stress-mediated neuronal damage and reduce histopathological injury following cerebral ischemia-reperfusion.

METHODS

Ethics

The experimental protocol was approved by the Local Ethics Committee for Animal Experiments at Kırıkkale University (Date: 21.10.2021, Decision No: 35). All procedures were conducted in accordance with the ARRIVE guidelines and the national/international guidelines for the care and use of laboratory animals.

Experimental Animals

A total of 40 adult female Sprague-Dawley rats weighing between 250 and 300 g were included in the study. Animals were housed under standardized laboratory conditions under controlled temperature and humidity with a 12-hour light/dark cycle and had free access to standard laboratory chow and water throughout the experimental period.

Inclusion and Exclusion Criteria

Healthy adult female Sprague-Dawley rats without neurological deficits or systemic disease were included in the study. Animals that developed intraoperative complications, inadequate reperfusion, anesthesia-related mortality, or technical failure during the ischemia-reperfusion procedure were excluded from the study.

Randomization and Experimental Groups

Female rats were preferred in order to reduce inter-animal physiological variability during the ischemia-reperfusion process. However, potential sex-related differences in ischemic injury response should be considered when interpreting the findings.

Animals were randomly assigned into five groups (n=8/group):

- Ischemia-reperfusion control group (C), in which carotid artery occlusion and reperfusion procedures were performed without antioxidant treatment
- Low-dose edaravone group (LDE)
- High-dose edaravone group (HDE)
- Low-dose ascorbic acid group (LDA)
- High-dose ascorbic acid group (HDA)

Histopathological analyses were performed under blinded conditions.

Experimental Cerebral Ischemia-Reperfusion Model

General anesthesia was induced using ketamine hydrochloride (90 mg/kg) and xylazine hydrochloride (10 mg/kg) administered intraperitoneally. A unilateral carotid artery occlusion model was preferred to investigate acute oxidative stress-mediated ischemia-reperfusion injury under controlled experimental conditions. Following a midline cervical incision, the left common carotid artery was carefully isolated and clamped for 90 minutes to induce ischemia. Reperfusion was subsequently established by removal of the vascular clamp. Treatments were administered 60 minutes after ischemia induction and immediately before reperfusion based on previously published experimental cerebral ischemia-reperfusion studies evaluating antioxidant administration during the pre-reperfusion period.[20,23,26] The selected edaravone and ascorbic acid doses were based on previously published experimental cerebral ischemia-reperfusion studies demonstrating antioxidant and neuroprotective efficacy.[20,23,26,32] Edaravone and ascorbic acid solutions were prepared in sterile saline and administered intraperitoneally. Equivalent volumes of sterile saline were administered intraperitoneally to the ischemia-reperfusion

control group following carotid artery occlusion and reperfusion procedures. Reperfusion was maintained for an additional 150 minutes. This reperfusion duration was selected to evaluate early oxidative stress-mediated biochemical and histopathological alterations during the acute reperfusion phase. At the end of the experimental period, blood and brain tissue samples were collected under deep anesthesia for biochemical and histopathological analyses.

Biochemical Analysis

Serum MDA and NO levels were measured using commercially available ELISA kits according to the manufacturer's instructions. MDA values were expressed as nmol/ml and NO values as µmol/L.

Histopathological Evaluation

Brain tissue samples were fixed in 10% formalin solution and embedded in paraffin blocks. Sections of 4-5 µm thickness were stained with hematoxylin-eosin and examined under light microscopy for neuronal degeneration and histopathological injury findings. Histopathological examinations were performed by a blinded histopathologist unaware of group allocation. Representative histopathological images were selected from sections demonstrating characteristic neuronal degeneration findings. Degenerative neurons characterized by eosinophilic cytoplasm, pyknotic nuclei, and loss of Nissl substance were considered atrophic neurons. Atrophic neuron percentage (ANP) was calculated by evaluating 100 pyramidal neurons at ×200 magnification in each section (**Figure**). ANP was calculated in randomly selected microscopic fields. Histopathological evaluation was performed in all animals (n=8 per group), and representative sections demonstrating characteristic neuronal degeneration findings were selected for figure preparation.

