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The efficacy of MLC 901 in neuron-specific enolase, functional outcome and cerebral infarct volume for acute ischemic stroke model in rats

Ilsa Hunaifi[1,7], Andi Kurnia Bintang[2], Jumraini Tammasse[2]*, Isra Wahid[3], Rizka Vidya Lestari[4], Triana Dyah Cahyawati[5] and Iman Surya Pratama[6]

[1]Department of Neurology, Medical Faculty and Health Sciences University of Mataram, Pendidikan 37 Street, Mataram, Indonesia
[2]Department of Neurology, Faculty of Medicine Hasanuddin University, Perintis Kemerdekaan Km 10 Street, Makassar, Indonesia
[3]Department of Parasitology, Faculty of Medicine Hasanuddin University, Perintis Kemerdekaan Km 10 Street, Makassar, Indonesia
[4]Department of Histology, Medical Faculty and Health Sciences University of Mataram, Pendidikan 37 Street, Mataram, Indonesia
[5]Department of Radiology, Medical Faculty and Health Sciences University of Mataram, Pendidikan 37 Street, Mataram, Indonesia
[6]Bachelor in Pharmacy Program, Medical Faculty and Health Sciences University of Mataram, Pendidikan 37 Street, Mataram, Indonesia
[7]Doctoral Program, Faculty of Medicine Hasanuddin University Perintis Kemerdekaan Km 10 Street, Makassar, Indonesia

Abstract: **Background**: Ischemia causes neuronal death and releases Neuron-Specific Enolase (NSE). Thrombolysis is a standard therapy for ischemic stroke, but only 10-20 percent of patients receive thrombolysis. It is necessary to develop a treatment to increase neuroprotection by administering MLC 901 to influence NSE levels. **Objectives**: The study investigates the efficacy of MLC 901 on NSE levels, functional outcome and infarct volume in the stroke model of rats. **Methods**: Male Wistar rats were divided into acute ischemic stroke with MLC 901 43.2 mg/body weight, acute ischemic stroke with MLC 901 21.6 mg/body weight and acute ischemic stroke with CMC-Na for placebo-all treatment for 14 days. The NSE level was determined by ELISA, functional outcome determined by motoric score and infarct volume using NIH Image J. **Results**: NSE level increased at 24 hours after stroke. There was no difference in administering the dose of MLC 901 to improve functional outcome and reduce cerebral infarct volume. **Conclusion**: MLC 901 improved functional outcomes and reduced the volume of cerebral infarction in acute ischemic stroke but did not affect NSE levels.

Keywords: Acute ischemic stroke; Functional outcome; Infarction volume; MLC 901; Neuron-specific enolase

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INTRODUCTION

Ischemic stroke is the leading cause of mortality and disability in the world (Feigin *et al*., 2021). The American Heart Association (AHA) reports that 87% of stroke patients in the United States experience disability, resulting in impairment of daily and social activities, thus reducing the quality of life (Tsao *et al*., 2022). Brain ischemia is a condition that indicates blood supply interruption or blockage to the brain and leads to some change or damage to brain tissue resulting in stroke or brain infarction (Salaudeen *et al*., 2024). This will result in brain cell damage that determines the prognosis of the patient. Brain tissue damage results in the release of Neuron-Specific Enolase (NSE), which is an enzyme released by neuronal cells and neuroendocrine cells. NSE is related to the volume of the brain infarct after stroke. NSE levels will increase two hours after a stroke and remain for 48 hours. NSE levels are also associated with stroke outcomes and mediate axonal damage and neuronal death by mediating the activation of inflammatory cytokines, chemokines and other inflammatory mediators that cause axonal damage. Regulation of NSE plays an important role in controlling inflammation and degeneration processes in the central nervous system (Freitas *et al*., 2024). The standard treatment for ischemic stroke is thrombolysis with alteplase with an onset of less than 4.5 hours (Powers *et al*., 2019). Evidence shows that thrombolysis administration is only around 9.1 percent (Gajurel *et al*., 2023). Thus, many treatment strategies have been developed to increase neuroprotection and reduce brain cell death. Research shows that herbal medicine improves brain microcirculation and protects against ischemic/reperfusion by reducing oxidative stress, neuroinflammation and modulating microglia polarization (Xiong *et al*., 2018). MLC 901 is herbal medicine; it contains nine active components, including *Astragalus membranaceus radix*, *Salvia miltiorrhizae radix*, *Paeoniae lactiflora rubra radix*, *Ligusticum chuanxiong rhizoma*, *Angelicae sinensis radix*, *Carthamus tinctorius flos*, *Prunus persicae semen*, *Polygalae tenuifolia radix* and *Acori tatarinowii rhizome* (Theadom *et al*., 2018). *Astragalus membranaceus radix* with the active compound Astragaloside IV (AST-IV) can decrease NSE levels in spinal cord injury models (Zhou *et al*., 2018; Anjum *et al*., 2024). *Salvia miltiorrhizae radix* contains Salvionic Acid B (SAB), which can promote neurogenesis through specific proteins such as NSE, Tau, Nestin and Glial Fibrillary Acidic Protein (GFAP) (Xin *et al*., 2020; Zhou *et al*., 2018). Administration of MLC 901,

