Med & Health Jan 2026; 21(1): 128-144 https://doi.org/10.17576/MH.2026.2101.06

ORIGINAL ARTICLE

Exploring the Therapeutic Potential of Stingless Bee Honey as a Novel Therapeutic Approach for Middle Cerebral Artery Occlusion Stroke Models

MISYA AFIQAH NOOR TUAH[1], NUR ATHIRAH AZLAN[2], NIK NASIHAH NIK RAMLI[2], MOHD ZULKIFLI MUSTAFA[3], HUSSIN MUHAMMAD[4], LIAO PING[5], CHEN BO[5], MUHAMMAD DANIAL CHE RAMLI[1]*

[1]Department of Diagnostic and Allied Health Science, Faculty of Health and Life Sciences, Management and Science University, 40100 Shah Alam, Selangor, Malaysia

[2]School of Graduate Studies, Management and Science University, 40100 Shah Alam, Selangor, Malaysia

[3]Department of Neuroscience, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

[4]Toxicology & Pharmacology Unit, Herbal Medicine Research Centre, Institute for Medical Research, National Institutes of Health, 40170 Shah Alam, Selangor, Malaysia

[5]National Neuroscience Institute, 11 Jalan Tan Tock Seng, 308433, Singapore

Received: 20 September 2024 / Accepted: 04 February 2025

ABSTRAK

Strok merupakan antara penyumbang utama kematian dan kecacatan di seluruh dunia dan kira-kira 87% kes dikaitkan dengan strok iskemia. Pola yang membimbangkan terhadap peningkatan kes strok dalam populasi warga emas dilihat semakin berleluasa di Malaysia. Malah, diramalkan bahawa Malaysia akan menjadi negara tua menjelang 2030 yang memerlukan lebih perhatian. Oleh hal yang demikian, terdapat peningkatan terhadap minat dalam menggunakan produk semula jadi untuk menyembuhkan strok. Dalam kajian ini, kami merungkai potensi pengaruh neuroprotektif madu lebah tanpa sengatan (madu kelulut) dalam merawat model strok 'middle cerebral artery occlusion' (MCAO). Selepas mengoklusikan (penyumbatan arteri) tikus dengan strok melalui MCAO model, tikus tersebut dirawat dengan madu kelulut untuk tempoh 14 hari. Sampel otak tikus yang diwarnai dengan 2,3,5-triphenyltetrazolium chloride bagi model strok iskemia menunjukkan kehadiran infark yang jelas, mengesahkan kebolehpercayaan kaedah MCAO dalam menginduksi strok. Selepas pengesahan, pemarkahan 'Modified Neurological Severity Score' digunakan untuk menilai defisit neurologi yang menunjukkan peningkatan ketara selepas 14 hari rawatan. Analisis tingkah laku yang merangkumi prestasi kognitif dan sensorimotor turut dijalankan menggunakan beberapa ujian yang menunjukkan perubahan positif yang ketara. Tambahan pula, kajian histologi mempamerkan morfologi neuron yang lebih baik, pengurangan kehilangan neuron serta penurunan tahap keradangan. Kajian ini memberikan hasil yang menggalakkan dalam peningkatan gangguan neurologi dan tisu otak, menonjolkan potensi manfaat neuroprotektif dalam madu kelulut terhadap rawatan strok iskemia.

Kata kunci: *MCAO; madu kelulut; negara yang menua; strok iskemia*

Correspondence: Muhammad Danial Che Ramli. Department of Diagnostic and Allied Health Science, Faculty of Health and Life Sciences, Management and Science University, Off Persiaran Olahraga 40100 Shah Alam, Selangor, Malaysia. Tel: +6013 6969173 E-mail: muhddanial_cheramli@msu.edu.my

ABSTRACT

Stroke has been the leading cause of death and disability worldwide and approximately 87% of cases are attributed to ischemia. A worrying trend towards a rise in cases of stroke in an ageing population is seen and strokes have become more prevalent in Malaysia. In fact, it is predicted that Malaysia will become an ageing country by 2030 which warrants attention. In the present study, new insights on the potential neuroprotective influences of stingless bee honey (SBH) in treating middle cerebral artery occlusion (MCAO) stroke model was tested. After occluding rats with stroke via MCAO, the rats were treated with SBH for a period of 14 days. The 2,3,5-triphenyltetrazolium chloride-stained brain for ischemic stroke model showed a distinct presence of infarction confirming the reliability of MCAO method. Following confirmation, Modified Neurological Severity Score scoring was used to assess neurological deficits exhibiting a significant improvement after 14 days. Behavioural analysis encompassing cognitive and sensorimotor performance was conducted using several tests exhibiting significant positive changes. Furthermore, histological studies exhibit improved neuronal morphology, reduced neuronal loss and decreased inflammation. These studies provide encouraging results in the improvement of neurological impairments and brain tissue, highlighting the potential neuroprotective benefits of SBH in the setting of ischemic stroke.

Keywords: Ageing country; MCAO; ischemic stroke; stingless bee honey

INTRODUCTION

Stroke is the most common cause of mortality and disability in the world, and ischemia is responsible for around 87% of instances (Zhu et al. 2021). Therefore, this study's primary goal is to assess the potential value of stingless bee honey (SBH) in the management of ischemic stroke. Following the phase of ischemia reperfusion, blood flow to the ischemic tissue is restored, and reactive oxygen species (ROS) generation rises, resulting in oxidative stress and oxidative stress-related deoxyribonucleic acid (DNA) damage, local inflammatory response and cell death. Controlling both inflammation and oxidative stress could thus be very advantageous (Xu et al. 2022). The sole Food and Drug Administration (FDA) approved treatment for ischemic stroke at the moment is tissue plasminogen activator (tPA). However, fewer than 10% of patients are suitable for tPA therapy due to its constrained therapeutic window and possibility for haemorrhagic complications. Due to this, there is increasing interest in using natural remedies to treat strokes since certain natural substances seem to have an excellent chance for preventing ischemia reperfusion harm to the brain (Tao et al. 2020).

The stingless bee is a member of the Meliponinae subfamily of bees and is distinguished from other bees by its inability to sting and its honey contains greater quantities of reducing sugars, moisture and acidity. It contains a complex matrix made up of carbohydrates, water, minerals, proteins and vitamins obtained from nectar, pollen or the maturation of honey (Pimentel et al. 2022). Recent research has revealed SBH has a variety of health benefits, which are attributed to the substance's high levels of flavonoids and phenolic chemicals, which have anti-inflammatory and antioxidant properties (Zulkifli et al. 2023) which are extremely beneficial for controlling oxidative stress, reducing neuroinflammation and improving mental function (Tao et al. 2020). Compounds that can be found in SBH, like phenylalanine, may enhance memory by acting on brain-derived neurotrophic factor (BDNF) pathways, whereas flavonoids diminish neuroinflammation by reducing proinflammatory cytokine and free radical generation. Given that SBH has stronger antioxidant activity than honey from *Apis* sp., it could be more beneficial therapeutically. However, little study has been done on their impact on ischemic stroke (Zulkifli et al. 2023).