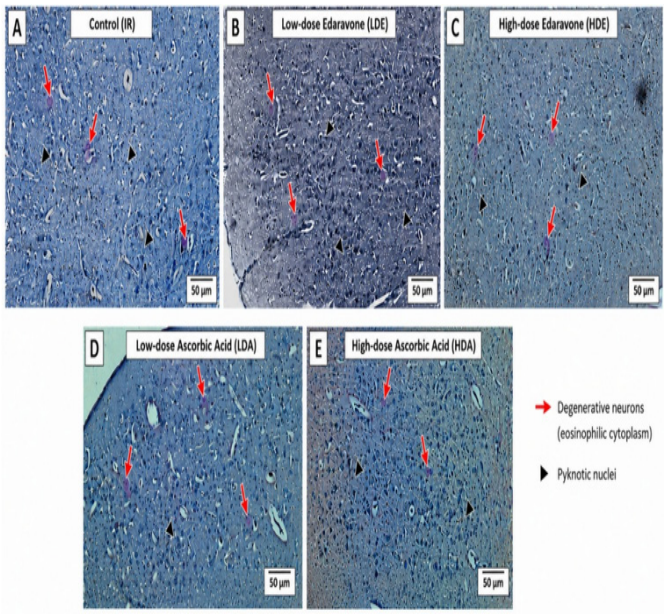

Figure. Representative histopathological findings of cerebral ischemia-reperfusion injury among experimental groups. (**A**) ischemia-reperfusion control group (C); (**B**) low-dose edaravone group (LDE); (**C**) high-dose edaravone group (HDE); (**D**) low-dose ascorbic acid group (LDA); (**E**) high-dose ascorbic acid group (HDA). Brain tissue sections were stained with hematoxylin-eosin and examined under light microscopy at ×200 magnification. Red arrows indicate degenerative neurons characterized by eosinophilic cytoplasm, whereas black arrowheads indicate pyknotic nuclei. Scale bars: 50 µm.

Statistical Analysis

The data analyses were performed using IBM SPSS Statistics version 21.0. Data were expressed as mean±standard deviation (SD). Normality distribution was assessed using the Shapiro-Wilk test. Intergroup comparisons biochemical parameters (MDA and NO) were performed using one-way analysis of variance (ANOVA) followed by Tukey post hoc analysis for pairwise subgroup comparisons. Tukey post hoc analysis was preferred for biochemical subgroup comparisons in order to control for multiple testing across normally distributed biochemical variables. Histopathological comparisons were additionally evaluated using Bonferroni correction for multiple comparisons. In response to reviewer recommendations, direct subgroup comparisons between low-dose and high-dose treatment groups were additionally analyzed using post hoc pairwise comparison tests. A p-value <0.05 was considered statistically significant. Adjusted p-values <0.0083 were considered statistically significant for Bonferroni-corrected histopathological multiple comparisons. Sample size was determined based on previously published experimental cerebral ischemia-reperfusion studies with similar biochemical and histopathological endpoints. A formal a priori power analysis was not performed because of the exploratory nature of the study. A group size of eight animals per group was considered methodologically appropriate while minimizing animal use in accordance with ethical principles and the principles of experimental animal welfare.

RESULTS

Biochemical Findings

Mean biochemical parameter values for all experimental groups are summarized in (**Table 1**). Serum MDA levels demonstrated significant differences among the experimental groups. Compared with the control group, MDA levels were significantly lower in both the low-dose ascorbic acid group (p=0.040) and the high-dose ascorbic acid group (p=0.002). No statistically significant reduction in MDA levels was observed in the edaravone-treated groups.

Table 1. Serum malondialdehyde (MDA) and nitric oxide (NO) levels among experimental groups

Group	MDA (nmol/ml) mean±SD	NO (µmol/L) mean±SD
Control	0.97±0.17	80.01±14.74
LDE	0.85±0.28	77.84±17.98
HDE	1.03±0.46	80.20±12.05
LDA	0.80±0.13*	69.18±12.96
HDA	0.71±0.10**	63.31±11.94*#

Values are presented as mean±standard deviation (SD)
Abbreviations: C: Control, SD: Standard deviation, LDE: Low-dose edaravone, HDE: High-dose edaravone, LDA: Low-dose ascorbic acid, HDA: High-dose ascorbic acid, MDA: Malondialdehyde, NO: Nitric oxide
*p<0.05 versus control, **p<0.01 versus control, #p<0.05 versus HDE group

Serum NO levels were significantly lower in the high-dose ascorbic acid group compared with the control group (p=0.026). Although lower NO levels were also observed in the low-dose ascorbic acid group, this difference did not reach statistical significance. Similarly, no statistically significant biochemical improvement was observed in the edaravone-treated groups under the present experimental conditions.