**Corresponding author:* e-mail: jumraini@med.unhas.ac.id

two capsules (0.4 g/capsule) three times daily, improves the quality of life (cognitive domain) within 3–6 months after a traumatic brain injury (Theadom *et al*., 2018). Currently, there is no established standard dosage of MLC 901 for treating acute ischemic stroke, and the effects of MLC 901 on NSE and the improvement of neurological deficits in this condition are still limited, necessitating further investigation.

MATERIALS AND METHODS

Animal preparation

Adult male Wistar rats (n = 15), approximately 90 days old (200–300 g weight), were obtained from the colony of the Anatomy Department in the Medical Faculty of the University of Mataram. All rats were well treated at a temperature of 25°C ± 2°C with a humidity of 60 ± 10%, and free access to food and water was also provided. All experiments were carried out at the Drug Research Laboratory of the Medical Faculty of the University of Mataram, Mataram, West Nusa Tenggara, Indonesia. The experimental protocol was approved by the Ethical Committee of the Medical Faculty, University of Mataram, No. 120/UN18.F8/ETIK/2023. All efforts were made to minimize animal suffering and reduce the number of animals per group.

Drug preparation and cerebral ischemia induction

The stock solution was prepared by suspending 0.25% w/v Natrium-Carbomethyl Cellulosa (CMC-Na) with an appropriate amount of MLC 901 (Moleac, Singapore, batch number C022072) powder. The stock solution was prepared less than 30 minutes before oral administration. The stock solution was further diluted to the desired concentration with 0.25% w/v CMC-Na.

The rats were randomly divided into three groups (n = 5 per group): (i) group one treated with 43.2 mg/200 g body weight MLC 901 (doses 1), (ii) group 2 treated with 21.6 mg/200 g body weight MLC 901 (doses 2) and (iii) group three was given CMC-Na as control. Ninety minutes after the establishment of the Common Carotid Artery Occlusion (CCAO) model, the control group received CMC-Na 0.25% w/v, while the treatment groups received MLC 901. The drugs and CMC-Na were administered orally once daily for 14 days. There is no positive control because it clinically resembles (mimics) use in patients with acute ischemic stroke. The drug is clinically used after the golden period of acute ischemic stroke to improve neuroprotection. The experimental design is shown in fig. 1.

The ischemic stroke was induced by the Unilateral Carotid Artery Occlusion (UCAO) method, as described in a previous study (Indra & Gasmara, 2016; Ulya *et al*., 2021). To develop the UCAO model, rats were anesthetized intraperitoneally using a mixture of xylazine 10 mg/kg of weight and ketamine 80 mg/kg of weight. Anesthetized rats were placed on a surgical table in the supine position. The rats were dissected using surgical instruments through a small incision approximately 2-3 cm above the neck midline to isolate the left common carotid artery. The bulldog clamp blocked the isolated artery for 180 minutes. After 180 minutes, the clamp was released for reperfusion.

