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Centella asiatica (L.) Urb. as Neuroprotection Agents to Ischemic Stroke: A Scoping Review

Valentina B.M. Lumbantobing [1,3,*], Nurul Darmawulan [2], I Ketut Adnyana [1,*]

[1] Pharmacology and Clinical Pharmacy Department, School of Pharmacy, Institut Teknologi Bandung, Indonesia
[2] Pelni Hospital, Jakarta, Indonesia
[3] Fundamental Nursing Department, Faculty of Nursing, Universitas Padjadjaran, Bandung, Indonesia
* Correspondence: valentina@unpad.ac.id (V.B.M.L); adnyana@itb.ac.id (I.K.A);

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Abstract: Ischemic stroke is a severe brain problem that can result in neuronal damage and cognitive deficits. *C. asiatica* contains various triterpene compounds that hold potential therapeutic effects for ischemic stroke. This study aims to elucidate the effects of *C. asiatica*, including neuroprotective mechanisms, optimal doses, advantages, and potential limitations for ischemic stroke patients. A scoping review was conducted, employing the PRISMA-ScR framework to ensure standardized reporting. The search strategy utilized Boolean operator keywords, which were explored in CINAHL, PubMed, and Google Scholar databases. Inclusion criteria encompassed articles published in English between 2012 and 2022, employing experimental or quasi-experimental study designs, with a primary focus on stroke and *C. asiatica*. The review obtained 15 articles that met the criteria after undergoing quality assessment using critical appraisal tools. Results suggest *C. asiatica* is promising to be an alternative treatment for neurological conditions, especially in ischemic stroke. Animal studies have demonstrated neuroprotective effects independent of dosage and range of doses (6 to 300 mg/kg). However, the optimal dosage for ischemic stroke patients remains uncertain, and variations in extract composition may affect efficacy. Future research is needed to determine the safety and efficacy of *C. asiatica* and its active components in humans.

Keywords: *C. asiatica*; ischemic stroke; neuroprotection.

1. Introduction

Stroke has become the second leading cause of death in the world these days. Not only that, stroke also causes high morbidity rates, where 50% of patients endure disabilities [1]. The World Health Organization (WHO) stated that the incidence of stroke annually reaches 13.7 million cases, and 87% of deaths and disabilities due to stroke occur in low to middle-income countries [2]. The prevalence of stroke in Indonesia tends to increase every year. Ghani *et al*. [3] said that in 2007, the prevalence of stroke was only 8.3%, but it increased to 12.1% in 2013. This indicates that Indonesia still has to focus on the prevention and treatment of stroke.

Research on stroke management has been carried out in the last few decades, especially in ischemic stroke. But so far, t-PA (tissue-plasminogen activator) is the only approved ischemic stroke treatment, has a limited time window, and even has a high risk of causing hemorrhagic complications [4,5]. Therefore, the need to find an alternative treatment that

focuses on neuroprotection is very important. Current stroke drug discovery has focused on natural compounds such as traditional medicinal herbs because they have promising potential and therapeutics for the treatment of neurological disorders, one of which is cerebral ischemia [6,7].

C. asiatica, also known as Gotu kola, Asiatic pennywort, or Indian pennywort, has emerged as a promising candidate for neuroprotection against ischemic stroke. This belongs to the family *Apiaceae* and sub-family *Mackinlayoideae*. *C. asiatica* generally grows in southern African countries crossed by the equator and is an endogenous plant that grows in Australia and Indonesia [8]. Research reports that *C. asiatica* provides beneficial neurobehavioral effects. The results showed that *C. asiatica* contains various *triterpene* compounds, including *asiaticoside*, *madecassoside*, and *Asiatic acid*, which have neuroprotective effects on animal disease models [9]. This herbal plant has the potential as an alternative treatment for neurological conditions, including ischemic stroke [10].

A significant attribute of *C. asiatica* is its capability to traverse the blood-brain barrier, enabling direct interaction with central nervous system tissues [1,11]. This characteristic strongly emphasizes its potential as a neuroprotective agent in ischemic stroke. Moreover, *C. asiatica* demonstrates versatility in dosage [12]. However, there is a lack of comprehensive literature that discusses the neuroprotective mechanism and the safe dosage of *C. asiatica*. Furthermore, the previous literature only discussed the effects of *C. asiatica* on different diseases without addressing the safe dosage and potential drawbacks that stroke patients might experience [13]. Further research is still needed to determine the neuroprotective mechanism that occurs and the safe dose of *C. asiatica*. This study aims to explore *C. asiatica* holistically regarding how neuroprotective mechanisms occur, safe dosage, advantages, and disadvantages of *C. asiatica* for the treatment of ischemic stroke.

2. Materials and Methods

2.1. Study design.

This scoping review was conducted between June and July 2022 using Arksey and O'Malley's methodological framework and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR) checklist as a writing guide [14]. The main idea of this review was to identify the effects of *C. asiatica* regarding neuroprotective mechanisms, doses, advantages, and inadequacies for patients with ischemic stroke.

2.2. Eligibility criteria.

This study found the relevant articles using the PCC framework, namely population "Patients with Stroke", concept "Neuroprotection", and context "*Centella asiatica".* This study included articles that were published between 2012 and 2022, experimental or quasi-experimental studies, topics relevant to the specific objectives of the review about stroke and *C. asiatica*, and published in English. Duplicates and non-full-text articles were excluded.

2.3. Search strategy.

Articles were obtained from electronic databases, including CINAHL, PubMed, and Google Scholar, using Boolean Operators OR and AND along with the keywords *"Stroke"* OR *"Ischemic Stroke"* AND *"Neuroprotection"* AND *"Centella asiatica"*.