Direct Subgroup Comparisons

In response to reviewer recommendations regarding subgroup-based dose comparisons, additional direct comparisons were performed between low-dose and high-dose treatment groups. Comparisons between low-dose and high-dose edaravone groups did not demonstrate statistically significant differences in MDA or NO measurements. Similarly, direct comparison between low-dose and high-dose ascorbic acid groups did not reveal statistically significant differences for these biochemical parameters, although lower mean MDA and NO values were observed in the high-dose ascorbic acid subgroup.

Direct comparison between the high-dose edaravone and high-dose ascorbic acid groups demonstrated significantly lower NO levels in the high-dose ascorbic acid group (p=0.014). In addition, MDA values were lower in the high-dose ascorbic acid group compared with the high-dose edaravone group, although this difference did not reach statistical significance.

Histopathological Findings

Histopathological examination demonstrated varying degrees of neuronal degeneration among the experimental groups. Degenerative neurons characterized by eosinophilic cytoplasm, pyknotic nuclei, and loss of Nissl substance were predominantly observed in the control and edaravone-treated groups. The lowest neuronal injury findings were observed in the high-dose ascorbic acid group. Bonferroni-corrected multiple comparison analysis demonstrated significantly lower neuronal injury in the high-dose ascorbic acid group compared with the control group (p=0.002). Although lower neuronal injury values were also observed compared with the low-dose ascorbic acid group, this difference did not remain statistically significant after Bonferroni correction (p=0.013).

Although mild histopathological improvement was also observed in the edaravone-treated groups, these findings did not reach statistical significance after correction for multiple comparisons. Representative histopathological images are presented in **Figure**. Histopathological comparison results are summarized in **Table 2**.

Table 2. Histopathological comparison of atrophic neuron percentage (ANP) values among experimental groups

Group	ANP (%) mean±SD
Control	42.6±1.7
LDE	41.3±1.8
HDE	41.0±1.3
LDA	42.1±2.3
HDA	39.1±1.1**'

Values are presented as mean±standard deviation (SD).
Abbreviations: ANP: Atrophic neuron percentage, LDE: Low-dose edaravone, HDE: High-dose edaravone, LDA: Low-dose ascorbic acid, HDA: High-dose ascorbic acid
**p<0.01 versus control, 'p<0.0083 versus low-dose ascorbic acid group after Bonferroni correction

DISCUSSION

The present experimental study investigated the neuroprotective effects of different doses of edaravone and ascorbic acid in a rat model of cerebral ischemia-reperfusion (I/R) injury. The principal finding of the study was that high-dose ascorbic acid exerted the most prominent neuroprotective effects both biochemically and histopathologically. High-dose ascorbic acid significantly reduced serum MDA and NO levels and attenuated neuronal degeneration compared with the control group. In contrast, edaravone treatment did not produce statistically significant biochemical improvements under the conditions of the present experimental model. Among the evaluated treatment protocols, high-dose ascorbic acid was associated with comparatively more favorable biochemical and histopathological findings under the present experimental conditions.

AIS initiates a complex pathophysiological cascade involving excitotoxicity, oxidative stress, inflammatory activation, endothelial dysfunction, mitochondrial injury, and apoptosis.[5-7,15-17] Although reperfusion therapies are essential for restoring cerebral blood flow and salvaging ischemic penumbral tissue, reperfusion itself may paradoxically aggravate neuronal injury through excessive production of ROS and RNS.[5-7] Oxidative stress therefore remains one of the major mechanisms contributing to secondary neuronal injury after ischemic stroke.

In the present study, serum MDA levels were significantly reduced in both low-dose and high-dose ascorbic acid groups compared with the control group. MDA is a well-established marker of lipid peroxidation and oxidative tissue injury.[11-14] Elevated MDA levels reflect free radical-mediated membrane degradation and neuronal cellular injury. Therefore, the reduction in MDA levels observed after ascorbic acid treatment suggests attenuation of oxidative membrane damage during cerebral ischemia-reperfusion injury. These findings are consistent with previous experimental studies demonstrating antioxidant and neuroprotective properties of ascorbic acid in ischemic brain injury models.[26,27,32]

NO has both physiological and pathological roles in cerebral ischemia. Excessive NO production during ischemia contributes to formation of peroxynitrite and other cytotoxic metabolites that promote mitochondrial dysfunction, DNA oxidation, and neuronal apoptosis.[8-10] In the present study, high-dose ascorbic acid significantly reduced NO levels compared with the control group, indicating suppression of nitrosative stress pathways. The concurrent reduction of both MDA and NO levels strongly supports the hypothesis that antioxidant therapy attenuates oxidative neuronal injury following cerebral ischemia-reperfusion. However, direct subgroup comparisons between low-dose and high-dose ascorbic acid groups did not demonstrate statistically significant differences for MDA and NO measurements, although lower mean values were observed in the high-dose subgroup. Therefore, the present findings should not be interpreted as definitive evidence of a dose-dependent neuroprotective effect.