MATERIALS AND METHODS

Study Design

The experimental procedure was conducted in the Animal House, located in Management and Science University (MSU) after obtaining ethic approval for animal study from MSU Ethics Committee (MSU-RMC-021-FR01/08/C3/017). A total of 50 Sprague Dawley (SD) rats in good condition were obtained in collaboration with the Institute of Medical Research (IMR), Kuala Lumpur. The rats, aged 8 weeks with weights ranging from 200-250 g, were used in the study. Table 1 provided detailed information on the grouping of rats for the study. The study was performed for a period of 14 days to evaluate the potential effects of SBH in treating middle cerebral artery occlusion (MCAO) rat stroke model. The experimental rats underwent the MCAO procedure to occlude the stroke and was treated with SBH via oral gavage for 14 days. Behavioural analyses, focusing on cognitive and locomotor functions, were performed throughout the experiment. At the conclusion of day 14, the rats were euthanised and their brains were obtained for further investigation of histopathological changes. A thorough chronological summary of the major occurrences and activities of the research is provided in Table 2.

The sample size was calculated using G*Power software, version 3.1.1The calculation was based on an assumed medium effect size (Cohen's f = 0.25), a significance level of = 0.05, and a desired statistical power of 80%. Based on these parameters, a total sample size of 40 rats was determined to be sufficient for this study (Faul et al. 2009). From this value, 20% attrition were expected and the corrected sample size may be explained in the form of structured formula i.e;

Corrected sample size (N_{adj})
= Sample size/ (1 [% attrition/100])
(N_{adj})= 40/ (1 [0.2])
(N_{adj})= 50

Hence, 50 rats were included in the study, with six animals allocated in five experimental groups; normal, negative and three treatments groups, respectively (Azwan et al. 2025).

SBH Sample & Dosage

A commercial MUSTAFA-hive's SBH (*Meliponini* sp.; developed by Dr. Mohd Zulkifli bin Mustafa and his team, USM, Kampus Kesihatan, Kota Bharu Kelantan) was simply transferred into a sampling bottle that was given. The sample taken was maintained at 4°C and shielded from the sun. The material was cooled to room temperature prior to being diluted for the experimental group's treatment. The experimental rats were grouped based on their respective treatments (n=10), i.e; (i) Group 1: Normal control rats treated with distilled water; (ii) Group 2: Negative control rats treated

TABLE 1: Rat grouping information for ischemic stroke study

Group	Concentration (mg/kg)	Gender	No. of Animals	Procedure
Normal	N/A	Male	6	Not occluded by MCAO procedure
Negative Control	N/A		6	Not occluded by MCAO procedure
Experimental 1	500 mg/kg SBH		6	Occluded by MCAO procedure
Experimental 2	750 mg/kg SBH		6	Occluded by MCAO procedure
Experimental 3	1000 mg/kg SBH		6	Occluded by MCAO procedure

TABLE 2: Summary of experimental procedures and timeline evaluation of stingless bee honey (SBH) in treating middle cerebral artery occlusion (MCAO) in a stroke model

	Normal	Negative Control	Experimental 1 (500 mg/kg)	Experimental 2 (750 mg/kg)	Experimental 3 (1000 mg/kg)
Study Initiation	1 February 2023				
Quarantine	6 Feb 2023 - 12 Feb 2023		6 Mac 2023 - 12 Mac 2023		
Acclimitisation	13 Feb 2023 - 19 Feb 2023		13 Mac 2023 - 19 Mac 2023		
Pre-Behavioural Study	20 Feb 2023 - 24 Feb 2023		20 Mac 2023 - 24 Mac 2023	27 Mac 2023 - 31 Mac 2023	3 April 2023 - 7 April 2023
MCAO Procedure	N/A	25 Feb 2023	25 Mac 2023	1 April 2023	8 April 2023
Treatment	N/A	26 Feb 2023 - 11 Mac 2023	26 Mac 2023 - 8 April 2023	2 April 2023 - 15 April 2023	9 April 2023 - 22 April 2023
Behavioural Study	26 Feb 2023 - 11 Mac 2023		26 Mac 2023 - 8 April 2023	2 April 2023 - 15 April 2023	9 April 2023 - 22 April 2023
Sacrifice	11 March 2023		8 April 2023	15 April 2023	22 April 2023
Histological Study	11 Mac 2023 - 17 Mac 2023		8 April 2023 - 14 April 2023	15 April 2023 - 21 April 2023	22 April 2023 - 28 April 2023
Study Completion	8 May 2023				

distilled water; (iii) Group 3: MCAO rats treated with 500 mg/kg of SBH; (iv) Group 4: MCAO rats treated with 750 mg/kg of SBH; and (v) Group 5: MCAO rats treated with 1000 mg/kg of SBH

The selection of doses for SBH treatments were according to previous study where treatment dosage up to 1000 mg/kg for SBH have been reported to produce therapeutic effect towards rats with neurological disorder (Shaikh et al. 2024). Hence, to compare the effect of different dosage of treatments, low and medium doses (500 mg/kg & 750 mg/kg) of SBH treatment were included for this study alongside with the high dosage (1000 mg/kg). The treatments were given using oral gavage for a period of 14 days, as suggested from Organization for Economic Co-operation and Development (OECD) guideline for acute study. Moreover, multiple studies involving natural product have shown therapeutic window throughout 14 days of treatments (Koppula et al. 2012; Pakaprot et al. 2024).

Middle Cerebral Artery Occlusion Procedure

All surgical instruments were autoclaved and sanitised. The rat was anaesthetised via intraperitoneal injection with a combination of ketamine (80 mg/kg; Pfizer Inc., New York, USA) and xylazine (10 mg/kg; Bayer AG, Leverkusen, Germany), with doses adjusted according to body weight. The fur on the ventral neck region was then clipped using an electric razor to reveal the skin. An iodine solution was then applied to clean and sterilise the surgical site (Che Ramli et al. 2020; Morris et al. 2016).