2.4. Data collection and analysis.

After determining articles from three databases, the findings will be initially screened by the first author. This study selection process was conducted by i) Removing duplicate articles, ii) Screening titles, abstracts, and article summaries, iii) Determining articles that met inclusion criteria, and iv) Analyzing the reference lists from the results of identified studies. We obtained 1134 articles that were published in English over the last 10 years, including PubMed 3 studies, CINAHL 1 studies, and Google Scholar 1130 studies. After screening titles and abstracts, 35 articles will be further selected. However, we reviewed the full text of the articles. There were 3 duplicate articles, and the rest of the 18 articles were excluded due to their being irrelevant to the topic and purpose of this study. Finally, a total of 15 articles were included. The flow of the study selection process is right down below (Figure 1).

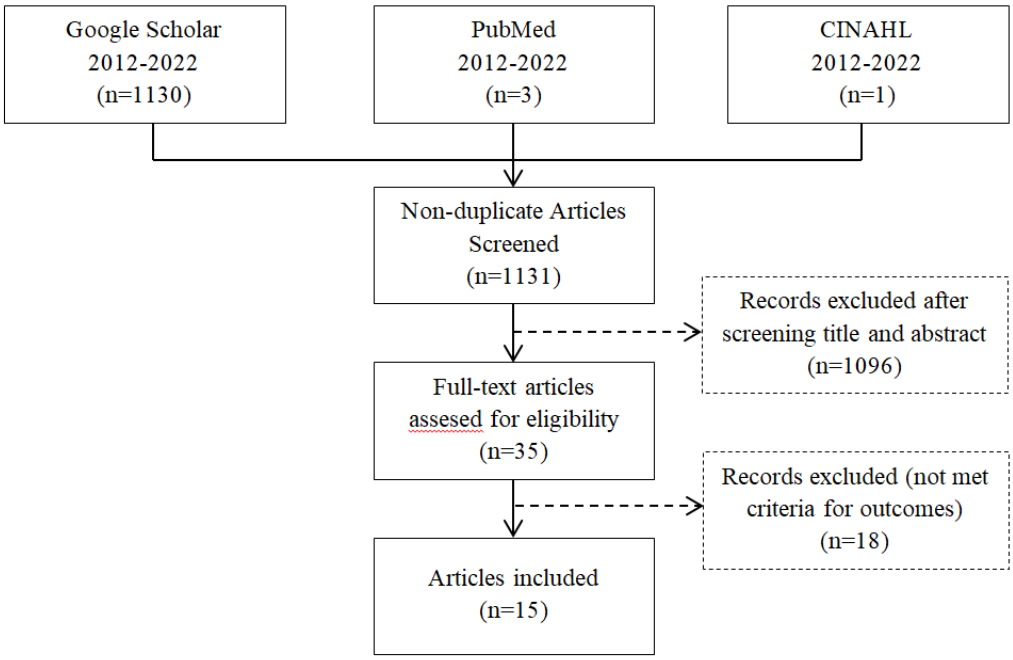

Figure 1. PRISMA flow diagram for Scoping Review. This figure was created by the authors based on the PRISMA-ScR guidelines and has not been previously published.

The articles that meet the criteria will be synthesized into a table. This study recorded data from each study regarding the author, year of publication, research objectives and method, location of study, sample, and research findings. Subsequently, the articles will be read and analyzed intensively using content analysis to map out key themes in order to address the research questions by the first author independently.

2.5. Quality appraisal.

The quality of the articles was evaluated using the critical appraisal checklist tools for experimental studies by the Joanna Briggs Institute (JBI) to ensure a comprehensive assessment of the study's design, conduct, and analysis [14]. This checklist for experimental studies

consisted of nine criteria to determine the potential risk of bias and the answer choices with "Yes", "No", "Unclear" or "Not applicable". "Yes" was assigned a value of 1, "No" a value of 0. The total score ranged from 0 to 9. The total score was graded using categories, namely poor <50%, moderate 50-80%, and good >80%, but was not used to exclude studies [15] (Table 1).

Table 1. Quality assessment using JBI.

Author	Q1*	Q2*	Q3*	Q4*	Q5*	Q6*	Q7*	Q8*	Q9*	%	Quality rating
Luo *et al.*, 2014 [16]	Yes	Yes	Yes	No	Un-clear	Un-clear	Yes	Yes	Yes	(6/9) 67%	Moderate
Lee *et al.*, 2014 [17]	Yes	Yes	Yes	Un-clear	Yes	Yes	Yes	Yes	Yes	(8/9) 88%	Good
Islam *et al.*, 2013 [18]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	(9/9) 100%	Good
Lee *et al.*, 2012[19]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	(9/9) 100%	Good
Zhang *et al.*, 2020[20]	Yes	Yes	Yes	Yes	Yes	Un-clear	Yes	Yes	Yes	(8/9) 88%	Good
Chen *et al.*, 2014[21]	Yes	Yes	Yes	Yes	Un-clear	Un-clear	Yes	Yes	Yes	(7/9) 77%	Moderate
Gray *et al.*, 2014[22]	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	(8/9) 88%	Good
Kuswati *et al.*, 2019[23]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	(9/9) 100%	Good
Yuliani & Linar, 2019[12]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	(9/9) 100%	Good
Farhana *et al.*, 2016 [24]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	(9/9) 100%	Good
Luo *et al.*, 2017 [25]	Yes	Yes	Yes	Un-clear	Yes	Yes	Yes	Yes	Yes	(8/9) 88%	Good
Sun *et al.*, 2015 [26]	Yes	Yes	Yes	Yes	Un-clear	Yes	Yes	Yes	Yes	(8/9) 88%	Good
Qi *et al.*, 2014 [27]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	(9/9) 100%	Good
Farida *et al.*, 2018 [28]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	(9/9) 100%	Good
Gofir *et al.*, 2021 [29]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	(9/9) 100%	Good

*Q1-Q9 indicates questions 1 to 9 based on the JBI assessment.