Histopathological findings further supported the biochemical results. The lowest ANP was observed in the high-dose ascorbic acid group, indicating reduced neuronal degeneration after ischemia-reperfusion injury. Degenerative neuronal changes, including eosinophilic cytoplasm, pyknotic nuclei, and loss of Nissl substance, were

substantially less prominent in the high-dose ascorbic acid group. Furthermore, lower ANP values observed in the high-dose compared with the low-dose ascorbic acid group suggest comparatively greater histopathological improvement in the high-dose ascorbic acid subgroup. Bonferroni-corrected multiple comparison analysis demonstrated significantly lower neuronal injury findings in the high-dose ascorbic acid group compared with the control group. Although lower neuronal injury values were also observed compared with the low-dose ascorbic acid group, this difference did not remain statistically significant after Bonferroni correction (p=0.013). These histopathological findings support the biochemical results and suggest that attenuation of oxidative stress may contribute to reduced neuronal degeneration following reperfusion injury.

Ascorbic acid is a potent water-soluble antioxidant capable of scavenging free radicals within intracellular and extracellular compartments.[25,27] In addition to its antioxidant activity, ascorbic acid contributes to preservation of endothelial integrity and modulation of inflammatory pathways. Previous studies demonstrated reduced infarct volume and attenuation of neuronal injury following ascorbic acid administration in focal cerebral ischemia models.[26,32] The present findings further support the potential neuroprotective role of high-dose ascorbic acid in ischemia-reperfusion injury. In addition, alternative antioxidant strategies involving dehydroascorbic acid have also been proposed because of improved blood-brain barrier transport characteristics.[31] Experimental antioxidant pretreatment studies have similarly demonstrated attenuation of ischemic neuronal injury.[33,34]

Edaravone is another established free radical scavenger that has demonstrated neuroprotective efficacy in both experimental and clinical studies.[18-24,28] Previous reports showed that edaravone suppresses oxidative tissue injury, inflammatory responses, and endothelial dysfunction following cerebral ischemia.[20-24] Oishi et al.[29] demonstrated attenuation of ischemia-induced metabolic alterations with edaravone treatment, while Zhang et al.[30] reported reduced oxidative injury in myocardial reperfusion models. However, unlike previous reports, statistically significant biochemical improvements were not observed in edaravone-treated groups in the present study.

Several factors may explain these findings. The neuroprotective efficacy of edaravone may be highly dependent on timing of administration, ischemia duration, and tissue bioavailability.[2,23] Variability in experimental protocols may substantially influence treatment efficacy. Additionally, ischemia-reperfusion injury involves multiple overlapping molecular pathways beyond oxidative stress alone, and suppression of free radical production may be insufficient to completely prevent neuronal injury under certain conditions. Several methodological and experimental factors may therefore have contributed to the relatively limited biochemical response observed in the present study, including treatment timing, reperfusion duration, dose selection, and characteristics of the selected ischemia-reperfusion model.

Interestingly, although edaravone-treated groups did not demonstrate statistically significant biochemical improvements, histopathological neuronal degeneration appeared slightly reduced compared with the control group. This discrepancy may indicate partial local neuroprotective effects that were not fully reflected by serum oxidative stress markers alone. Interestingly, the HDE group demonstrated numerically higher MDA levels compared with the ischemia-reperfusion control group, although this difference did not reach statistical significance. The relatively high standard deviation observed in the HDE group may reflect biological variability within the experimental model and may have contributed to the absence of statistically significant biochemical improvement.

In response to reviewer recommendations, direct subgroup comparisons were additionally performed between low-dose and high-dose treatment groups. Comparisons between low-dose and high-dose edaravone groups did not demonstrate statistically significant differences in MDA or NO measurements. Direct comparison between the high-dose edaravone and high-dose ascorbic acid groups demonstrated significantly lower NO levels in the high-dose ascorbic acid group.

