OPEN ACCESS
Advances in Animal and Veterinary Sciences

Research Article

Brain Histopathological Changes After Treatment Using Calabash Fruit (*Crescentia cujete* L.) in Rat Model with Artificially Induced Ischemic Stroke

Jasir Hakim Hidayah[1,2], Yos Adi Prakoso[2], Sitarina Widyarini[3*]

[1]Master Program in Veterinary Sciences, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia; [2]Department Pharmacology, Faculty of Veterinary Medicine, University of Wijaya Kusuma Surabaya, East Java, 60225, Indonesia; [3]Department Pathology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia.

Abstract | The brain is an essential neurological system. Brain function can be disturbed through circulatory system disturbance, such as thrombus. An ischemic stroke is one of the brain diseases caused by a thrombus. Ischemic stroke therapy is commonly using antiplatelet, and it causes various side effects. An alternative treatment must be developed to mitigate the side effects of antiplatelet drugs against stroke, such as using herbs, including calabash (*Crescentia cujete* L.). This study aimed to analyze the efficacy of calabash fruit against histopathology of the brain in a mouse model with artificially induced ischemic stroke. As many as fifty – rat models were used in this study. The rats were separated into P1 (sham-operated), P2 (ischemic stroke model (IS)), P3 (IS + 0.74 mg/kg BW of calabash), P4 (IS + 1.48 mg/kg BW of calabash), P5 (IS + 2.96 mg/kg BW of calabash). The P2 – P4 groups were artificially induced ischemic stroke using common carotid artery ligation for 4 hours. After 24 hours, the rats were treated twice daily using various doses of calabash for seven days. On day 8, the brains were collected and processed against histopathology. The data was analyzed using SPSS version 26. The result indicated that the most efficacious dose of calabash was 2.96 mg/kg BW from the P5 group. It is supported by the minimization of infarct area in the P5 group compared to the others. Further, this dose showed a decrease in a histopathological score of necrosis neuronal, microgliosis, neuronal oedema, secondary haemorrhage, and perivascular oedema compared to the others. This study proved that calabash can be an alternative therapy against ischemic stroke. In advance, the experimental intoxication of this fruit and its processing product must be elucidated.

Keywords | Brain, Calabash, Choline, Histopathology, Ischemic stroke, Neuron.

Received | August 03, 2023; **Accepted** | September 05, 2023; **Published** | November 25, 2023
***Correspondence** | Sitarina Widyarini, Department Pathology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia; **Email:** w.sitarina@gmail.com
Citation | Hidayah JH, Prakoso YA, Widyarini S (2023). Brain histopathological changes after treatment using calabash fruit (*Crescentia cujete* l.) In rat model with artificially induced ischemic stroke. Adv. Anim. Vet. Sci. 11(12): 2003-2009.
DOI | http://dx.doi.org/10.17582/journal.aavs/2023/11.12.2003.2009
ISSN (Online) | 2307-8316

INTRODUCTION

The brain is an essential neurological system in humans and animals. Brain function can be destroyed through circulatory system disturbance, such as thrombus. Thrombus causes a significant decrease in the blood supply that not to carry nutrition, oxygen and other blood components to the brain tissue. Further, they promote severe stress within the neuronal tissue and cause neuronal death (Kuriakose and Xiao, 2020). In advance, it triggers partial infarction within brain tissue and causes ischemic stroke. An ischemic stroke is the highest prevalence in humans,

reaching 52.9% (Herpich and Rincon, 2020). Ischemic stroke triggers neurological deficits and paralysis and decreases the human quality of life. The clinical signs of ischemic stroke commonly occur in a couple of hours and often show a high fatality and death (Murphy and Werring, 2020).

The most common treatment for the patient with ischemic stroke is using clopidogrel (Yang et al., 2021). Clopidogrel is a member of the antiplatelet drug group, and it is essential to inhibit the platelet P2Y12 adenosine diphosphate receptor (Kuszynski and Lauver, 2022). This drug has 50% bioavailability, and only 15% became active via esterase hydrolysis with the CYP enzyme. Furthermore, clopidogrel has several side effects, such as skin rash, hypersensitivity, allergy, and thrombocytopenia, and it can cause bleeding with various degrees of severity (Patti et al., 2020). An alternative treatment must be developed to mitigate the side effects of antiplatelet drugs against stroke, such as using herbs, including calabash (*Crescentia cujete* L.).