3. Results and Discussion

Based on the results of the article analysis, studies were conducted in China (n=5), Indonesia (n=5), Korea (n=2), India (n=1), Tiongkok (n=1), and USA (n=1). All articles were quantitative studies, with 13 articles being experimental studies and 2 articles using quasi-

experiments. Besides, there are 14 studies performed using animal disease models as their sample, which are divided into 3 experimental methods, namely *in vitro* (n=6), *in vivo* model (n=6), and *ex vivo* experimental methods (n=2) (Table 2). *In vitro* studies describe the procedure of conducting experiments in a controlled environment outside of the living organism's body. On the other hand, in vivo studies imply experiments that use a whole part of a living organism, and *ex vivo* studies reverse this to *in* vivo [30]. Meanwhile, another study was arranged in post-ischemic stroke patients, with a total of 48 patients involved (Table 3).

Table 2. Characteristics of the study.

Author and year of publication	Research objectives	Country	Sample	Research design	Results
Luo *et al*., 2014 [16]	Investigate the effectiveness of *Madecassoside* in ischemia-reperfusion-induced disturbances in cerebral focal rats	China	Sprague-Dawley rats with a weight of 240-270 g	*Experimental study*	Treatment of MA[1] can protect the brain from post-ischemia-reperfusion damage, preserve cell structure, reduce interstitial edema, and reduce nerve cell necrosis
Lee *et al*., 2014 [17]	Identify the effect of AA[2] coadministered with t-PA[3] in a rat model of focal embolic stroke and evaluate the histological outcome and its function	Korea	320-380 g Male Wistar Rats (Male Wistar Rats) as a model of embolic stroke 250-300 g Male Sprague-Dawley Rats were used ex vivo	*Experimental study*	*Asiatic acid* reduced infarct volume and neurologic deficits
Islam *et al*., 2013 [18]	Examine the effects of *C. asiatica* extract on the prevention of rat brain ischemia	India	Male Wistar Rats	*Experimental study*	*C. asiatica* can increase neurobehavioral activity and reduce infarct volume along with histological morphology of the recovered brain in MCAO[4] mice.
Lee *et al*., 2012[19]	Determine pharmacokinetics, dose-response relationships, therapeutic time windows, and efficacy using multiple stroke models and exploring potential mechanisms of *Asiatic acid*	Korea	Sprague-Dawley rats male, female, and male spontaneously hypertensive rats weighing 250-300 g	*Experimental study*	*Asiatic acid* showed significant neuroprotection even when administered 12 hours post-ischemia.
Zhang *et al*., 2020[20]	Investigate the effects and mechanisms of *asiaticoside* on cerebral ischemia-reperfusion injury	China	Sprague-Dawley rats weighing 300-350 g	*Experimental study*	*Asiaticoside* alleviated cerebral ischemia-reperfusion injury through inhibition of inflammation and oxidative stress
Chen *et al*., 2014[21]	Investigate the effects of Asiaticoside on memory impairment and inflammatory cytokines expression induced by transient cerebral ischemia-reperfusion in rats	China	18-22 g Male ICR Rats	*Experimental study*	Treatment with *Asiaticoside* can attenuate/reduce memory impairment.
Gray *et al*., 2014[22]	Identify the components associated with the neuroprotective activity of *C. asiatica*	USA	MC65[5] cells and SH-SY5Y[6] cells	*Experimental study*	Few *dicaffeoylquinic acid* (CQA) found in *C. asiatica* showed efficacy in protecting MC65 cells from Aβ-induced cell death

Author and year of publication	Research objectives	Country	Sample	Research design	Results
Kuswati *et al*., 2019[23]	Determine the anti-apoptotic effect of gotu kola extract by decreasing Bax expression in the prefrontal cortex of Sprague-Dawley rats with chronic stress restraint intervention.	Indonesia	25 Sprague-Dawley Rat-brain Tissue	*Experimental study*	*C. asiatica* can reduce the expression of Bax in the prefrontal cortex, thereby reducing the number of cells that are infected. undergo apoptosis
Yuliani & Linar, 2019[12]	Examine the effect of gotu kola extract on caspase 3 expression in pyramidal cells in the hippocampus	Indonesia	30 adult Sprague-Dawley rats weighing 180-200 g	*Experimental study*	*Asiaticoside* blocks cell death by reducing intracellular free radical concentrations and protects mitochondria from oxidative stress
Farhana *et al*., 2016 [24]	Investigate the effectiveness of *C. asiatica* in improving cognitive function in patients with VCI (Vascular Cognitive Impairment)	Indonesia	Ischemic Post-Stroke Patients	*Quasi-experiment*	Gotu kola extract was effective in reducing cognitive impairment after stroke infarction.
Luo *et al*., 2017 [25]	Examine the role of the TLR4/MyD88 *(Toll-like Receptor 4/Myeloid Differentiation Factor 88/Nuclear Factor-Kappa (NF-kB)) pathway* in the neuroprotective and anti-inflammatory effects of *Madecassoside*	China	BV2[7] microglia cells	*Experimental study*	*Madecassoside* protected BV2 microglia against OGD/R[8] induced cytotoxicity
Sun *et al*., 2015 [26]	Identify Asiaticoside in terms of its neuroprotective mechanism in cerebral ischemia	China	Primary cultured newborn Sprague Dawley Rats	*Experimental study*	*Asiaticoside* has a protective effect on ischemic-hypoxic nerve cells *in vitro* and also reduces cell apoptosis.
Qi *et al*., 2014 [27]	Identify the protective effect of *Asiaticoside* on cultured rats' cortical neurons with an attempt to uncover the underlying mechanisms involved	Tiongkok	Primary cortical neuron cultures were taken from rat embryonic brains.	*Experimental study*	*Asiaticoside* is protective against injury induced by NMDA[9].
Farida *et al*., 2018 [28]	Determine the neurotherapeutic effect of the *Acalypha Indica* (AI)-*Centella Asiatica* (CA) combination in increasing hippocampal neuronal injury in post-hypoxic rats	Indonesia	36 Sprague-Dawley rats weighing 250-275 g	*Experimental study*	The combination of AI–CA extracts decreased the neuronal damage in the hypoxia-induced hippocampal injury animal model.
Gofir *et al*., 2021 [29]	Identify the effect of the combination of *C. asiatica* and *Curcuma Longa L.* extracts in improving memory performance in stroke models and acute toxicity rats	Indonesia	25 Wistar rats underwent bilateral temporary common artery occlusion weighing 150-200 g	*Quasi-experiment*	*C. asiatica* and *asiaticoside* were reported to be tolerated with good, where more than 1 g/kg BW *asiaticoside* does not cause toxic effects