A midline incision (less than 2 cm) in the centre of the neck was made using a surgical blade. The superficial cervical fascia (SCF), omohyoid muscles (OHM), deep cervical fascia (DCF) and carotid sheath were bluntly divided using two sterilised cotton buds to expose the underlying common carotid artery (CCA), external carotid artery (ECA) and internal carotid artery (ICA). Once the arteries had been located, the vagus nerve located next to the CCA, fascia and nearby nerves was carefully detached from the arteries. Next, the ECA was permanently ligated with silk sutures 4/0 near the carotid bifurcation and the CCA was also permanently ligated further down the carotid bifurcation. Then, CCA near

the carotid bifurcation was loosely ligated and a micro vessel clip was positioned on the ICA to prevent blood flow from entering the CCA. A small incision in the artery also known as arteriotomy was performed between the two ligation that surrounds the CCA using a micro scissor. The silicone-coated filament was inserted into the arteriotomy and progressed into the ICA. The loose ligation near the CCA was then permanently ligated and the micro vessel clip was removed. The silicone-coated filament was inserted further to the direction of the MCA until the filament was fully in. After 120 minutes of occlusion time, the filament was removed slowly and the permanent ligation around the CCA near the carotid bifurcation was secured. Lastly, the neck incision was stitched (Morris et al. 2016: Zhang et al. 2021). Rats were recuperated for 48 hours in their own cages, which were single-housed and soft food pellets were provided to aid in their recovery (Morris et al. 2016).

2,3,5-triphenyltetrazolium Chloride Staining

Infarct volume was assessed via 2,3,5-triphenyltetrazolium chloride (TTC) staining (Sigma-Aldrich, St. Louis, MO, USA). Rats were sacrificed using chloroform and ensured death by cervical dislocation and the brain was obtained, washed with saline and cooled at 4°C to harden the organ. Ten coronal sections of the brain with 2 mm thickness were cut and immersed for 20 minutes into 2% TTC solution at room temperature. The ischemia damage in the infarcted hemisphere was observed in stained brain sections (Yu et al. 2022).

Behavioural Study

(i) Modified neurological severity score test

The modified neurological severity score (mNSS) test was used to assess the neurological functioning deficits. The tests were conducted on day 1 and day 14 following the MCAO. The scale included a range of 0 (normal score) to 18 points (maximum deficit). The motor and sensory tests were all included in the mNSS. If the rat failed the test or failed to exhibit the predicted response, one point was given; hence, the greater the score, the greater the damage (Long et al. 2020).

(ii) Cognitive test (T-maze test)

A maze consisted of a long central corridor and a perpendicular arm extending from the middle of the corridor in the shape of a 'T' was used. One arm was designated as the 'correct' arm which led to a reward while the other arm was designated as the 'incorrect' arm and led to an aversive stimulus. Prior to surgery, the rats were trained for 3 days and the results obtained before surgery were used as a pre-surgery baseline. As for post-surgery, the test consisted of 4 trials for 2 minutes with a 30 seconds interval for each trial on days 1, 3, 7 and 14. The number of correct choices (CC) as well as total number of trials (N) were recorded and the Working Memory Index (WMI) calculation was used (Zlatanova et al. 2022).

$$WMI = \frac{CC}{N} \times 100$$

CC = Correct Choices
N = Total number of trials

(iii) Cognitive test (Morris-Water Maze)

The Morris Water Maze (MWM) was used in this study to measure the cognitive ability of rats. It consisted of a circular pool of water with a hidden platform from which the rats could escape onto. The rats were placed at the starting point and were assigned to find a hidden platform placed distal to the starting point and 1 cm below the water and the escape latency of each rat were recorded. The rats were given 30 seconds to recover on the platform during the experiment. The rats were manually directed to the platform if they weren't able to locate the platform in 120 seconds (Yao et al. 2021). Prior to surgery, the rats were trained for 3 days and the results obtained before surgery were used as a pre-surgery baseline. As for post-surgery, the tests were conducted on days 1, 3, 7 and 14.

(iv) Motor coordination test (Rotarod)

The rotarod test (Ugo Basile, Gemonio, Italy) was used to determine balance and motor coordination of rats. In this test, the rats underwent four trials at an increased speed of 4 to 40 revolutions per minute (RPM) over the course of 2 minutes. The average engagement time and latency to fall throughout the trials was recorded and calculated (Shi et al. 2021). Prior to surgery, the rats were trained for 3 days and the results obtained before surgery were used as a pre-surgery baseline. As for post-surgery, the tests were conducted on days 1, 3, 7 and 14.

(v) Motor coordination test (Pole test)

Pole test was used to evaluate motor deficits associated with stroke. The test consisted of 5 trials within 2 minutes. In this study, the rats were placed at the top of the vertical pole with 50 cm in length and the time taken for each rat to descend as well as the frequency of fall was recorded (Li et al. 2021b). Prior to surgery, the rats were trained for 3 days and the results obtained before surgery were used as a pre-surgery baseline. As for post-surgery, the tests were conducted on days 1, 3, 7 and 14.

Cresyl Violet Staining

At the end of 14 days, cresyl violet (Sigma-Aldrich, St. Louis, MO, USA) staining was used to determine the severity of ischemia-induced damage. After sacrifice, the brains were removed and fixed in 4% buffered formaldehyde for 72 hours. Samples underwent tissue processing, deparaffinised, rehydrated and stained with cresyl violet. After 3 minutes of cresyl violet staining, graded ethanol dehydration of 70% alcohol, 95% alcohol and 100% alcohol were used and finally xylene for 5 minutes was performed. Microscopic examination of brain slices was then performed using light microscope (Umbar et al. 2025; Yao et al. 2021).

Data Analysis

Data normality was assessed using the Shapiro-Wilk test, while homogeneity of variances was evaluated using Levene's test. Statistical analysis was performed using SPSS version 29 (IBM Corp., Armonk, NY, USA). For normally distributed data, differences among groups were analysed using one-way analysis of variance (ANOVA) followed by Games–Howell post hoc analysis. A p-value < 0.05 was considered statistically significant. Results were presented as the average and standard error of the average (mean $\pm$ SEM). P-values lower than 0.05 were regarded as statistically significant.

RESULTS

2,3,5-triphenyltetrazolium Chloride Staining

Through TTC staining, the presence of infarct in the brains of rats that had suffered an ischemic stroke following a 24-hour reperfusion was demonstrated. When compared to the normal group, the TTC-stained ischemic stroke brain data from the current study shown in Figure 1 demonstrated a clear distinction among the infarcted (white) versus non-infarcted (red) regions, indicating that the MCAO treatment was successful in causing ischemic stroke.