Calabash is a tropical fruit that can be found in Indonesia. This fruit, also known as buah maja, buah bila, and berenuk (Rivera et al., 2008), become part of the legendary Majapahit Kingdom. Every part of this plant can be utilized as an antimicrobial, antifungal, and antioxidant. Its fruit and leaves contain alkaloids, flavonoids, saponin, tannin, polyphenols, and triterpenoids (Das et al., 2014). Wilujeng et al. (2023) described that this fruit also consists of choline, an eminent neurotransmitter. A previous study described that using choline in the prenatal period in rats increases cognitive performance, size and response of neurons, and neuroprotectant effects from the neurotoxin agents (Derbyshire and Obeid, 2020). Based on that, this study aimed to analyze the efficacy of calabash fruit against brain histopathology in a mouse model with ischemic stroke.

MATERIALS AND METHODS

Ethic approval

The ethical clearance committee from the Faculty of Dentistry, Universitas Airlangga, Indonesia, has approved this study. The clearance number was: 463/HRECC.FOD-M/V/2023. This study was conducted from March until July 2023 in the Laboratory of Pharmacology, Faculty of Veterinary Medicine, University of Wijaya Kusuma Surabaya.

Herbal preparation

This study used calabash. The calabash species was determined in Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional (B2P2TO-OT), Tawangmangu, Central Java, Indonesia. The species of calabash was *Crescentia cujete* L, and it was registered with voucher number: KM.04.02/2/1248/2022. The fruit was peeled, and its pulp was collected. The fruit was fermented for 30 days using the following formulae, water: fruit pulp: sugar: pectinase (100: 40: 8: 4). The fermented calabash was stored in a fermentation bottle and stirred once daily. After 30 days, the fermented calabash was filtered and stored inside the bottle at four °C using a fridge (Wilujeng et al., 2023).

Choline standardization

The choline level of fermented calabash was measured using liquid chromatography-mass spectrometry/ mass spectrometry (LC-MS/MS). The choline level was measured using the demonstrated procedure in the previous study (Wilujeng et al., 2023). The result of LC-MS/MS indicated that the level of choline was 110.33 mg/kg.

Artificially induced ischemic stroke (IS) in rat model

The artificial ischemic stroke in rats was performed using common carotid artery (CCA) occlusion using simple interrupted ligation. Before occlusion, the rat was anaesthetized using ketamine® (50 mg/kg BW) and xylazine® (4 mg/kg BW) (Bhatia et al., 2022). Further, the rat was shaved on their neck and disinfected using 70% isopropyl alcohol. The skin neck was incised as long as 2 cm, and its neck muscle was retracted to expose the CCA. The CCA was ligated using silk suture for four hours. After occlusion, the knot was cut to sustain the blood reperfusion on the brain tissue. The skin was then closed. The analgesia was given using buprenorphine® (0,1 mg/kg BW).

Experimental design

Fifty Sprague Dawley rats (male, 3 months, 254.25±8.18 grams) were used as a model for artificially induced ischemic stroke. The rats were separated into five groups (10/ each) as follows: P1 (sham-operated), P2 (IS without treatment), P3 (IS + 0.74 mg/kg BW of calabash), P4 (IS + 1.48 mg/kg BW of calabash), P5 (IS + 2.96 mg/kg BW of calabash). After 24 hours, the rats were treated twice daily using various doses of calabash for seven days. The treatment was given at 06.00 (a.m. and p.m).

Specimen collection

On day 8, the rats were euthanized using lethal doses of ketamine® (300 mg/kg BW). The skull was opened and brain was collected. The cerebrum was divided into two parts, the first part for 2% triphenyl tetrazolium chloride (TTC) staining and the second part for histopathology using hematoxylin and eosin (H&E) staining.

Neurodeficit score

The neurodeficit score was observed from the animal models. The observation was conducted 24 hours after artifi-

cially induced ischemic stroke, day four and day seven after treatment. The scoring system followed the demonstrated procedure in a previous study, including 0 = normal, 1 = inability of contralateral limb, 2 = circle behaviour, 3 = spontaneous circling, 4 = no spontaneous movement with a low consciousness, and 5 = death (Althurwi et al., 2022).