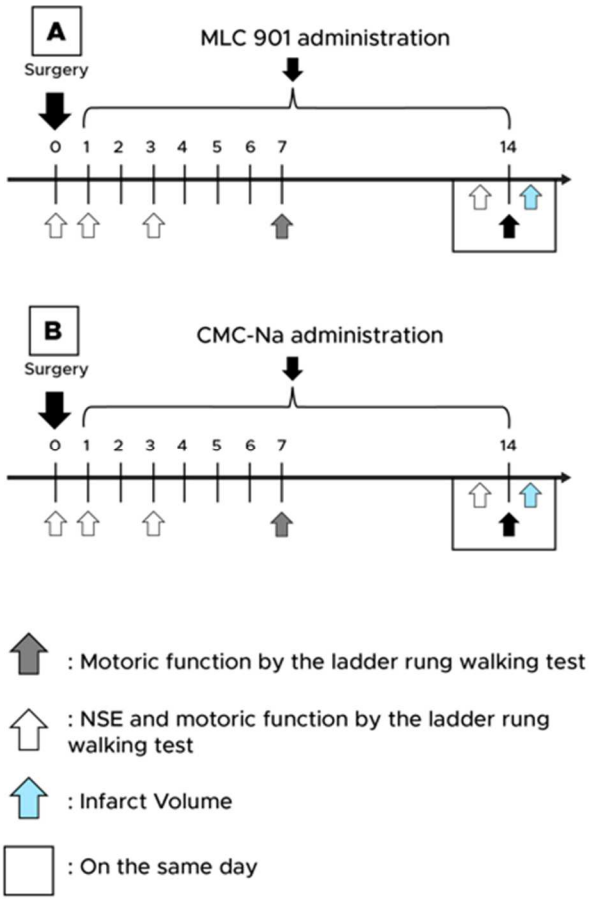

Fig. 1: Schematic illustration of the experimental design

Evaluation of the functional outcome

The rats were tested for neurological deficit using The Ladder Rung Walking Test apparatus (Fig. 2) before surgery, on days 1, 3, 7 and 14 after stroke surgery. Each rat was allowed to walk the cylindrical stairs, which were set in 1-meter courses at different distances. Every step the right rat made in its hindlimb movement was observed. That was recorded on camera. Rats that slipped had reduced or impaired motor function. Based on the shape of the error and where the foot was placed on the rungs, a seven-category scale was used to evaluate the placement (Metz & Whishaw, 2002). Each animal had five rounds of training and testing for every session. Subsequently, the average error score was examined. The percentage of errors in each trial and the quality of the placement of the right forelimb and hindlimb were examined. Errors were recorded for scores of 0, 1 and 2 (paw slip or fall), which were averaged over five trials using the following formula:

number of errors/number of steps X 100. Data on error scores were displayed as a percentage of 100 (Metz & Whishaw, 2009).

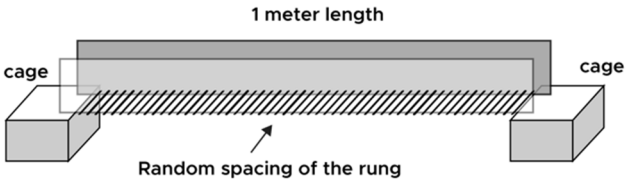

Fig. 2: Ladder rung walking test apparatus for measurement of functional outcome

Analysis of Neuron-Specific Enolase (NSE)

Blood samples from each rat were taken to measure NSE levels before surgery and on days 1, 3 and 14 after stroke surgery. The serum was separated by centrifugation, and if it could not be tested immediately, it was stored at -20°C. NSE levels were measured using the ELISA method using the mouse NSE Enzyme-linked Immunosorbent Assay (ELISA) kit (FineTest Biotech Inc, Wuhan, China, Catalogue Number EM1242) as per the manufacturer's instructions. ELISA procedure in accordance with a previous study (Hardiany *et al.,* 2024). The NSE assay was performed at the Mataram Teaching Hospital Clinical Pathology Laboratory with the Multiskan Sky Thermo Scientific ELISA Reader, United States of America (USA), and the biochemist who performed it was blinded to groups.

Measurement of cerebral infarction volume

At 14 days after stroke surgery, all rats were euthanized. The animal brain was removed and cut into coronal sections using a rat brain slicer and placed in a 2% solution of dye 2,3,5 Triphenyltetrazolium Chloride (TTC) developed at 37°C for 30 minutes. The infarction was designed as a white area and measured with National Institutes of Health (NIH)/FIJI Image J software (Weber *et al*., 2024).

Statistical analysis

All data were analyzed with the Statistical Package for the Social Sciences version 26. The normality of the data distribution was confirmed by the Shapiro–Wilk test. Data were presented as mean ± SD. The relationship between NSE levels, functional outcome and cerebral infarction volume was analyzed by the Spearman correlation test. The effect of MLC 901 administration on NSE and infarction volume using the Kruskal Wallis test followed by Mann Whitney post hoc test, while functional outcome using the One Way Annova and Least Significant Difference (LSD) post hoc test; differences were considered significant whenever $p \leq 0.05$.