Modified Neurological Severity Score

The results of the mNSS analysis shown in Figure 2 showed that the therapy had a substantial impact on motor deficits in the groups that received the treatment. The results of the ANOVA showed a very significant difference ($p<0.001$) between the treatment groups, indicating that the therapies clearly had an effect on the rats' motor performance. The Levene test was also used to evaluate the homogeneity of variances, and it too produced a very significant result ($p<0.001$). This showed that there was a significant difference in the motor score variance between the treatment

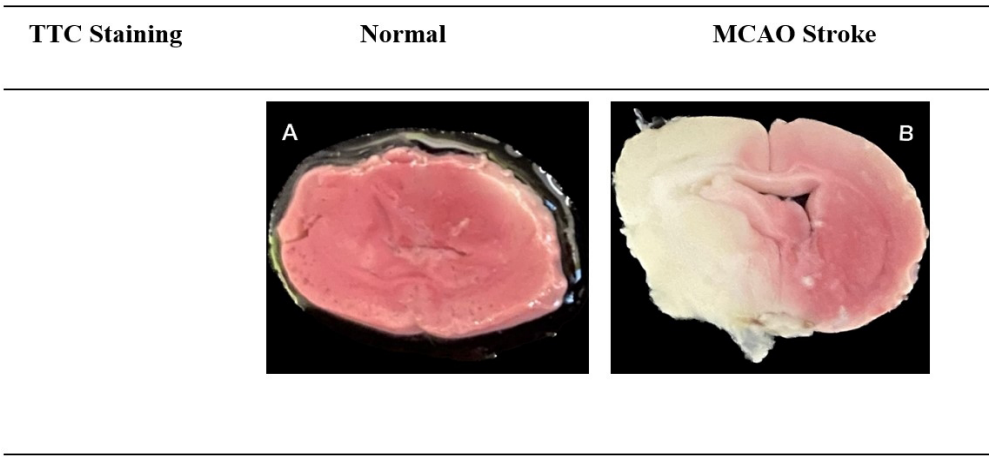

FIGURE 1: Representative images of 2,3,5-triphenyltetrazolium chloride (TTC) stained brain slices; (A) The normal group's rat brain had no differences in colouring; (B) The infarcted (white) and non-infarcted (red) areas of the rat brain were clearly separated by the MCAO stroke model

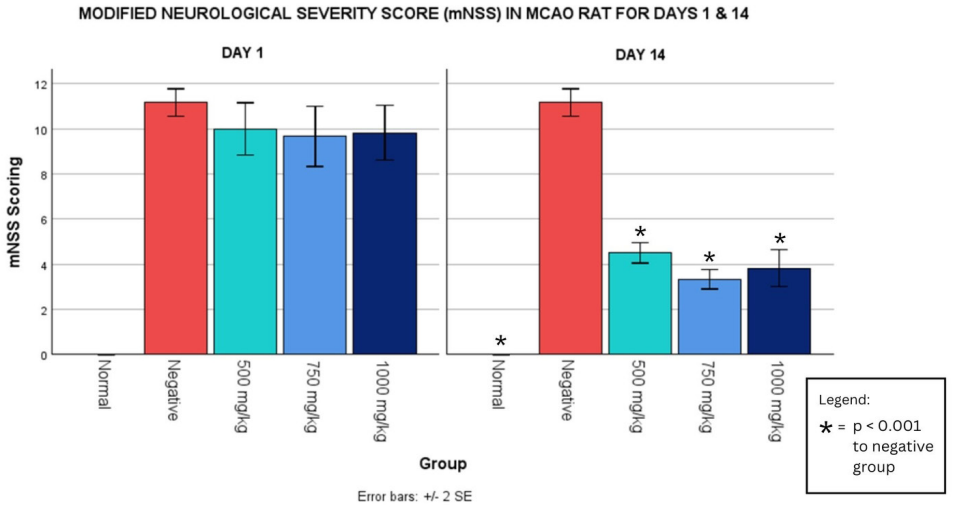

FIGURE 2: Modified Neurological Severity Score (mNSS) at Day 1 and Day 14. The bar graph shows the mean mNSS scores for the MCAO stroke rat at Day 1 and Day 14 at two different time periods. The groups were shown on the x-axis, while the mNSS scores were shown on the yaxis. Standard error was shown by the error bars. The results showed a noticeable decline in scoring from Day 1 to Day 14, which suggested that SBH may be effective in improving neurological impairments ($p<0.001$).

groups. The findings' validity was strengthened by the significant results from the ANOVA and Levene test, which helped to support the idea that the treatments had a significant impact on the motor deficit shown in the experimental rats. The observed variations in motor scores across the treatment groups were improbable to be the result of chance, as indicated by the low p-values, which also reflected an elevated level of statistical significance.

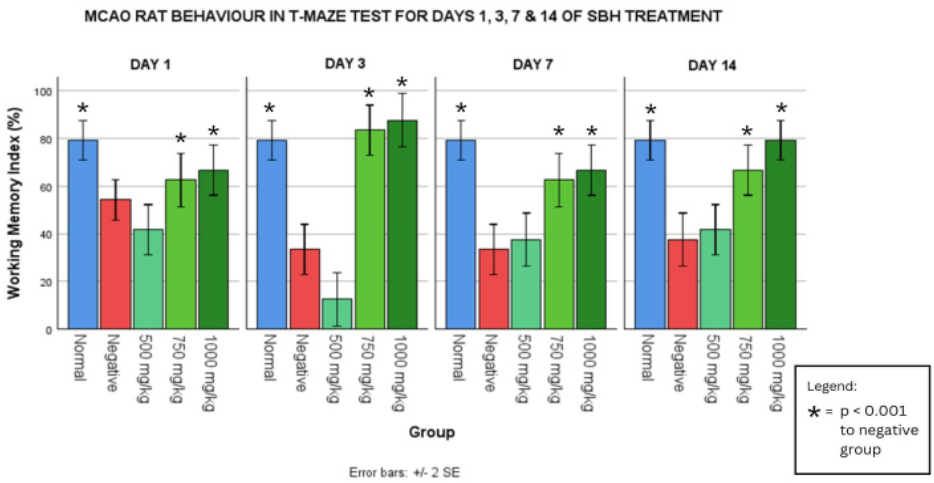

FIGURE 3: Comparison of working memory index values (%) between control groups and groups treated with stingless bee honey (3 different doses) in the T-maze test on days 1, 3, 7 and 14. There was a significant difference in mean WMI between all groups, according to the results of the overall ANOVA ($F = 50.767$, $p<0.001$). The normal group showed no significant changes between the 750 mg/kg and 1000 mg/kg groups, indicating equal performance levels and the effectiveness of SBH treatment in recovering spatial learning abilities

Cognitive Test - T-Maze

The T-Maze was done to evaluate rat's competency in spatial learning and memory after treatment with SBH. Figure 3 showed the result of WMI values in the T-maze test. Two control (normal and negative) groups and three treatment groups were included in the analysis. The homogeneity of variances assumption using Levene' test was first examined and the results of the test showed that the variances were significantly different among the groups ($p<0.05$), indicating that the assumption of homogeneity of variances were not met. Therefore, the Games-Howell post-hoc test was used, which did not assume equal variances, to compare means between the groups. The overall ANOVA was found to be significant ($F = 50.767$, $p<0.001$), indicating that there was a significant difference in mean WMI among the 5 groups. Post-hoc comparisons using Games-Howell showed all significant differences had a p-value < 0.05 except for the normal group with 750 mg/kg and 1000 mg/kg groups, where p-values were 0.052 and 0.772, respectively. This suggested that there may not be significant differences between the normal group and 750 mg/kg and 1000 mg/kg groups.