[1]MA (*Madecassoside*); [2]AA (*Asiatic acid*); [3]t-PA *(Tissue-Plasminogen Activator*); [4]MCAO (Middle Cerebral Artery Occlusion); [5]MC65 (human neuroblastoma cell line); [6]SH-SY5Y (thrice-subcloned cell line derived from the SK-N-SH neuroblastoma cell line); [7]BV2 (microglial cell derived from C57/BL6 murine); [8]OGD/R (oxygen-glucose deprivation/reperfusion); [9]NMDA (N-methyl-D-aspartate)

Table 3. Summary of findings.

Author and year of publication	Intervention method	Findings
Luo *et al*., 2014 [16]	*Madecassoside* (6, 12, or 24 mg/kg intravenously) was administered 1 hour after the start of reperfusion. Neurological deficit scores and infarct volume were evaluated 24 hours afterward.	• The results showed that *Madecassoside* (MA) at any dose reduced neurological deficit scores ($p<0.01$) • MA in any dose can also reduce infarct volume ($p<0.01$) • Treatment of MA 12 mg/kg can protect the brain from post-ischemia-reperfusion damage, preserve cell structure, reduce interstitial edema, and reduce nerve cell necrosis (because the dose of MA 12 and 24 mg/kg exerted a marked cytoprotective effect, a dose of 12 mg/kg was used in all subsequent trials.)
Lee *et al*., 2014 [17]	Male rats were treated with AA (75mg/kg) alone, low-dose t-PA *(Tissue-Plasminogen Activator)* (2.5 mg/kg) alone, or a combination of AA and low-dose t-PA at 3 hours after inducing embolic stroke	• AA (75 mg/kg body weight, 3 hours after MCAO[1]) significantly reduced infarct volume ($p=0.025$) and neurologic deficits ($p=0.001$) • Combination treatment of low-dose AA and t-PA *(Tissue-Plasminogen Activator)* resulted in a significant reduction in infarct volume ($p=0.018$) and neurological score ($p=0.004$) • The results found that the combination of AA and t-PA increased the neuroprotective effect both histologically and functionally
Islam *et al*., 2013 [18]	Rats were randomly assigned to 6 groups: 1. False group: Control given saline 2. MCAO: Ischemic group given saline 3. MCAO: Ischemic group treated with C(100mg/kg body weight once daily for 21 days) 4. MCAO: Ischemic group treated with C(200mg/kg once daily for 21 days) 5. MCAO: Ischemic group treated with C(300mg/kg body weight once daily for 21 days) 6. Pretreatment with C(300 mg/kg body weight once daily for 21 days) in a sham group	• *C. asiatica* improved the grip strength and motor coordination significantly in C200+MCAO (61.58 and 37.04%; $p<0.05$), C300+MCAO (89.63 and 95.69%; $p<0.01$) in a dose-dependent manner. Lower dose C100+MCAO didn't show significant changes compared to the MCAO group. Then, the C300+S and sham groups have no significant difference • *C. asiatica* can increase neurobehavioral activity and reduce infarct volume, along with the histological morphology of recovered brains in MCAO mice. • The antioxidant activity of *C. asiatica* can be attributed to the content of *triterpenes*, *Asiatic acid*, *asiaticoside*, *madecassic acid*, and *madecosside*, which are clinically useful for the prevention of ischemic stroke
Lee *et al*., 2012[19]	**Intervention 1: Pharmacokinetics.** Male SD[2] rats were divided into 4 groups (Fake, AA 10 mg/kg, 25, and 75 mg/kg). Blood samples were taken before administration and at 15 minutes, 1 hour, 3, 6, 12, and 24 hours after administration. AA concentrations were measured using high-performance liquid chromatography/mass spectroscopy. **Intervention 2: Dose-Response.** Male SD rats were treated 30 min before ischemia (sham, AA 50 mg/kg, and AA 75 mg/kg) and 6, 9, and 12 hours (AA 75 mg/kg and FAL) after ischemia onset in the pMCAO model. **Intervention 3: Gender Influence.** Female SD mice were treated with AA (75 mg/kg) or sham group at 6 hours after ischemia onset **Intervention 4: Effect of Hypertension.** Hypertensive mice were subjected to pMCAO for 1 hour and treated with AA 75 mg/kg or sham group 6 hours after ischemia onset.	• Administration of *Asiatic Acid* (AA) (50 or 75 mg/kg, 30 min before MCAO) had a dose-dependent protective effect, attenuating the infarct volume of pMCAO[3] at 24 h by 37%-52.5%, respectively. • In tMCAO[4], infarct volume was significantly reduced with AA treatment at 6 or 9 hours, 53.1%-52.3%, respectively. • AA treatment (75 mg/kg) in female rats 6 hours after treatment reduced infarct volume by 56.4% • AA treatment (75 mg/kg) 6 hours after ischemia in hypertensive rats reduced infarct volume by 25.6% • AA reduced volume infarction by 36.3% for 14 days and significantly improved functional outcomes and motor, sensory, and reflex impairment at 1, 3, 7, and 14 days after ischemia • AA showed significant neuroprotection even when administered 12 hours post-ischemia.