The translational implications of the present findings are clinically important. Despite advances in thrombolytic therapy and mechanical thrombectomy, ischemia-reperfusion injury remains a major contributor to secondary neuronal damage after AIS.[3,4] Antioxidant therapies targeting oxidative stress pathways may therefore provide additional therapeutic benefit following successful reperfusion. Antioxidant supplementation has also demonstrated protective effects in extracerebral ischemia-reperfusion models, including myocardial reperfusion injury and cardiac surgery.[30,35]

Although middle cerebral artery occlusion (MCAO) remains one of the most widely used and accepted experimental models for focal cerebral ischemia, recent reviews emphasize that different animal models may be selected according to the specific mechanistic endpoint being investigated, including oxidative stress, reperfusion-related biochemical alterations, and early neuronal injury. The unilateral carotid artery occlusion model used in the present study provided a reproducible experimental environment for evaluating acute oxidative stress-mediated ischemia-reperfusion injury and early biochemical alterations under controlled conditions. Nevertheless, consistent with recent discussions on the limitations of experimental stroke models, this model may not fully reproduce the complex vascular, thromboembolic, and pathophysiological characteristics of human focal ischemic stroke.[36,37]

Overall, the present findings suggest that high-dose ascorbic acid was associated with significant biochemical and histopathological improvement following cerebral ischemia-reperfusion injury. Recent literature continues to support oxidative stress as a central mechanism in cerebral ischemia-reperfusion injury and highlights antioxidant-based neuroprotective strategies as an active area of experimental stroke research. However, although direct subgroup comparisons were additionally performed in response to reviewer recommendations, the present study was not primarily designed or statistically powered to establish a

definitive dose-response relationship between antioxidant treatment intensity and neuroprotective efficacy. Therefore, further studies incorporating functional neurological assessment, molecular inflammatory/apoptotic markers, infarct volume analysis, and larger experimental cohorts are required to clarify the mechanisms and translational relevance of antioxidant-based neuroprotective strategies in ischemic stroke.[38,39]

Limitations

Several limitations of the present study should be acknowledged. Infarct volume measurements, cerebral edema evaluation, and brain weight analysis were not performed. In addition, advanced immunohistochemical analyses, including apoptotic and inflammatory markers such as caspase-3, TUNEL, GFAP, and Iba-1 were not evaluated. Therefore, the molecular and mechanistic basis of the observed neuroprotective findings could not be fully clarified. The relatively short reperfusion period may not fully reflect delayed neuronal injury and long-term neuroinflammatory processes observed in clinical stroke. Therefore, delayed apoptotic and inflammatory responses occurring during later reperfusion stages could not be comprehensively evaluated in the present study. In addition, functional neurological assessment tools, including Bederson and Garcia neurological deficit scores, were not evaluated in the present study. Therefore, the translational and clinical relevance of the observed neuroprotective findings could not be fully determined. Furthermore, although the experimental model reproduced important aspects of oxidative neuronal injury, it may not completely represent the complex pathophysiology of human AIS. In addition, a separate sham-operated group was not included in the present experimental design. Therefore, the isolated effects of anesthesia, surgical manipulation, and vascular exposure could not be evaluated independently from ischemia-reperfusion injury.

CONCLUSION

As a result, the present study demonstrated that high-dose ascorbic acid was associated with significant biochemical and histopathological improvement against cerebral ischemia-reperfusion injury. Reduction of oxidative stress markers and attenuation of neuronal degeneration suggest that high-dose antioxidant therapy may represent a promising strategy for limiting secondary neuronal injury after AIS. Further translational studies are warranted to determine the optimal therapeutic timing, dosage, and clinical applicability of antioxidant agents in ischemic stroke management.

ETHICAL DECLARATIONS

Ethics Committee Approval

The experimental protocol was approved by the Local Ethics Committee for Animal Experiments at Kırıkkale University (Date: 21.10.2021, Decision No: 35).

Informed Consent

Since this study involved animal experimentation, informed consent was not required.

Peer Review Process

This manuscript was subject to external peer review.

Conflict of Interest

The authors declare no conflicts of interest related to this study.

Financial Disclosure

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Author Contributions

Conceptualization: PB, OE; Methodology: PB, OE, YŞ; Data Collection: PB, EC, MZ, ÜK; Formal Analysis: PB; Writing–Original Draft: PB; Writing–Review and Editing: OE, TD.

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