Cognitive Test - Morris Water Maze

The MWM was done to evaluate rats competency in spatial learning and memory after treatment with SBH. Figure 4 showed the result of time taken to reach the platform (second) in the MWM test. Two control (normal and negative) groups and three treatment groups were included in the analysis. The homogeneity of variances assumption using Levene' test was first examined and the results of the test showed that the variances were significantly different among the groups ($p<0.001$), indicating that the assumption of homogeneity of variances were not met. Therefore, the Games-Howell post-hoc test was used, which did not assume equal variances, to compare means between the groups. The overall ANOVA was found to be significant ($F = 99.216$, $p<0.001$), indicating that there was a significant difference in time taken to reach the platform among the 5 groups. Post-hoc comparisons using

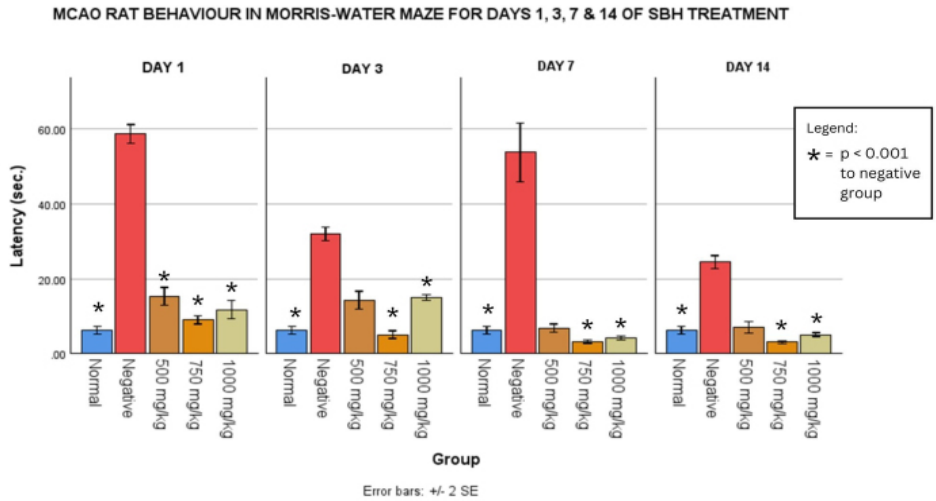

FIGURE 4: Comparison of time taken to reach the platform (second) between control groups and groups treated with stingless bee honey (3 different doses) in the Morris water maze test on days 1, 3, 7 and 14. The results of the overall ANOVA revealed a significant difference in how long it took each group to get to the platform ($F = 99.216$, $p<0.001$). The normal group showed no significant changes between the 750 mg/kg and 1000 mg/kg groups, indicating equal performance levels and the effectiveness of SBH treatments in recovering spatial learning abilities

Games-Howell showed that normal groups had significant differences with negative and 500 mg/kg groups ($p<0.001$) but not with 750 mg/kg ($p = 0.302$) and 1000 mg/kg ($p = 0.086$) groups. The negative group had significant differences with all the other groups ($p<0.001$). The 500 mg/kg group had significant differences with all the other groups ($p<0.001$) but not with the 1000 mg/kg group ($p = 0.640$). The 750 mg/kg group had significant differences with all the other groups ($p<0.05$) except for the normal group ($p = 0.302$). The 1000 mg/kg group had significant differences with negative and 750 mg/kg groups ($p<0.05$) but not with normal ($p = 0.086$) and 500 mg/kg ($p = 0.640$) groups.

Motor Coordination Test - Rotarod

The Rotarod was done to evaluate rats competency in motor coordination after treatment with SBH. Figure 5 showed the result of latency to fall (s) throughout 4 different speeds of RPM (4, 10, 15, 20) over the course of 2 minutes in the Rotarod test. Two control (normal and negative) groups and three treatment groups were included in the analysis. The homogeneity of variances assumption using Levene' test was first examined and the results of the test showed that the variances were significantly different among the groups ($p<0.001$), indicating that the assumption of homogeneity of variances were not met. Therefore, Welch's test was used to compare the means between the groups, which was also found to be significant ($p<0.001$) for all groups. The overall ANOVA was found to be significant ($p<0.001$), indicating that there was a significant difference in latency to fall among the five groups over four different speeds of RPM. To further explore the differences between the treatment groups in different speeds of RPM, post-hoc tests using Games-Howell were used. Games–Howell post hoc analysis indicated that several treatment groups differed significantly from the normal group ($p < 0.05$). In contrast, comparisons between the normal group and the 750 mg/kg group for RPM4, RPM10 and RPM15 did not reach statistical significance ($p = 1.000$, 0.325, 0.073 and 0.772, respectively).

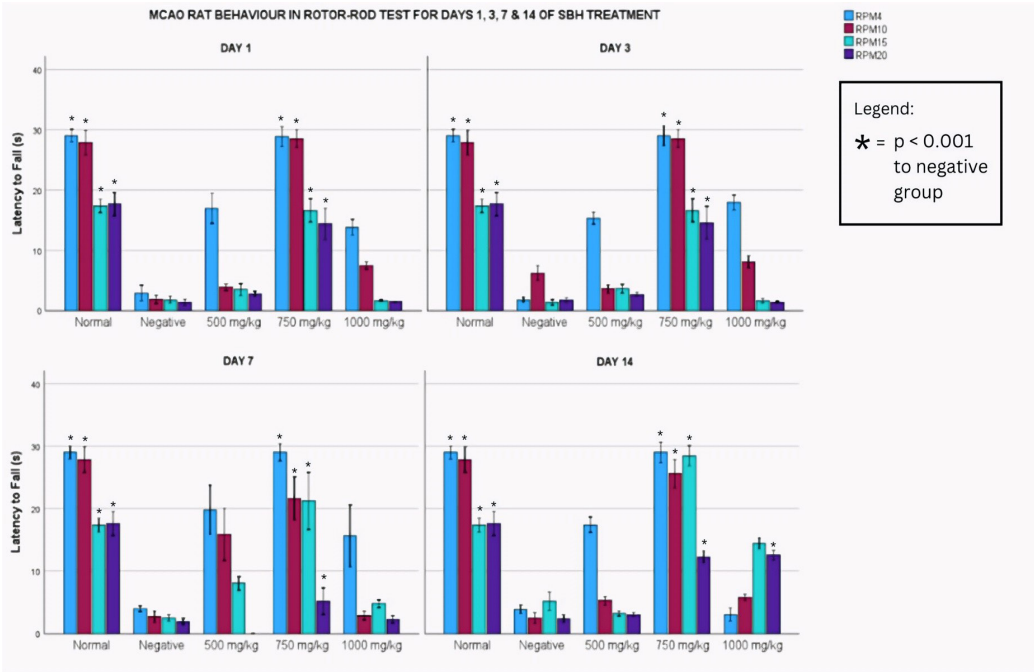

FIGURE 5: Comparison of latency to fall (s) between control groups and groups treated with stingless bee honey (3 different doses) in the rotarod test on days 1, 3, 7 and 14. The overall ANOVA was found to be significant ($p<0.001$), indicating that there was a significant difference in latency to fall (s) among all groups