Brain infarct measurement and morphometry

The brain infarct measurement was performed using 2% TTC staining. The staining was conducted by immersion of the cerebrum within TTC for 30 minutes. After 30 minutes, the cerebrum was drained using towel tissue. The cerebrum was then photographed using a camera (Santo et al., 2023). The white area indicated the infarct area, measured using Image J Software. The infarct area was measured as the percentage area of the brain.

Histopathology and morphometry

For histopathology, the brain was fixed using 10% neutral buffer formalin. Following the previous study, the brain was processed using routine histopathological H&E staining (Hristu et al., 2021). Before the staining, the organ was dehydrated using alcohol and xylene. The organ was blocked using liquid paraffin at 62°C. The organ was cut using a microtome in 4 µm. The section was then cleared using xylene, dehydrated using alcohol, and stained using hematoxylin. The eosin was used as the counterstain of the tissue section. Further, the section was dehydrated using alcohol and cleared using xylene. The tissue was mounted using Entelan and covered with cover glass. The slide was analyzed by a pathologist under a blindfold condition. The histopathology was reported as a score as follows: 1 = normal, 2 = mild, 3 = moderate, and 4 = severe (Rahaman and Del Bigio, 2018).

Analysis data

The data analysis was conducted using SPSS software version 26. The data, which was expected and homogeny, was analyzed using ANOVA. However, the abnormal and heterogeny data was analyzed using Kruskal – Wallis and Mann – Whitney U test (Percie du Sert et al., 2020). The significance value was 0.05.

RESULT

This study showed that there is no infarct area was observed in sham-operated group (Figure 1a). There is a significant occurrence of infarct area from group P2 (Figure 1b) compared to the sham-operated (P1) group ($P \leq 0.05$). Moreover, there is a significant decrease in infarct area from group P5, treated with 2.96 mg/kg BW of calabash (Figure 1d), compared to P2, P3, and P4 ($P \leq 0.05$). Groups P3 and P4 did not show differences against the parameters of infarct area and microgliosis ($P \geq 0.05$). Moreover, groups P3 and P4 showed better necrosis neuronal scores than the P2 group ($P \leq 0.05$). The P1 group showed a similar histopathological score on the microgliosis parameter compared to the P5 group ($P \geq 0.05$). No one from the treated group shows similar results against infarct area and neuronal necrosis compared to the sham-operated group (Table 1). Nevertheless, the group treated with 2.96 mg/kg BW of calabash showed that there is a repair mechanism against ischemic stroke.

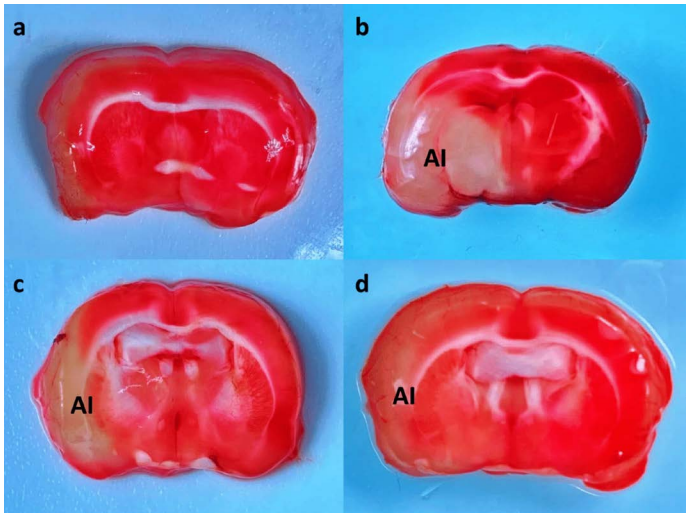

Figure 1: Brain macroscopy of rat model with artificially induced ischemic stroke after treatment. The sham-operated group showed there is infarct area was observed (a); ischemic stroke without treatment showed a large infarct area (AI) with white color (b); however, there is a decrease of infarct area (AI) from the group treated with 1.48 mg/kg BW of calabash (c); and pale infarct area (AI) from the group treated with 2.96 mg/kg BW of calabash (d). Macrophotograph, 2% TTC staining.

Furthermore, groups P1 and P5 showed no differences against oedema neuronal, secondary haemorrhage, and perivascular oedema ($P \geq 0,05$). In contrast, the other treated groups with lower doses of calabash showed significant differences compared to the sham-operated group ($P \leq 0,05$). Group P3 did not differ from P2 in all parameters ($P \geq 0,05$). However, group P4 showed differences against oedema neuronal and secondary haemorrhage ($P \leq 0,05$) (Table 2). It indicates that 2.96 mg/kg BW of calabash potentially promotes the repairing of brain tissue. However, the lower dose (1.48 mg/kg BW) still has minimal potential benefit.