Author and year of publication	Intervention method	Findings
	Intervention 5: Determination of long-term histological and behavioral outcomes. Male SD rats were divided into 3 random groups. The sham group underwent surgery but was not targeted by tMCAO. The other 2 groups (AA 75 mg/kg and the sham group) at 6 hours after the onset of ischemia. **Intervention 6: Possible Mechanisms of Action.** Male SD mice were divided into 2 random groups and given tMCAO. Then, treated with AA (75 mg/kg) and the sham group at 6 hours after the onset of ischemia	
Zhang *et al*., 2020[20]	The mice were grouped into an experimental group given different concentrations of *Asiaticoside* orally (20, 40, and 60 mg/kg), a contrast-positive group given nimodipine (20 mg/kg), a sham group, and a model control group. The sham group and the control model were given the same volume of saline.	• The results showed that *Asiaticoside* was superior to nimodipine in reducing functional injury. Nerves, brain edema, and infarct size in rats. • Findings found that *Asiaticoside* alleviated cerebral ischemia-reperfusion injury through inhibition of inflammation and oxidative stress ($p<0.05$ when compared with the MCAO group) • The protective mechanism of *Asiaticoside* was investigated. The MAPK[5] signaling pathway is involved in many cellular processes, including cell differentiation, cell apoptosis, and cell proliferation. The MAPK pathway has been reported to be activated by oxidative stress and inflammation. NF-kB[6] is a vital pathway in the regulation of inflammatory factor release. MAPK is a positive regulator of the NF-kB signaling pathway. Furthermore, NF-kB is triggered by NOD2[7], which has been reported to enhance the innate inflammatory response by activating NF-kB signaling. These results suggest that the NOD2/MAPK/NF-kB signaling pathway protein is upregulated in vivo and in vitro in a mouse model of ischemia-reperfusion injury. NOD2/MAPK/NF-kB signaling pathway protein induced by MCAO and OGD/R reduced *Asiaticocide*.
Chen *et al*., 2014[21]	Rats were anesthetized with 10% chloral hydrate (350 mg/kg) and underwent *transient cerebral hypoperfusion* with minor modifications. *Transient cerebral hypoperfusion* was induced by tBCCAO (*transient bilateral common carotid artery occlusion*) with aneurysm clipping for 10 minutes, followed by 10 minutes of reperfusion. The aneurysm clip is clipped for 10 minutes. Blood flow is restored by removing the clip. Treat sham controls undergoing the same surgical operation without carotid artery clipping. Rats were given doses of 20, 40, or 60 mg/kg *Asiaticoside* orally 24 hours after surgery, then once daily for a week. The sham control group and the model control group were treated with distilled water instead.	• Treatment with *Asiaticoside* can attenuate/reduce memory impairment. • 60 mg/kg *Asiaticoside* treatment reduced latency and increased error number induced by tBCCAO[8] ($p<0.05$). Meanwhile, 40 mg/kg *Asiaticoside* treatment enhanced latency but failed to reduce the number of errors ($p<0.05$) • The results of this study indicate that cerebral ischemia induced by tBCCAO in mice can increase microglial activation and trigger an inflammatory response, which is manifested in increased expression of Iba1[9], levels NO[10], and expression of cytokines including IL-1y[11], IL-6[12], and TNF-y[13]. Treatment with AS may decrease the increase in abnormal microglial activation. Furthermore, the increase in NO and inflammatory cytokines was reduced dramatically with AS treatment, suggesting that AS has neuroprotective activity through its anti-inflammatory properties
Gray *et al*., 2014[22]	Cells were harvested, and RNA was extracted using Tri-Reagent. RNA was reverse transcribed with superscript III First-Strand Synthesis Kit (Invitrogen) to generate cDNA according to the	• 100 µg/mL *C. asiatica* reduced cell death of MC65 cells ($p<0.05$). Pretreatment of *C. asiatica* also able to prevent significant cell death after 48 hours caused by Aβ peptide treatment in SH-SY5Y cells ($p<0.05$).