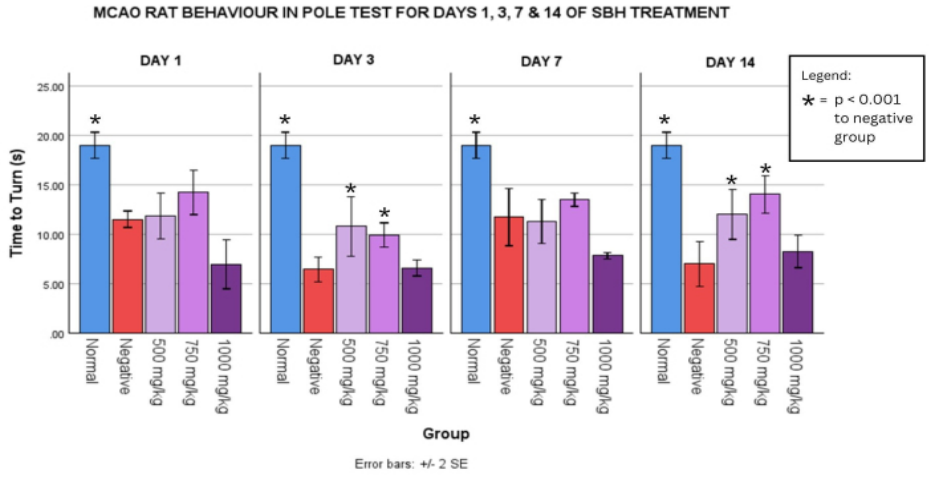

FIGURE 6: Comparison of time to descend (s) between control groups and groups treated with stingless bee honey (3 different doses) in the pole test on days 1, 3, 7 and 14. The overall ANOVA was found to be significant ($p < 0.001$), indicating that there was a significant difference in time to turn (s) among all groups

This suggested that there may not be significant differences between the normal group and 750 mg/kg groups.

Motor Coordination Test - Pole Test

The pole test was done to evaluate rat's competency in motor coordination after treatment with SBH. Figure 6 showed the result of time to descend (s) values in the pole test. Two control (normal and negative) groups and three treatment groups were included in the analysis. The homogeneity of variances assumption using Levene' test was first examined and the results of the test showed that the variances were significantly different among the groups ($p<0.001$), indicating that the assumption of homogeneity of variances were not met. Therefore, the Welch T-test and Games-Howell post-hoc test was used, which did not assume equal variances, to compare means between the groups. The overall ANOVA was found to be significant ($F = 73.075$, $p<0.001$), indicating that there was a significant difference in time to turn among the five groups. Post-hoc analysis revealed that 500 mg/kg had a significant effect on pole test but the effect was not significantly different from the negative control group. However, 500 mg/kg had a significantly lower effect as compared to the 750 mg/kg group showing better performance in the 750 mg/kg group.

Cresyl Violet Staining

Based on Figure 7, the normal group's histological examination revealed no obvious pathological alterations. The granular cell layer (GCL) in the hippocampus contained neurons that were organised in an organised manner. The cortical neurons have distinct structures with rounded, big, and regular nuclei as well as a number of glial cells were observed. The negative group emphasised severe abnormalities in both the hippocampus and cortex, including disorganised cellular organisation, disorderly tissue construction, and a number of pyknotic nuclei in the cortex, along with atrophy, neuronal loss, and loose neuronal organisation in the hippocampus. These alterations point to significant cellular malfunction in the areas of the studied brain. When contrasted with the control group, the hippocampus and cortex of the 500 mg/kg group underwent substantial histopathological alterations. The hippocampal GCL exhibited more pronounced structural improvements in the 750 mg/kg treatment group, accompanied by an increase in neuronal density within the cortex. Lastly, the hippocampus and cortex showed similar alterations in the histological examination of the 1000 mg/kg group compared to the 750 mg/kg group, while the 750 mg/kg dosage had a more significant therapeutic effect on both brain areas' histopathological features.

DISCUSSION

In the present study, TTC staining was applied to definitively show that an infarction had occurred in the brain of the rat that had undergone an ischemic stroke following MCAO. When compared to the normal group depicted in Figure 1(B), the TTC-stained ischemic stroke brain had a clear separation among the infarcted (pale) versus non-infarcted (red) regions. The distinction between the pale-white and red areas shows that prior studies have also documented a considerable infarct volume (Hou et al. 2018). Next, current findings showed that the mNSS scores on day 1 as well as day 14 following SBH treatment differed significantly. These results suggest that SBH treatment has a significant long-term effect on motor impairment recovery. The enhancement in motor performance that has been noticed implies that SBH may be a treatment option for enhancing motor recovery after an ischemic stroke. These findings are in line with other research that looked at how comparable therapies affected motor deficits in stroke model organisms treated stem cell exosomes (Liu et al. 2021). By way of example, Shi et al. (2023) at time periods between day 1 and day 8 after 5-aza-2'-deoxycytidine therapy found comparable gains in motor abilities in the MCAO rat model (Shi et al. 2023).