The histopathological score was supported by their histopathology qualitatively. The sham-operated group showed a clear, typical neuron with a nucleus and nucleolus in this study. Moreover, the microglial cells all still presented at low density. The blood vessel from the sham-operated group showed an intake vessel wall without increasing Virchow Robin space (Figure 2a). It is different from ar

Table 1: Histopathology score of infarct area, neuronal necrosis, and microgliosis of the brain from rat model with artificially induced ischemic stroke.

Group	Area infarct (%)	Neuronal necrosis	Microgliosis
P1	1,90 ± 0,48[a]	1,25 ± 0,50[a]	1,25 ± 0,50[a]
P2	34, 48 ± 8,58[b]	3,75 ± 0,50[b]	3,75 ± 0,50[b]
P3	24,76 ± 4,13[c]	3,25 ± 0,95[b]	3,25 ± 0,50[b,c]
P4	21,76 ± 9,01[c]	2,75 ± 0,50[c]	2,75 ± 0,50[c]
P5	6,78 ± 4,69[d]	2,25 ± 0,50[d]	2,00 ± 0,81[a]

[a,b,c,d] different superscripts on the same row showed a significant difference ($P \leq 0,05$).

Table 2: Histopathology score of oedema neuronal, secondary haemorrhage, and perivascular brain oedema from rat model with artificially induced ischemic stroke.

Group	Oedema neuronal	Secondary haemorrhage	Perivascular oedema
P1	1,50 ± 0,57[a]	1,00 ± 0,00[a]	1,25 ± 0,50[a]
P2	3,50 ± 0,57[b]	3,25 ± 0,50[b]	3,25 ± 0,50[b]
P3	3,50 ± 0,57[b]	3,00 ± 0,81[b]	2,50 ± 0,57[b,c]
P4	2,50 ± 0,57[c]	2,25 ± 0,50[c]	2,50 ± 0,57[b,c]
P5	1,50 ± 0,57[a]	1,75 ± 0,94[a,c]	1,75 ± 0,50[a]

[a,b,c,d] different superscripts on the same row showed a significant difference ($P \leq 0,05$).

tificially induced ischemic stroke without treatment. The untreated group showed neuronal necrosis, microgliosis, oedema neuronal, secondary haemorrhage, and inflammation with high severity (Figure 2b – d). Group P3 showed moderate to severe histopathological changes (Figure 3a – b), while group P4 showed mild to moderate histopathological changes (Figure 3c). However, group P5 showed better brain histopathology than the other calabash doses (Figure 3d).

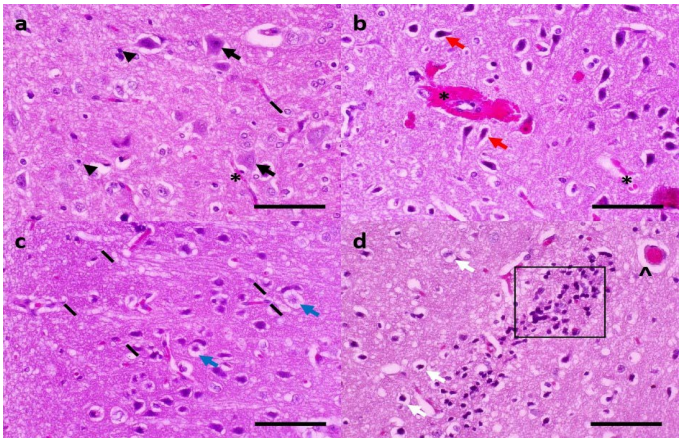

Figure 2: Brain histopathology of rat model with artificially induced ischemic stroke. Typical brain histology with an apparent nucleus and nucleolus are presented (black arrow), microglia (line), satellite cell (arrowhead), and intake blood vessels (*) from a sham-operated group (a); ischemic stroke group without treatment showed secondary haemorrhage (*) inside the Virchow Robin space and tissue, neuronal necrosis that marked by pyknosis neuron (red arrowhead) (b); most of the neuron suffered oedema (blue arrow) marked by pale cytoplasm and cleft that suspected by the crystalline inclusion, microgliosis (line) (c); moreover, the brain also showed perivascular oedema (^), oedema neuronal (white arrow) with neutrophilic and lymphocytic inflammation, and satellitosis (box) (d). H&E, 400×, skala bar: 50µm (a-d).