Author and year of publication	Intervention method	Findings
	manufacturer's instructions. mRNA expression. Relatives were determined using the TaqMan Gene Expression Master Mix (Invitrogen) and commercially available TaqMan primer (Invitrogen) for Tau, encoded by the microtubule-associated Tau protein (MAPT) gene and GAPDH[14]. Quantitative PCR was performed on a StepOne Plus machine (Applied Biosystem) and analyzed using the delta method. 6 replicas were analyzed for each treatment condition.	• This study identified several *caffeoylquinic acids* that exhibit neuroprotective activity against Aβ toxicity, intracellular and extracellular. • The CQA found was present in CA but not in aqueous extracts specifically. *Chlorogenic acid*, *Cryptochlorogenic acid*, *Neochlorogenic acid*, and *Isochlorogenic acids* A and B were all identified in the alcoholic extract of *C. asiatica*
Kuswati *et al*., 2019[23]	This brain tissue was derived from 25 mouse models divided into groups P1, P2, P3, P4, and P5. Group P1 intervened with restrain stress; P2 was intervened by stress and ethanol extract of *C. asiatica* 150 mg/kg BW/day; P3 was intervened by stress and ethanol extract of *C. asiatica* 300 mg/kg BW/day; P4 was intervened by stress and ethanol extract of *C. asiatica C. Asiatica* 600 mg/kg BW/day; P5 was intervened by stress and fluoxetine 10 mg/kg BW/day.	• In the P1 group., the average number of Bax-expressing cells = 72.54%. Bax expression in this group was higher than the other groups who were intervened with restrain stress and given *C. asiatica* or *Fluoxetine* (groups P2, P3, P4, and P5). P2=44.6%; P3 = 28.11%; P4 = 28.97%; P5 = 7.76% (p=0.01) • The administration of ethanolic extract of *C. asiatica* at doses of 300 and 600 mg/kgBW/day for 21 days can reduce the expression of Bax in the prefrontal cortex, thereby reducing the number of cells that are infected. undergo apoptosis • *C. asiatica* prevents a decrease in ATP[15], increases basal oxygen consumption, inhibits the reduction of mitochondrial respiration, and inhibits the slowing of metabolic rate.
Yuliani & Linar, 2019[12]	The treatment was carried out for 35 days. Rats were randomly divided into 6 groups, each consisting of 5 rats. The normal group was given oral CMC-Na[16] solution and intraperitoneal injection of 0.9% saline. The negative control group was given oral CMC-Na solution and TMT[17] dissolved in 0.9% saline. The positive control group was given citicoline 200 mg/kg body weight orally and TMT and the extract group was each given *C.Asiatica* extract 100, 200, and 300 mg/kg BW orally and TMT. The extract and citicoline solution were administered on day 1 to day 35 of the experiment, while TMT chloride was injected as a single dose of 8 mg/kg BW on day 8 of the experiment. On the 36th day, the rats were sacrificed using CO2 gas inhalation, and their brains were taken for immunohistochemical observations.	• The percentage of the CA1 and CA2 CA3 pyramidal cells expressing protein caspase 3 in the TMT group was higher than the normal group ($p<0.05$). Administration of gotu kola extract at doses of 100, 200, and 300 mg/kg BW can reduce the percentage of pyramidal cells CA1 and CA2-CA3 expressing protein caspase 3 • Administration of citicoline can also prevent caspase 3 expression in both cells CA1 and CA2-CA3 • Asiaticoside blocks cell death by reducing intracellular free radical concentrations and protects mitochondria from oxidative stress
Farhana *et al*., 2016 [24]	The study subjects were divided into 3 groups: 17 subjects were treated with 1000 mg/day of *C.Asiatica* extract, 17 others with 750 mg/day of *C.Asiatica* extract, and the remaining 14 with 3 mg/day of folic acid for 6 week	• Average MoCA-Ina[18] *(Montreal Cognitive Assessment-Indonesian Version)* for 6 weeks with a dose of CA 1000 mg 5.6±4.61 ($p<0.001$), CA 750 mg 4.94±2.16 ($p<0.001$), and 3 mg folic acid 4.06±3.11 ($p<0.001$). • This study found that gotu kola extract doses of 750 mg and 1000 mg and folic acid 3 mg per day for 6 weeks were effective for reducing cognitive impairment after stroke infarction.
Luo *et al*., 2017 [25]	Microglial cells were exposed to OGD (ischemia) for 6 hours, followed by reperfusion 24 hours later. Microglia were treated with different *Madecassoside* concentrations for 30 min before ischemia.	• MA may reveal its neuroprotective properties against OGD/R through its effect against microglia-mediated neuroinflammation. Its anti-inflammatory and neuroprotective mechanisms stem from the inhibition of TLR4 and MyD88 activation in BV2 microglia and blockage of nuclear translocation of NF-kB p65, which results in reduced cytokine expression

Author and year of publication	Intervention method	Findings
		• 200 µM *Madecassoside* pretreatment significantly attenuated ODG/R-induced toxicity (P<0.05) compared to OGD/R group meaning that *Madecassoside* protected BV2 microglia against OGD/R induced cytotoxicity
Sun *et al*., 2015 [26]	The cells were randomly divided into 4 groups: 1. Normal control, cultured normal cortical neurons; 2. Hypoxic ischemia group; 3. *Asiaticoside* treatment group (10nmol/L); 4. The *Asiaticoside* intervention group (100 nmol/L). Hypoxic incubator cells were added with different concentrations of asiaticoside (soluble in medium) and incubated in a serum-free medium for 24 hours.	• The MTT[19] test results showed that compared to the hypoxic-ischemic cell group, the *Asiaticoside* treatment group showed higher cell survival rates. High (P<0.05). In addition, the survival rate increased significantly after increasing the dose. This shows that AS increases the proliferative capacity of ischemia-hypoxia cells • AS has a protective effect on ischemic-hypoxic nerve cells *in vitro* and also reduces cell apoptosis. • Lactate dehydrogenase detection found that asiaticoside can inhibit the release of lactate dehydrogenase by ischemic hypoxic cells (P<0.05)
Qi *et al*., 2014 [27]	Cortical neurons cultured for 7 days in vitro were divided into three groups: control, NMDA treatment (200 mol/L NMDA and 10 mol/L glycine, NMDA receptor co-activator) and *Asiaticoside* pretreatment (*Asiaticoside* (less than 1%) treatment initial treatment for 24 hours, then treatment of NMDA (200 mol/L) and glycine (10 mol/L) for another 30 minutes) The cells were then returned to the original culture medium for 24 hours	• *Asiaticoside* pretreatment improved the cell viability of neurons in a dose-dependent manner. Neuroprotection was most effective at 10 µmol/L *Asiaticoside* (P<0.01) compared to the NMDA treatment group • *Asiaticoside* pretreatment (10 µmol/L) prevented cell damage caused by NMDA treatment as fewer cells were positive for propidium iodide staining (14.61 ± (P<0.01) compared to NMDA treatment group means *Asiaticoside* is protective against injury-induced by NMDA. • In pathological conditions such as stroke, traumatic damage, and inflammation, excessive accumulation of cytoplasmic Ca^{2+} through the NMDA receptor triggers excitotoxicity and neuronal damage. In this regard, the NMDA-stimulated increase in Ca^{2+} was inhibited by asiaticoside, suggesting inhibition of calcium influx through the NMDA receptor contributes, at least in part, to neuroprotection by *Asiaticoside*.
Farida *et al*., 2018 [28]	Rats were grouped into six and placed in a hypoxia chamber for 7 days. After that, they were moved to normoxia cages and treated for 7 days as follows: control group without treatment as a negative control; treatment groups were administered citicoline 50 mg/kgBW as a positive control; three different dose combinations of AI150–CA150, AI200–CA150, and AI250–CA150 mg/kgBW, respectively.	• Treatment with citicoline significantly reduced nerve cell damage (30, 8%, p=0.027); The combination of AI-CA extracts of AI150-CA150, AI200-CA150, and AI250-CA150 also significantly reduced nerve cell damage (36%, p=0.003; 36.4%, p=0.002; and 30.4%, p=0.029, respectively) compared to control mice (10, 4%) • The AI-CA group showed a higher reduction in cell damage than the citicoline group.
Gofir *et al*., 2021 [29]	Rats were divided into 5 groups, namely Group 1, given a dilution of NaCMC[20]. Group 2 was given donepezil 0.7 mg/kg BW/day, and groups 3, 4, and 5 were given a combination extract at doses of 59, 118, and 236 mg/kg BW/ day, respectively.	• According to the OECD21 Guideline, the fixed-dose method was used to estimate the LD50[22] in acute toxicity testing on rats. There was a difference significantly in the delta percentage among the tested group (p<0.05) • *C. asiatica* and *asiaticoside* were reported to be tolerated well, whereas more than 1 g/kg BW *asiaticoside* does not cause toxic effects. In Wistar rats, *C. Asiatica* doses of more than 10,000 mg/kgBW once and 1,000 mg/kgBW for 90 days did not cause toxic effects, either in acute or sub-chronic use. • This study revealed that the LD50 of *C. asiatica* and the combination of C.Longa L. extract was more than 2,000 mg/kg BW physically, macroscopically, and microscopically. This indicates that the dose used in this study was safe