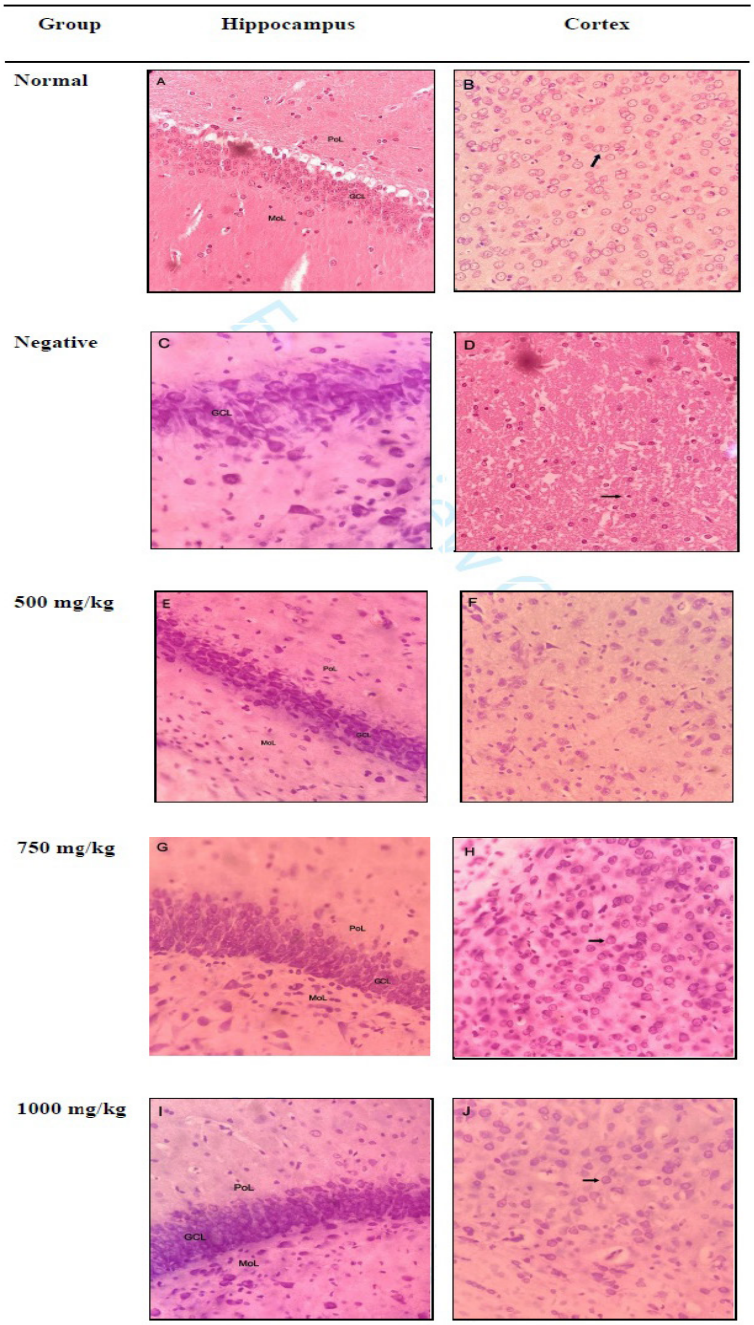

FIGURE 7: Histology of hippocampus and cortex in normal, negative, 500 mg/kg, 750 mg/kg and 1000 mg/kg rats under 40X magnification. (A-B) Normal group showed normal tissue structure. (C) Negative group showed loose arrangement of neurons, obvious atrophy and loss of neurons. (D) Negative group showed cells were arranged disorderly, loose tissue structure and pyknotic nuclei. (E-F) 500 mg/kg group showed distorted cellular structures and neuronal loss. (G-J) 750 mg/kg group and the 1000 mg/kg group showed positive changes in the granular cell layers of the hippocampus and increased neurons in the cortex were observed

Further into the study, ischemic stroke rats treated with a low dosage (500 mg/kg) and negative control group showed substantial abnormalities in spatial learning and memory in comparison to untreated rats, showing poor spatial memory. However, as compared to untreated and low dosage treated rats, SBH-treated rats at mid-dose (750 mg/kg) and high-dose (1000 mg/kg) exhibited a substantial enhancement in the WMI. In-depth analysis of the findings showed observable trends in T-maze performance over the course of the testing days. The MWM test was used in conjunction with the T-maze test in an effort to further support the results on spatial learning ability. According to the significant ANOVA ($F = 99.216$, $p<0.001$), there was a substantial overall difference in how long it took for all groups to arrive at the platform in the MWM test, which is consistent with previously documented spatial learning abilities in the T-maze test. Surprisingly, in contrast to the normal group, there were no discernible changes when comparing the 750 mg/kg and 1000 mg/kg groups. This data suggests that SBH treatments at dosages of 750 mg/kg and 1000 mg/kg may have a possible beneficial effect on the recovery of spatial learning capacities in the MWM test, which is a promising discovery. The T-maze and MWM tests supported the SBH treatment's effectiveness in regaining spatial learning abilities at comparable levels. Previous study involving intravenous administration of *Achyranthes bidentata* polypeptide had shown similar improvement to spatial and memory function towards ischemic stroke rats (Shen et al. 2013). It is suggested that the SBH treatments at these doses successfully attenuated or eliminated the spatial learning impairments brought on by the experimental settings since there were no substantial distinctions among the 750 mg/kg and 1000 mg/kg groups in comparison to the normal group in either test.

Research has shown that the phenolic content of SBH has the ability to enhance memory, learning and cognitive function in the brain (Sardooi et al. 2020). Its antioxidant capabilities are one potential mechanism. Consuming antioxidant compounds has been found to reduce the level of oxidative stress in the brain and improve cognitive function (Zulkifli et al. 2023). Therefore, as can be observed in the current work, SBH may safeguard neurons and sustain cognitive function by lowering oxidative damage. By virtue of its anti-inflammatory properties, another putative pathway exists. Another important factor in brain injury and cognitive impairment following a stroke is inflammation. SBH has been proven to have anti-inflammatory characteristics, and flavonoid compounds like quercetin and polyphenols stimulate SBH's actions. SBH may assist in protecting neurons, maintaining synaptic plasticity, and preserving cognitive function by reducing the inflammatory cascade (Zulkifli et al. 2023).

Next, the current results showed varied performance patterns for the rotarod at various RPM settings and timing intervals. Results demonstrated that across all tested rpms, ischemic stroke rats had substantially shorter latencies to fall than normal rats. Shorter latency to fall in the context of stroke denotes that the rats were incapable to retain their balance for an extended period of time before falling off. Essentially, this denotes a lack of motor coordination, unsteadiness, or muscle strength, which are signs of motor deficiencies which are mentioned by prior study (Shi et al. 2021). It is interesting to note that as rpm increased, the motor function losses became more significant, showing that the ischemic stroke rats had trouble adapting to greater spinning speeds. According to similar research by Ahmed et al. (2021), motor-impaired rats are unable to maintain balance on a revolving rod for more than 3 minutes as the RPM increases. In contrast, healthy rats can do so. The current research is consistent with that of Ahmed et al. (2021), which also showed comparable results on days 7 and 14. Both investigations noted a considerable increase in motor coordination on those two days, pointing to the treatment's beneficial effects on the signs and symptoms of motor impairment (Ahmed et al. 2021). In the current investigation, a modest modification to the original pole test procedure

was used, in which a shorter time to descend denotes poorer motor performance and a longer time to descend denotes better motor performance. This is so because a longer time spent on the pole signifies more stability and grip power. The treatment groups' performance on the pole test continued to improve on day 14 across the board. Significantly, across all days, the mid-dose treatment group consistently had the longest time to descend, indicating that the mid-dose treatment of SBH could have a more positive effect on maintaining motor coordination in the later phases of recovery. A study using propofol, an anaesthetic agent commonly associated with anti-inflammatory properties like SBH also have been reported to exhibit an improvement in balancing time on the revolving rod (Zhou et al. 2013).