RESULTS

The efficacy of MLC 901 on the NSE level

The mean level of NSE showed a significant increase at 24 hours after stroke surgery ($p = 0.009$; Fig. 3). There were no significant differences in the efficacy of MLC 901 on NSE levels at 24 hrs, 72 hrs and day 14 between groups (p= 0,093 at 24-hrs, p = 0,229 at 72-hrs, p= 0,543 at day 14).

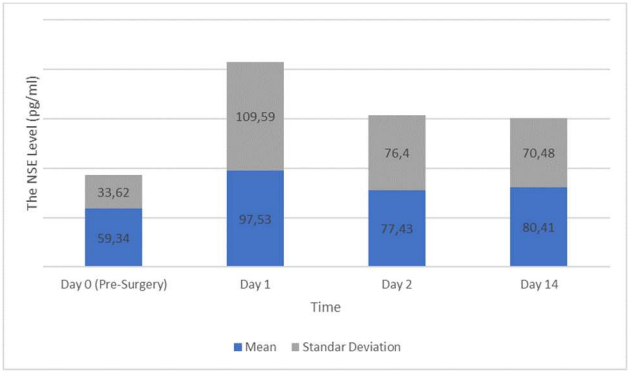

Fig. 3: The effect of MLC 901 on NSE Levels.

The efficacy of MLC 901 on functional outcome

Functional outcome was assessed by examining motor function with the ladder rung walking test. The rats with MLC 901 doses 1 and 2 showed an improvement in motor function compared to the control groups. On days 7 and 14 after stroke surgery, there was a significant difference between group 1 and group 2 versus the control group. This investigation shows that there were significant differences in the administration of MLC 901 in groups 1 and 2 on the neurological deficits score in the acute phase (day 7) and the subacute phase (day 14) (Fig. 4). Furthermore, there was a significant difference in the neurological deficits between the administration of MLC 901 in groups 1 and 2 compared to the control group. This study shows that the administration of MLC 901 can improve neurological deficits in rats compared to the control group.

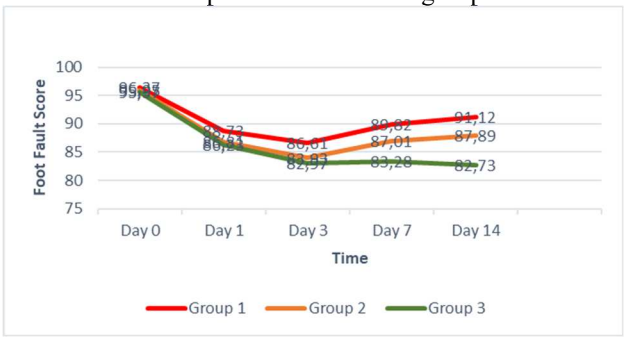

Fig. 4: The efficacy of MLC 901 on the functional outcome.

LSD post hoc analysis on day 7 showed $p = 0.001$ for group 1 vs. control and p = 0.026 for group 2 vs. control; on day 14, $p = 0.00$ (<0.001) for group 1 vs. control and p = 0.000 (<0.001) for group 2 vs. control.

The efficacy of MLC 901 on cerebral infarction volume

The volume of cerebral infarction of the three groups is presented in fig. 5. There was a relationship between the

MLC 901 administration and cerebral infarction volume (p=0.004). Administration of MLC 901 doses 1 and 2 reduced the volume of cerebral infarction compared to the control group, and there were no significant differences between groups 1 and 2 (Fig. 5).

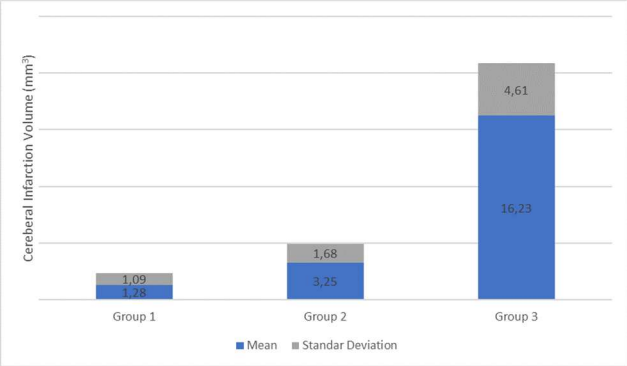

Fig. 5: The efficacy of MLC 901 on cerebral infarction volume. Mann-Whitney Post Hoc Analysis: group 1 vs group 2 (p=0.095); group 1 vs control, (p=0.008); group 2 vs control (p=0.008).