[1]MCAO (middle cerebral artery occlusion); [2]SD (Sprague-Dawley); [3]pMCAO (permanent middle cerebral artery occlusion); [4]tMCAO (transient middle cerebral artery occlusion); [5]MAPK (mitogen activated protein kinases); [6]NF-kB (Nuclear factor kappa-light-chain-enhancer of activated B cells); [7]NOD2 (nucleotide-binding oligomerization domain protein 2); [8]tBCCAO (transient bilateral common carotid artery occlusion); [9]Iba1 (ionized calcium binding adaptor molecule 1); [10]NO (nitric oxide); [11]IL-1y (interleukin 1); [12]IL-6 (interleukin 6);

[13]TNF-y (tumor necrosis factor); [14]GAPDH (glceraldehyde-3-phosphate dehydrogenase); [15]ATP (adenosine triphosphate); [16]CMC-Na (sodium carboxymethyl cellulose); [17]TMT (tandem mass tag); [18]MoCA-Ina (Montreal Cognitive Assessment-Indonesian Version); [19]MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide); [20]NaCMC (sodium carboxymethyl cellulose); [21]OECD (organization for economic co-operation and development); [22]LD50 (median lethal dose)

3.1. The effects of C. asiatica in patients with ischemic stroke.

Mostly, stroke was divided into two categories: ischemic and hemorrhagic stroke. However, 80% of stroke cases were categorized as ischemic stroke. Stroke occurs when the loss of oxygen and nutrients supplied to particular parts of the cerebral system causes a sudden loss of body function and disability [1,5]. The most vulnerable area in the brain to hypoxia is the hippocampus, which contains stem cells from the nervous system and is located in the dentate gyrus [31].

When an ischemic attack occurs, cerebral cells try to maintain cell metabolism and reduce the function of the penumbral tissue. If the supply of ATP (adenosine triphosphate) and Na^+/K^+ transporters is reduced, excess Ca^{2+} enters the cell and causes cell apoptosis [32]. Excitotoxicity that occurs due to the influx of Ca^{2+} is caused by the activation of NMDA (N-methyl-D-aspartate) glutamate and AMPA (a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors that trigger nerve damage [33,34]. In addition, increased production of oxygen radicals initiates oxidative stress and inflammatory responses, leading to cerebral edema [4]. Based on the above explanation, there are several targets for post-stroke neuroprotection, including cell apoptosis, excitotoxicity, ATP deficiency, oxidative stress, and inflammatory response.

Stroke management is conventionally carried out by administering thrombolytics such as aspirin and controlling blood pressure. Meanwhile, the treatment of ischemic stroke is carried out with t-PA, which is only effective if given within 3 hours of the stroke. However, t-PA also carries a risk of adverse effects [4,35]. Herbal plants are considered therapeutic agents that provide neuroprotective and neurodegenerative effects for the treatment of ischemic stroke.

Based on the results of the article analysis, *C. asiatica* is effective in enhancing neurobehavioral activity, reducing infarct volume, reducing cell apoptosis, increasing basal O2 consumption, inhibiting decreased mitochondrial respiration, inhibiting cell metabolism rate, and reducing oxidative stress [36–38]. *C. asiatica* consists of *triterpenes*, including *asiaticoside*, *madecassoside*, and *Asiatic acid*, which play an important role in antioxidant activity [20,36].

An *in vivo* study found that *Asiaticoside* itself was effective in reducing cerebral ischemia-reperfusion injury by inhibiting the inflammatory response and oxidative stress in experimental rats. *Asiaticoside* was also able to reduce neurologic decline, brain edema, infarct size, and cell apoptosis [10]. In line with the results of *in vivo* studies, *Asiaticoside* was found to be effective in reducing memory impairment and has neuroprotective activity through its anti-inflammatory properties [39].

In another experiment conducted on a rat model of embolic stroke, *Asiatic acid* was found to be effective in mediating neuroprotection by protecting mitochondria and inhibiting the induction and activation of matrix *metalloproteinase-9* [40]. In addition, *Asiatic acid*, in combination with low-dose t-PA, enhances neuroprotective effects, both histologically and functionally, because it reduces infarct volume and neurologic score [41].

Furthermore, *Madecassoside* in 2 *in vitro studies* on BV2 (microglia cells) was found to have neuroprotective properties against OGD/R conditions (Oxygen-Glucose Deprivation/Reperfusion) through its effect against microglia-mediated neuroinflammation. This anti-inflammatory mechanism stems from the inhibition of NF-kB (Nuclear Factor-Kappa B) activation, which results in a reduction in cytokine expression [6,40].