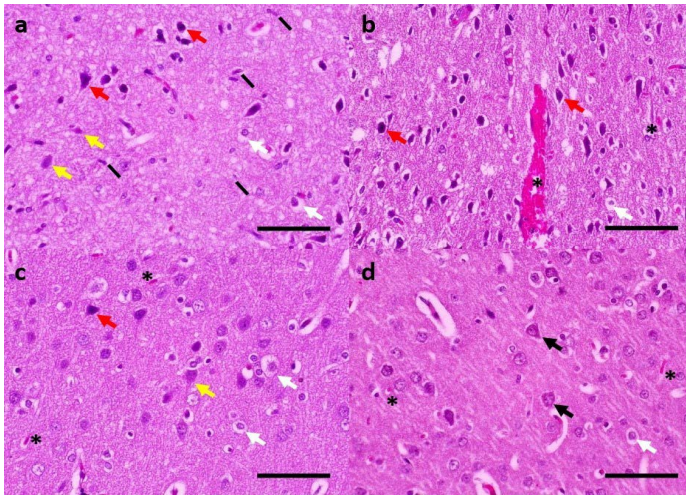

Figure 3: Brain histopathology of rat model with artificially induced ischemic stroke after calabash treatment. Group P3, treated with 0.74 mg/kg BW of calabash, showed moderate histopathological changes against neuronal necrosis (red arrow), microgliosis (line), and secondary haemorrhage (*) (a, b); group P4 that, treated with 1.48 mg/kg BW of calabash showed ghost cell (yellow arrow), oedema perineuronal (white arrow), pyknosis (red arrow), and secondary haemorrhage (*) (c); finally, the group P5 showed a dominate of typical neuron (black arrow), mild secondary haemorrhage (*), and mild oedema perineuronal (white arrow) (d). H&E, 400×, skala bar: 50µm (a-d).

The result of area infarction and histopathology was correlated to the neurodeficit score. After 24 hours of artificially induced ischemic stroke, all rats in groups P2 to P5 showed high neurological deficit scores that were not different from each other (P≥0,05). The score of neurodeficit is stagnant in groups P2 and P3 in all observation times. Groups P4 and P5 significantly decreased neurodeficit in days 4 and 7 after treatment. Moreover, group P5 indicated the lowest neurodeficit score among all the ischemic stroke groups compared to the others (P≤0,05) (Figure 4).

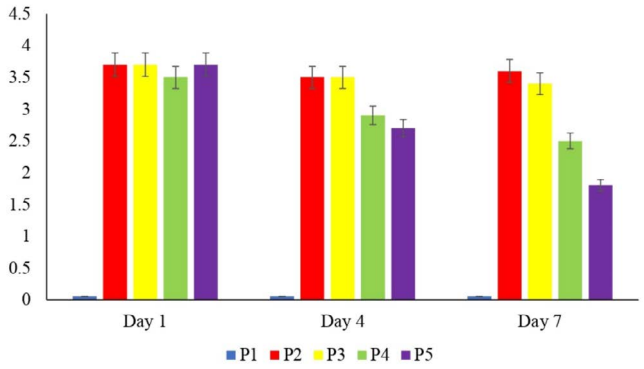

Figure 4: Neurodeficit score from rat model with artificially induced ischemic stroke.

DISCUSSION

Ischemic stroke is a degenerative disease caused by circulatory disturbance, including a thrombus. The circulatory disturbance impacts the blood supply to the brain. Brain circulatory disturbance significantly impacts neurons and triggers necrosis and other histopathological changes. Generally, the histopathological changes in ischemic stroke are secondary haemorrhage, neuronal necrosis, microgliosis, and satellitosis.

The untreated group showed the occurrence of secondary haemorrhage. Shao et al. (2022) explained that the secondary haemorrhage during ischemic stroke is caused by vascular anomaly, coagulopathy, and vasculitis. Secondary haemorrhage is a compensatory impact of intravascular pressure on the brain blood vessel. This mechanism allows the blood extravasation to penetrate the Virchow Robin space (Bautista et al., 2021). Further, oedema perivascular and perineuronal indicate a trapped fluid within the space in the brain tissue. Based on Jha et al. (2019), brain oedema is a fluid accumulation in the tissue because of an increase in intracranial pressure. During the stroke pathophysiology, the oedema is formed within 2 to 5 days after the stroke attack (Wu et al., 2018).