Relationship between NSE level with functional outcome

Neuron-specific enolase is a dimeric isoenzyme of the enzyme glycolytic enolase used as a marker of acute neuronal injury to assess the degree of short-term neurological deficit (Aritonang *et al*., 2018). This study found a relationship between NSE levels and functional outcomes. NSE levels 24 and 72 hours post-stroke surgery were associated with functional outcome (r=0.525, p=0.044) vs NSE 72 hours (r=0.586, p=0.022). On day 14, the examination of NSE levels was not associated with the functional outcome (r = 0.361, p=0.187) (table 1).

Relationship between NSE level and cerebral infarction volume

The volume of cerebral infarction is an indicator of brain cell damage due to acute ischemic stroke. In this study, the examination of the volume of cerebral infarction was carried out at the end of the study. The results showed significant differences between NSE levels measured at 24 hours and 72 hours with cerebral infarction volume, but there were no significant differences between NSE levels measured at day 14 with cerebral infarction volume. The results of the study are presented in table 2.

Relationship between motoric score with cerebral infarction volume

The severity of stroke indicates the death of many brain cells. The results of this study showed that the functional outcome of stroke severity measured by motoric score on day 14 was strongly associated with cerebral infarction volume (r = 0.879, p = 0.000 (<0.001). The smaller volume of cerebral infarction indicates a mild degree of the stroke.

DISCUSSION

Stroke is a neurological disorder characterized by neurological deficits. Cerebral ischemia causes brain cell death that will result in neuronal damage to the brain, resulting in the release of cell-specific markers in the blood, one of which is NSE (Mochetti *et al*., 2024). NSE can be a prognostic marker in acute ischemic stroke (Kurakina *et al*., 2021; Freitas *et al*., 2024). NSE is a marker of poor prognosis in patients undergoing resuscitation after cardiac arrest (Kim *et al*., 2023). Several studies have shown a relationship between NSE levels and cerebral infarction volume measured 12–72 hours post-stroke (Mochetti *et al*., 2024). In this study, NSE levels were found to increase within 24 and 72 hours after stroke induction. The highest level of increase in NSE occurred 24 hours after stroke. This is consistent with research that NSE levels peak at 24-48 hours after cerebral occlusion (Kurakina *et al*., 2021; Freitas *et al*., 2024). NSE levels will continue to increase in 96 hours and can last for 6 days (Kim *et al*., 2014). Zaheer *et al.* (2013) reported that NSE levels on the first day of a stroke were associated with the volume of cerebral infarction (Zaheer *et al*., 2013). Freitas *et al.* (2024) concluded that NSE levels more than 26.3 ng/ml were associated with unfavorable outcomes (Freitas *et al*., 2024). In this study, there was no relationship between NSE levels and cerebral infarction volume because the examination of cerebral infarction volume was carried out at the end of the study (day 14) when NSE levels had decreased.

The results of this study indicated that there was a relationship between NSE levels and cerebral infarction volume at 24 and 72 hours. Purroy *et al.* (2021) reported that NSE levels, NT-ProBnP, S100b, hs-CRP, hs-Troponin, and IL-10 24 hours after ischemic stroke onset were associated with cerebral infarct volume. Cerebral ischemia is a condition in which there is a deficiency of blood flow to the brain. This deficiency can cause damage to neurons and neuroconduction, resulting in various pathologies. These pathologies include neuronal edema, abnormal lipid and glucose metabolism, and damage to the blood-brain barrier, leading to cell death and increased levels of NSE (Liu *et al*., 2024). This may be due to the examination of cerebral infarction volume performed on day 14, where NSE levels have decreased. Quintard *et al.* (2014) reported that the administration of MLC 901 can prevent an increase in NSE and S100B levels in traumatic brain injury (TBI) (Quintard *et al*., 2014). The AST-IV content in MLC 901 is neuroprotective by reducing neuroinflammation and reducing the ferroptosis process through the Nrf/HO-1 signal pathway induced by stroke (Zhang *et al*., 2023). This study showed that NSE levels at 24 hours and 72 hours were strongly associated with functional outcome with increased motor score. This is in line with Saiko OV (2020), where an increase in NSE levels at the beginning of a stroke is associated with the severity of the degree of neurological deficit (Sm & Ov, 2020).