3.2. Neuroprotective mechanisms of C. asiatica compounds.

The results of an *in vitro* study conducted in China demonstrated that during excitotoxicity induced by exposure to NMDA in cultured cortical neurons, administration of *Asiaticoside* to neurons (10 mol/L) caused Ca^{2+} reduction. NMDA has 2 receptor subunits, namely NR2A and NR2B. Further findings showed that *Asiaticoside* attenuated the expression of the NR2B receptor subunit but not NR2A. NR2B plays a role in mediating excitotoxicity. Low NR2B expression is thought to cause shorter NMDA receptor openings so that less Ca^{2+} enters neurons. Therefore, *Asiaticoside* protects neurons from cell apoptosis and excess Ca^{2+} [42,43].

The effect of *Asiaticoside* has also been tested in mice with cerebral ischemia-reperfusion injury, where *Asiaticoside* was shown to reduce cell apoptosis and inflammation levels. The mechanism for the emergence of the *Asiaticoside* is caused by the NOD2/MAPK/NF-kB signaling pathway [44]. MAPK (mitogen-activated protein kinase) signaling pathway is involved in several cellular processes, such as cell differentiation, apoptosis, and cell proliferation, which is activated due to oxidative stress and inflammatory response [45]. Meanwhile, NF-kB is a regulatory pathway for the release of inflammatory factors. MAPK is a regulator of the NF-kB signaling pathway. NF-kB triggered by NOD2 (nucleotide-binding oligomerization domain 2) enhances the inflammatory response. In this study, the administration of *Asiaticoside* was able to reduce pro-inflammatory factors such as MCP-1, IL-6, IL-1β b, and TNF-α induced in OGD/R [20].

In addition to *Asiaticoside*, another article reveals the anti-inflammatory mechanism of *Madecassoside*, in which the compound plays a role in inhibiting the activation of TLR4 (Toll-Like Receptor 4, a type of transmembrane protein that plays an important role in the inflammatory response due to cerebral I/R conditions) and activation of MyD88 (Myeloid) [46]. Differentiation Factor 88 in BV2 microglia blocks NF-kB, which results in reduced release of inflammatory cytokine agents [6].

3.3. Dose-response of C. asiatica compounds.

C. asiatica extracts in doses of 100, 200, and 300 mg/kg are known to decrease the percentage of CA1 and CA2-CA3 pyramidal cells expressing caspase 3 protein [12]. In another study, the combination treatment of *Acalypha indica* (AI) extract with *C. asiatica* (CA) of AI150-CA150, AI200-CA150, and AI250-CA150 significantly reduced nerve cell damage by 36%, 36.4%, and 30.4%, respectively. The AI-CA group showed a higher reduction in cell damage than the citicoline group. Treatment with the AI200-CA150 dose gave a higher damage reduction effect than the other dose combination groups [31].

In other compounds, Luo *et al*. [47] revealed that treatment with *Madecassoside* (6, 12, or 24 mg/kg iv) in cerebral ischemia model rats at any dose reduced neurological deficit scores ($p<0.01$) and infarct volume ($p<0.01$). Treatment with *Madecassoside* 12 mg/kg and 24 mg/kg

can protect the brain from post-I/R damage, maintain cell structure, reduce interstitial edema, and reduce nerve cell necrosis [7].

Treatment of *Asiatic acid* (AA) in rat models of focal embolic stroke showed that AA 75 mg/kg BW within 3 hours after MCAO (Middle Cerebral Artery Occlusion) reduced infarct volume ($p=0.025$) and neurological deficits ($p=0.001$) [41]. A previous study found that administration of AA 50 or 75 mg/kg 30 minutes before MCAO reduced infarct volume in persistent MCAO at 24 h by 37% - 52.5%, respectively. In the case of transient MCAO, administration of AA at 6 or 9 hours reduced infarct volume significantly by 53.1% - 52.3%. In the same study, AA was also found to reduce infarct volume by 36.3% and improve motor and sensory function within 14 days [40].

The effectiveness and safety of these doses may vary depending on factors such as the specific experimental conditions, the type of stroke model used, and individual variability.

3.4. Advantages and inadequacies of C. asiatica compounds.

Based on the results of the analysis of the article, *Centella asiatica* has properties that are not dependent on dose, especially in the ability to increase the thickness of the pyramidal cell layer in the hippocampus. This is due to the increased ability of high doses of *C. asiatica* to stimulate the formation of pro-oxidants through free radicals in the body [12].

In addition, *Madecassoside* is known to be more effective than NIM (nimodipine) treatment because NIM does not affect serum glutathione (GSH) levels [6]. These results were confirmed by another compound, namely *Asiaticoside*, in the research of Zhang *et al*. [20], in which *Asiaticoside* is believed to be superior to NIM in reducing nerve injury, brain edema, and infarct size in rats [48]. On the other hand, AA has the advantage of providing significant neuroprotection even when administered 12 hours post-ischemia. The mitochondrial respiratory index showed that AA also protects mitochondria during ischemia [40]. Unfortunately, based on the results of a review of the article, the shortcomings of C. asiatica and its compounds are not explained in detail.

4. Conclusions

C. asiatica is *e*ffective in enhancing neurobehavioral activity as a neuroprotective property, evidenced by its ability to independently increase the thickness of the pyramidal cell layer and play an important role as an antioxidant. *C. asiatica* has been tested in mice with cerebral ischemia-reperfusion injury, and the results show that the mechanism for the emergence of *Asiaticoside* is caused by the NOD2/MAPK/NF-kB signaling pathway, such as MAPK (mitogen-activated protein kinase) and NF-kB, that can reduce pro-inflammatory factors. The effectiveness and safety of these doses may vary depending on factors such as the specific experimental conditions, which can range from 6 to 300 mg/kg. However, the optimal dose for patients with ischemic stroke is not yet clear because it is dependent on the ability of active components to cross the blood-brain barrier. Future research is needed to determine the safety and efficacy of *C. asiatica* and its active components in humans.

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Conflicts of Interest

The authors declare no conflict of interest.

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