Furthermore, circulatory disturbance impairs brain oxygenation and its nutritional supply, which initiates neuronal necrosis during ischemic stroke. One of the most common neuronal necrosis within brain tissue is marked by pyknosis. Based on Mora-Gutiérrez et al. (2021) study described that the clumping of the nucleus marks pyknosis as an impact of DNA condensation. It is similar to the result of this study that there is a forming of secondary haemorrhage with severe necrosis in the untreated group.

Brain tissue is also arranged by the satellite cell and is essential as a neuroprotector. However, its role is switching to become the progenitor for improving connective tissue during an ischemic mechanism (Hanani and Spray, 2020). Like the satellite cell, the microglia cell is a natural macrophage within brain tissue. In this study, microglia can be found in untreated and treated groups. As a natural macrophage, the microglia play a role in phagocytosis. Microgliosis is an indicator of inflammation and tissue defect. The previous study described that ischemic stroke causes a decrease in blood supply that promotes a quick demarcation of microglia to form microgliosis (Zhang et al., 2019). Therefore, satellitosis and microgliosis always occur during ischemic stroke, which is related to this study's result in the untreated group.

Histopathologically, it can be seen that there are no significant differences between the sham-operated group and the group treated with 2.96 mg/kg BW of calabash against various parameters, except for neuronal necrosis. This result indicated that the calabash acts as a healing promoter in ischemic stroke in a rat model. The difference between sham-operated and 2.96 kg/BW of the calabash group regarding neuronal necrosis explains that neuronal necrosis requires a more extended period to be repaired (Sekerdag et al., 2018).

The efficacy of calabash as a treatment against ischemic stroke is supported by its choline content. Because choline is an eminent neuroprotectant and neuro supplement (Filipska et al., 2020). This hypothesis is supported by Blusztajn et al. (2017), that described that choline has a potency to repair the neuron in human and animal studies during the prenatal period. Choline is also an essential food supplement if it is combined with folic acid and B12. Moreover, choline has neuroprotectant effects to decrease the risk of neuronal defect in human embryonic studies. Bekdash (2019) reported that choline is needed by the body to synthesise phosphatidylcholine, which is required in repairing membrane cell degradation. Phosphatidylcholine also beneficially depresses reactive oxygen species, lipid peroxidase and increases the function of neuro cholinergic dependent (Zhong et al., 2021).

Moreover, there is a different efficacy between each dose of calabash against ischemic stroke. On the doses, 0.74 mg/kg BW of calabash, oedema neuronal, secondary hemorrhage,

oedema perineuronal, and microgliosis were observed. However, the dose of 1.48 mg/kg BW showed a minimal lesion, such as oedema perineuronal, and its efficacy increased at 2.96 mg/kg BW of calabash, which showed mild hydropic swelling. During the treatment using the highest doses of calabash, the microgliosis was minimal but still can be found. It shows that microgliosis is compensatory for severe tissue damage. Qin et al. (2019) described that microgliosis becomes activated in brain inflammation. Microglia undergo changes in form and function, synthesis of cytokine, chemokine, and other co-factor that initiate cell regeneration in the surrounding tissues (Yadav et al., 2019). Further, the efficacy of choline increases along with the increase in doses, and it describes that the dose of choline is related to ischemic stroke repair. Higher choline synthesis of higher acetylcholine as a neurotransmitter promotes the modification of methylation of neuro DNA to change their gene expression and neuronal activity (Derbyshire and Obeid, 2020). The histopathological changes after therapy can be analyzed through the neurodeficit or neurological deficit of the patient. A high repair of neurons within the brain significantly affects the normalization of neurodeficit. Althurwi et al. (2022) demonstrated that neurological deficits can be repaired following the therapy using β-carotene. Like the β-carotene, choline has beneficial effects as a neuroprotectant potent against neuronal injury.

CONCLUSION AND RECOMMENDATIONS

This study proved that calabash can be an alternative therapy against ischemic stroke. Higher doses of calabash promote higher repairing mechanisms in brain tissue, especially in promoting neuronal protection.