Table 1: Relationship between NSE level and functional outcome

Variable	Correlation coefficient	*p* value*
NSE 24 h vs. functional outcome	0.525	0.044
NSE 72 h vs. functional outcome	0.586	0.022
NSE 336 h vs. functional outcome	0.361	0.187

**Spearman's correlation test*

Table 2: Correlation between NSE level and cerebral infarction volume

Variable	Correlation coefficient	*p* value*
NSE 24 h vs. cerebral infarction volume	0.525	0.044
NSE 72 h vs. cerebral infarction volume	0.586	0.022
NSE 336 h vs. cerebral infarction volume	0.361	0.187

**Spearman's correlation test*

Examination of motor deficits in experimental stroke can use the Foot Fault Score or defect score that is examined using the Ladder Rung Walking test (Metz & Whishaw, 2009). In this study, there was an improvement in neurological deficits on days 7 and 14 in group 1 and group 2 compared to the control group. Analysis showed that MLC 901 administration at dose 1 and dose 2 was able to improve the severity of stroke compared to the control group. Administration of both doses of MLC 901 was able to improve neurological deficits, but dose 1 was more significant after more than 7 days of stroke onset. Administration of MLC 901 to stroke model rats with occlusion of the middle cerebral artery for 60 minutes showed an improvement in the neurological scale and mortality rate (Widmann *et al*., 2018). In addition, the active compounds of *ligustilide, 3-n-buthylphyhalide*, and *ferulic acid* in the *Ligusticum chuanxiong rhizome* and *Angelicae sinensis radix*, which have anti-inflammatory effects on neurogenesis, angiogenesis, and anti-atherosclerosis, can improve neurological function and infarct volume in patients with an ischemic stroke (Han *et al*., 2021).

This study demonstrated that the control group exhibited a larger volume of cerebral infarction compared to the two treatment groups. In particular, MLC 901, even at dose 2, effectively reduced the volume of cerebral infarction compared to the control group. Interestingly, statistical analysis revealed that MLC 901 administration at different doses did not exhibit a significant difference in reducing the volume of cerebral infarction. These findings suggest that MLC 901 administration, regardless of dosage, can improve stroke severity and reduce the volume of cerebral infarction. Mechanically, MLC 901 exerts its protective effects by inhibiting the activation of astrocytes and microglia/macrophages, thus reducing neutrophil infiltration into the ischemic area and mitigating the production of pro-inflammatory mediators such as cytokines, chemokines and matrix metalloproteinases (Widmann *et al*., 2018). The potential clinical implications of MLC 901's efficacy at lower doses warrant further investigation.

CONCLUSION

Administration of MLC 901 with dose 1 or dose 2 can improve functional outcomes and reduce cerebral infarction volume in acute ischemic stroke. This dose may be applicable for administering MLC 901 to patients with acute ischemic stroke, with adjustments made for human dosing. However, MLC 901 does not affect NSE levels, indicating that its neuroprotective effects may not be mediated through NSE. Further research is needed to investigate the exact mechanisms underlying the neuroprotective effects of MLC 901 and to determine whether different doses of MLC 901 have different effects on NSE levels and functional outcomes. Furthermore, the relationship between NSE levels and functional outcomes after ischemic stroke needs to be further explored to determine its potential role as a biomarker for stroke severity and prognosis.

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Author's contribution
IH, ISP were involved in concepting and planning the research, IH, RVL and TDC performed the data acquisition/collection, IH, RVL, TDC and ISP calculated the experimental data and performed the analysis, AKB, JT and IW drafted the manuscript and designed the figures, ISP aided in interpreting the results. All authors took parts in the critical revision of the manuscript.

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Data availability statement
The data that supports the finding of this study are available in the supporting information of this article.

Ethical approval
The experimental protocol was approved by the Ethical Committee of the Medical Faculty and Health Sciences University of Mataram (No. 120/UN18.F8/ETIK/2023.)

Conflict of interest

The authos declare no conflict of interest.

REFERENCES

Anjum A, Yazid MD, Daud MF, Idris J, Hwei Ng, AM, Naicker AS, Ismail OH, Kumar RKA and Lokanathan Y (2024). Mechanical scratch injury on differentiated motor neuron of NSC-34 cells as an *in vitro* model for evaluation of neuroregeneration potential of NeuroAiD II (MLC901). *In vitro models,* **3**: 65-79.