An advanced study must be performed to analyze the other potency of this fruit against ischemic stroke accompanied by other comorbid factors. Further, the reports of toxicity of calabash and its processing product must be elucidated to guarantee the safety aspect of this herbal utilization.

ACKNOWLEDGMENTS

The author acknowledged all research assistants in the Laboratory of Pharmacology, FVM, UWKS, and the Laboratory of Pathology, FVM, and UGM for their support.

NOVELTY STATEMENT

This is the first report to elucidate calabash's potential benefit as an alternative therapy against ischemic stroke. Further, this study provides an initial dose of calabash against ischemic stroke in a rat model and may provide the first report regarding human doses.

AUTHORS CONTRIBUTION

JHH, YAP, and SW contributed to the design, performed the study and data analysis, and wrote the draft of the submitted manuscript.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

REFERENCES

Althurwi H. N., Abdel-Rahman R. F., Soliman G. A., Ogaly H. A., Alkholifi F. K., Abd-Elsalam R. M., et al. (2022). Protective effect of *beta*-carotene against myeloperoxidase-mediated oxidative stress and inflammation in rat ischemic brain injury. Antioxidants., 11(12): 2344. https://doi.org/10.3390/antiox11122344

Bautista W., Adelson P. D., Bicher N., Themistocleous M., Tsivgoulis G., Chang, J. J. (2021). Secondary mechanisms of injury and viable pathophysiological targets in intracerebral hemorrhage. Ther. Adv. Neurol Disord., 14: 17562864211049208. https://doi.org/10.1177/17562864211049208

Bekdash R. A. (2019). Neuroprotective effects of choline and other methyl donors. Nutrients., 11(12): 2995. https://doi.org/10.3390/nu11122995

Bhatia A., Saikia P. P., Dkhar B., Pyngrope H. (2022). Anesthesia protocol for ear surgery in Wistar rats (animal research). Anim. Mod. Exp. Med., 5(2): 183–188. https://doi.org/10.1002/ame2.12198

Blusztajn J.K., Slack B.E., Mellott T.J. (2017). Neuroprotective actions of dietary choline. Nutrients., 9(8): 815. https://doi.org/10.3390/nu9080815

Das N., Islam M.E., Jahan N., Islam M.S., Khan A., Islam M.R. et al. (2014). Antioxidant activities of ethanol extracts and fractions of *Crescentia cujete* leaves and stem bark and the involvement of phenolic compounds. BMC Complement Altern. Med., 14: 45. https://doi.org/10.1186/1472-6882-14-45

Derbyshire E., Obeid R. (2020). Neurological development and brain function: A Systematic Review Focusing on the First 1000 Days. Nutrients.. 12(6): 1731. https://doi.org/10.3390/nu12061731

Filipska-Blejder K., Wiśniewski A., Biercewicz M., Ślusarz R. (2020). Are depression and dementia a common problem for stroke older adults? A review of chosen epidemiological studies. Psychiatr. Q., 91(3): 807-817. https://doi.org/10.1007/s11126-020-09734-5

Hanani M., Spray D. C. (2020). Emerging importance of satellite glia in nervous system function and dysfunction. Nat. Rev Neurosci., 21(9): 485–498. https://doi.org/10.1038/s41583-020-0333-z

Herpich F., Rincon F. (2020). Management of acute ischemic stroke. Crit. Care Med., 48(11): 1654-1663. https://doi.org/10.1097/CCM.0000000000004597

Hristu R., Stanciu S. G., Dumitru A., Paun B., Floroiu I., Costache

M. et al. (2021). Influence of hematoxylin and eosin staining on the quantitative analysis of second harmonic generation imaging of fixed tissue sections. Biomed. Op. Express., 12(9): 5829–5843. https://doi.org/10.1364/BOE.428701

Jha R. M., Kochanek P. M., Simard J. M. (2019). Pathophysiology and treatment of cerebral edema in traumatic brain injury. Neuropharm., 145(Pt B): 230–246. https://doi.org/10.1016/j.neuropharm.2018.08.004

Kuriakose D., Xiao Z. (2020). Pathophysiology and treatment of stroke: present status and future perspectives. Int. J. Mol. Sci., 21(20): 7609. https://doi.org/10.3390/ijms21207609

Kuszynski D. S., Lauver D. A. (2022). Pleiotropic effects of clopidogrel. Purinergic Signal., 18(3): 253–265. https://doi.org/10.1007/s11302-022-09876-0

Mora-Gutiérrez A., Guevara J., Rubio C., Calvillo-Velasco M., Silva-Adaya D., Retana-Márquez S. et al. (2021). Clothianidin and thiacloprid mixture administration induces degenerative damage in the dentate gyrus and alteration in short-term memory in rats. J. Toxicol., 2021; 9983201. https://doi.org/10.1155/2021/9983201

Murphy S.J., Werring D. J. (2020). Stroke: causes and clinical features. Medicine (Abingdon)., 48(9): 561–566. https://doi.org/10.1016/j.mpmed.2020.06.002

Patti G., Micieli G., Cimminiello C., Bolognese L. (2020). The role of clopidogrel in 2020: A reappraisal. Cardiovasc. Ther., 8703627. https://doi.org/10.1155/2020/8703627

Percie du Sert N., Ahluwalia A., Alam S., Avey M. T., Baker M., Browne W. J. et al. (2020). Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. PLoS Biol., 18(7): e3000411. https://doi.org/10.1371/journal.pbio.3000411

Qin C., Zhou L.Q., Ma X.T., Hu Z.W., Yang S., Chen M., et al. (2019). Dual functions of microglia in ischemic stroke. Neurosci. Bull., 35(5): 921-933. https://doi.org/10.1007/s12264-019-00388-3

Rahaman P., Del Bigio M. R. (2018). Histology of Brain Trauma and Hypoxia-Ischemia. Acad. Forensic Pathol., 8(3): 539–554. https://doi.org/10.1177/1925362118797728

Rivera-Mondragón A., Caballero-George C., Pieters L., Foubert K. (2022). Validation of an UPLC-DAD Method for the quantification of phenolic acids, verbascoside and 6-epi-aucubin in *Crescentia cujete* fruit. Comb. Chem. High Throughput Screen, 25(7): 1148-1157. https://doi.org/10.2174/1386207323666201207114302

Santo B. A., Ciecierska S. K., Mousavi Janbeh Sarayi S. M., Jenkins T. D., Baig A. A., Monteiro A., et al. (2023). Tectonic infarct analysis: A computational tool for automated whole-brain infarct analysis from TTC-stained tissue. Heliyon., 9(4): e14837. https://doi.org/10.1016/j.heliyon.2023.e14837

Sekerdag E., Solaroglu I., Gursoy-Ozdemir Y. (2018). Cell death mechanisms in stroke and novel molecular and cellular treatment options. Curr. Neuropharmacol., 16(9): 1396–1415. https://doi.org/10.2174/1570159X16666180302115544

Shao L., Chen S., Ma L. (2022). Secondary Brain Injury by Oxidative Stress After Cerebral Hemorrhage: Recent Advances. Front Cell Neurosc, 2022; 16: 853589. https://doi.org/10.3389/fncel.2022.853589

Wilujeng S., Wirjaatmadja R., Prakoso Y.A. (2023). Effects of extraction, fermentation, and storage processes on the levels of choline derived from calabash fruit (Crescentia cujete L.). J. Res. Pharm., 27(2): 620-626. https://doi.org/10.29228/jrp.344

Wu S., Yuan R., Wang Y., Wei C., Zhang S., Yang X., et al. (2018). Early prediction of malignant brain edema after ischemic stroke. Stroke., 49(12): 2918-2927. https://doi.org/10.1161/STROKEAHA.118.022001

Yang Y., Huang Z., Zhang X. (2021). Efficacy and safety of clopidogrel and/or aspirin for ischemic stroke/transient ischemic attack: An overview of systematic reviews and meta-analysis. Medicine., 100(50): e27804. https://doi.org/10.1097/MD.0000000000027804

Zhang L., Li X., Wang, D., Lv, H., Si, X., Li, X., et al. (2019). Risk factors of recurrent ischemic events after acute noncardiogenic ischemic stroke. Curr Pharm, 2019; 25(45): 4827-4834. https://doi.org/10.2174/1381612825666191029103756

Zhong, C., Lu, Z., Che B., Qian S., Zheng X., Wang A., et al. (2021). Choline pathway nutrients and metabolites and cognitive impairment after acute ischemic stroke. Stroke, (3): 887-895. https://doi.org/10.1161/STROKEAHA.120.031903