Aritonang CRL, Retnaningsih R and Husni A (2018). Hubungan kadar neuron specific enolase serum terhadap luaran klinis neurologis pasien stroke iskemik akut. *Neurona*, **36**(1): 49-58.

Feigin VL, Stark BA, Johnson CO, Roth GA, Bisignano C, Abady GG, Abbasifard M, Abbasi-Kangevari M, Abd-Allah F, Abedi V, Abualhasan A, Abu-Rmeileh NME, Abushouk AI, Adebayo OM, Agarwal G, Agasthi P, Ahinkorah BO, Ahmad S, Ahmadi S and Murray CJL (2021). Global, regional and national burden of stroke and its risk factors, 1990-2019: A systematic analysis for the Global Burden of Disease Study 2019. *Lancet Neurol*, **20**(10): 1–26.

Freitas TE, Costa AI, Neves L, Barros C, Martins M, Freitas F, Noronha D, Freitas P, Faria T, Borges S, Freitas S, Henriques E and Sousa AC (2024). Neuron-Specific Enolase as a prognostic biomarker in acute ischemic stroke patients treated with reperfusion therapy. *Front. Neurol*. **15**: 1408111.

Gajurel BP, Nepal G, Jaiswal V, Ang SP, Nain P, Shama N, Ruchika FNU, Bohara S, Kharel S, Yadav JK, Medina J RT and Shrestha AB (2023). Utilization rates of intravenous thrombolysis for acute ischemic stroke in Asian countries: A systematic review and meta-analysis. *Medicine*, **102**(42): e35560.

Han Y, Chen Y, Zhang Q, Liu BW, Yang L, Xu YH and Zhao YH (2021). Overview of therapeutic potentiality of Angelica sinensis for ischemic stroke. *Phytomedicine*, **90**: 153652.

Hardiany NS, Dewi PKK, Dewi S and Tejo BA (2024). Exploration of neuroprotective effect from *Coriandrum sativum* L. ethanolic seeds extracts on brain of obese rats. *Sci Rep.,* **14**(1): 603.

Haque A, Capone M, Matzelle D, Cox A and Banik NL (2017). Targeting enolase in reducing secondary damage in acute spinal cord injury in rats. *Neurochem Res*, **42**(10): 2777–2787.

Indra RM and Gasmara PC (2016). UCAO (Unilateral Cerebral Artery Occlussion) method increases the level of MMP- 9 brain tissue in rats model of ischemic stroke. *MNJ.*, **2**(2): 46–50.

Kim BJ, Kim YJ, Ahn SH, Kim NY, Kang DW, Kim JS and Kwon SU (2014). The second elevation of neuron-specific enolase peak after ischemic stroke is associated with hemorrhagic transformation. *J Stroke Cerebrovasc Dis,* **23**(9): 2437–2443.

Kim YJ, Kim YH, Youn CS, Cho IS, Kim SJ, Wee JH, Park YS, Oh JS, Lee BK and Kim WY (2023). Different neuroprognostication thresholds of neuron-specific enolase in shockable and non-shockable out-of-hospital cardiac arrest: A prospective multicenter observational study in Korea (the KORHN-PRO registry). *Crit Care,* **27**: 313.

Kurakina AS, Semenova TN, Guzanova EV, Nesterova VN, Schelchkova NA, Mukhina IV and Grigoryeva VN (2021). Prognostic value of investigating neuron-specific enolase in patients with ischemic stroke. *Sovrem Tekhnologii Med,* **13**(2): 68-73.

Liu F, Li H, Hong X, Liu Y and Yu Z (2024). Research progress of neuron-specific enolase in cognitive disorder: A mini review. *Front hum neurosci*, **18**: 1392519.

Metz GA and Whishaw IQ (2002). Cortical and subcortical lesions impair skilled walking in the ladder rung walking test: A new task to evaluate fore- and hindlimb stepping, placing and co-ordination. *J Neurosci Methods*, **115**(2): 169-179.

Metz GA and Whishaw IQ (2009). The ladder rung walking task: A scoring system and its practical application. *J Vis Exp: JoVE*, **28**: 1204

Mochetti MM, Silva EGP, Correa AAF, Cabette MR, Perissinotti IN, E Silva, LOJ, Pessoa AS, de Oliveira RC, da Silva LFF, de Souza HP and de Alencar JCG (2024). Neuron-specific enolase at admission as a predictor for stroke volume, severity and outcome in ischemic stroke patients: A prognostic biomarker review. *Sci Rep.*, **14**(1): 2688.

Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, Biller J, Brown M, Demaerschalk BM, Hoh B, Jauch EC, Kidwell CS, Leslie-Mazwi TM, Ovbiagele B, Scott PA, Sheth KN, Southerland AM, Summers DV and Tirschwell DL (2019). Guidelines for the early management of patients with acute ischemic stroke: 2019 update to the 2018 guidelines for the early management of acute ischemic stroke: a guideline for healthcare professionals from the american heart association/american stroke association. *Stroke*, **50**(12): E344–E418.

Purroy F, Farré-Rodriguez J, Mauri-Capdevila G, Vicente-Pascual M and Farré J (2021). Basal IL-6 and S100b levels are associated with infarct volume. *Acta neurol Scan*, **144**(5): 517–523

Quintard H, Lorivel T, Gandin C, Lazdunski M and Heurteaux C (2014). MLC901, a traditional Chinese Medicine induces neuroprotective and neuroregenerative benefits after traumatic brain injury in rats. *Neuroscience*, **277**: 72-86.

Salaudeen MA, Bello N, Danraka RN, Ammani ML (2024). Understanding the pathophysiology of ischemic stroke: The basis of current therapy and opportunity for new ones. *Biomolecules*, **14**(3): 305.

Sm S and Ov S (2020). Neuron-specific enolaza as a marker of lesion cerebral tissue in patients with ischemic stroke. *Biomed J Sci & Tech Res*, **31**(1): 2020.

Theadom A, Barker-Collo S, Jones KM, Parmar P, Bhattacharjee R and Feigin VL (2018). MLC901 (NeuroAiD II^{TM}) for cognition after traumatic brain injury: A pilot randomized clinical trial. *Eur. J. Neurol.*, **25**(8): 1055-e82.

Tsao CW, Aday AW, Almarzooq ZI, Alonso A, Beaton AZ, Bittencourt MS, Boehme AK, Buxton AE, Carson AP, Commodore-Mensah Y, Elkind MSV, Evenson KR, Eze-Nliam C, Ferguson JF, Generoso G, Ho JE, Kalani R, Khan SS, Kissela BM and Martin SS (2022). Heart disease and stroke statistics-2022 Update: A report from the American Heart Association. *Circulation*, **145**(8): E153–E639.

Ulya T, Ardianto C, Anggreini P, Budiatin AS, Setyawan D and Khotib J (2021). Quercetin promotes behavioral recovery and biomolecular changes of melanocortin-4 receptor in mice with ischemic stroke. *J Basic Clin Physiol. Pharmacol.*, **32**(4): 349–355.

Weber RZ, Bernardoni D, Rentsch NH, Buil BA, Halliday S, Augath MA, Razansky D, Tackenberg C and Rust R (2024). A toolkit for stroke infarct volume estimation in rodents. *NeuroImage*, **287**: 120518.

Widmann C, Gandin C, Petit-Paitel A, Lazdunski M and Heurteaux C (2018). The traditional Chinese medicine MLC901 inhibits inflammation processes after focal cerebral ischemia. *Sci Rep.*, **8**(1): 18062

Xin M, Hao Y, Huang G, Wang X, Liang Z, Miao J, Ma D, and Feng J (2020). The efficacy and safety of salvianolic acids on acute cerebral infarction treatment: A protocol for systematic review and meta-analysis. *Medicine*, **99**(23): e20059.

Xiong XY, Liu L and Yang QW (2018). Refocusing Neuroprotection in Cerebral Reperfusion Era: New Challenges and Strategies. *Front Neurol.*, **9**: 249

Zaheer S, Beg M, Rizvi I, Islam N, Ullah E and Akhtar N (2013). Correlation between serum neuron specific enolase and functional neurological outcome in patients of acute ischemic stroke. *Ann Indian Acad Neurol*, **16**(4): 504.

Zhang C, Shi Z, Xu Q, He J, Chen L, Lu Z, Huan Q, Wang Y and Cui G (2023). Astragaloside IV alleviates stroke-triggered early brain injury by modulating neuro-inflammation and ferroptosis via the Nrf2/HO-1 signaling pathway. *Acta Cir Bras.*, **38**: e380723.

Zhou LY, Song Z, Zhou LW, Qiu Y, Hu N, Hu Y and Hu X (2018). Protective role of astragalus injection in spinal cord ischemia-reperfusion injury in rats. *Neurosciences*, **23**(2): 116-121.