It is crucial to take into account in the current investigation how motor coordination relates to the possible therapeutic advantages of SBH. The capacity of the body's muscles and nerves to cooperate seamlessly and effectively to carry out motions is referred to as motor coordination. Damage to the brain interferes with neuronal transmission, impairing motor coordination (Jones 2017). According to a previous study, SBH has been discovered to have anti-inflammatory, neuroprotective, and antioxidant characteristics, all of which may help it to enhance motor coordination (Pimentel et al. 2022). The antioxidant capabilities of SBH have been addressed in another study as being important in the context of motor coordination (Ooi et al. 2021). Therefore, SBH's antioxidant properties may lessen oxidative stress, safeguard neurons and maintain their functionality (Al-Hatamleh et al. 2020), thereby contributing to improved motor coordination. The anti-inflammatory qualities of SBH are also remarkable. According to a study, inflammation can lead to further neuronal damage and motor deficits and is involved in the pathogenesis of ischemic stroke (Jones 2017). SBH's anti-inflammatory properties may therefore assist reduce damage brought on by inflammation, improving motor coordination. Additionally, the neuroprotective qualities of SBH are important for motor coordination. SBH's capacity to increase neuronal survival, trigger nerve growth factors, and encourage neuronal plasticity may aid in the regeneration and repair of injured neurons (Zulkifli et al. 2023).

Current findings obtained showed unique histopathological alterations in the various dose groups, elucidating the dosage-dependent effects of SBH on the preservation of tissue and neuronal health. The hippocampus and cortex were the two areas of interest that were targeted via coronal sectioning of the brain samples. In order to ensure a thorough analysis of the histological components inside these locations, the sections were produced at a magnification of 40x. We found substantial histological changes in the hippocampus and cortex of the negative control group. These results are in line with earlier research that described comparable histological alterations after being subjected to the MCAO stroke model (Luo et al. 2019). Moreover, an altered cell organisation was seen in the hippocampus of the 500 mg/kg group, which may indicate aberrant neuronal organisation. These results provide additional evidence that the tissue structure and neuronal health in both brain areas are negatively impacted by the 500 mg/kg dose. Previous research examining the impact of comparable treatment interventions for brain damage at comparable doses have revealed similar histological changes (Li et al. 2021a). Remarkably, a better modification in the GCL of the hippocampus and cortex in the 750 mg/kg group was seen, which is characterised by a more ordered and organised cellular arrangement with more neurons demonstrating a favourable effect on neuronal density. The 1000 mg/kg group, on the other hand, had histopathological alterations similar to those of the 750 mg/kg group but with fewer protective effects. This implies that there could be a dose level at which raising the dosage has no further positive benefits. These results are in line with earlier research that showed comparable hippocampal and cortex histological improvements after exposure to comparable treatment interventions for treating brain damage at comparable doses (Luo et al. 2019).

In this study, no obvious adverse effect of SBH treatments were observed. However, previous report has shown some undesired side effect such as stomach ache, loss of appetite, skin rashes, throat irritation, nausea, heart burn and abdominal pain (Kiprono et al. 2022). However, these side effect may not be significant as it only reported in very minor incidents. The neuroprotective capacity of SBH treatments were distinctively shown throughout the behavioral study and histopathological analysis in this experiment. The exact underlying biological mechanisms of SBH's therapeutic ability towards neurological damage are still in the blur but the neuroprotective ability of SBH may depends on its antioxidant capacity to inhibit the nuclear factor kappa-light-chain-enhancer of activated B cells and mitogen-activated protein kinases. This action may control the responsible genes that influence the production of inflammatory signalling, ultimately leads to a reduction of chemokines biomarkers and pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP) and Interleukin-6 (IL-6) (Ja'afar et al. 2024). Besides, the abundant of phenolic contents may help to decrease the production of arachidonic acids, a by-product of phospholipid metabolism (Mitjavila & Moreno 2012). Reduction of arachidonic acid and their metabolites, such as prostaglandins, helps to reduce the release of free radical and inflammation (Di Meo et al. 2016). Thus, inhibition of these pathways alleviate the oxidative stress and damage towards the brain, which also promotes cell regeneration improve the overall motor and cognitive function (Kamal et al. 2023).

CONCLUSION

Overall, the findings of this study imply that SBH may show potential as an additional treatment approach for motor deficits brought on by ischemic stroke. The precise dose, and the length of the therapy used are only a few of the study's shortcomings that must be acknowledged. Despite these drawbacks, the current study adds to the limited amount of research that suggests SBH may be helpful in stroke recovery. The results emphasise the need for further research into all-natural treatments for neurological deficits in stroke, including SBH. Current work highlights the potential of SBH as a treatment tool in stroke rehabilitation by providing early evidence that it improves functional outcome in ischemic stroke. However, further research is required in order to fully elucidate its potential molecular mechanisms with longer time frame of treatments period and to study any possible neurotoxicity of SBH. Additional study in this area might deepen the comprehension of the underlying processes and guide the creation of cutting-edge treatment strategies to enhance motor recovery as well as cognitive results in stroke.

Author contributions: Lab work, writing-original draft: MA, NAA; Visualisation, formal analysis, data curation; NNNR, MDCR; Methodology: NNNR, LP, CB, MDCR; Conceptualisation, resources: MZM, HM; Writing-review and editing: LP, CB; MDCR; Supervision: MDCR. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors declare no conflicts of interest.

Funding: The article publication charge was funded by Management and Science University (MSU) Malaysia, Grant No: SG-009-022022-FHLS.

Acknowledgement: The authors are extremely grateful to all faculty members and laboratory staff from Management and Science University for their assistance and cooperation. This project is supported by Management and Science University.

Ethical statement: Ethical approval for this study was obtained from the Institutional Human Research Ethics Committee (Approval No.: EA-L3-01-FHLS-2024-12-0038), and all participants provided written informed consent

REFERENCES

Ahmed, H., Khan, M.A., Ali Zaidi, S.A., Muhammad, S. 2021. In silico and in vivo: Evaluating the therapeutic potential of kaempferol, quercetin, and catechin to treat chronic epilepsy in a rat model. *Front Bioeng Biotechnol* 9: 754952. https://doi.org/10.3389/fbioe.2021.754952

Al-Hatamleh, M.A.I., Boer, J.C., Wilson, K.L., Plebanski, M., Mohamud, R., Mustafa, M.Z. 2020. Antioxidant-based medicinal properties of stingless bee products: Recent progress and future directions. *Biomolecules* **10**(6): 923. https://doi.org/10.3390/biom10060923

Azwan, N.Z., Okunsai, B., Hein, Z.M., Che Mohd Nassir, C.M.N., Che Ramli, M.D. 2025. Neuroregenerative effects of Clitoria ternatea in sciatic nerve crush injury: Morphological, morphometric, and functional analysis in rat model. *Transl Res Anat* **40**: 100414. https://doi.org/10.1016/j.tria.2025.100414

Che Ramli, M.D., Sumari, N.A., Selamat, N.W., Muhammad, H., Sanusi, J. 2020. Effectiveness of flaxseed oil on peripheral nerve regeneration following crush injury of sciatic nerve in rat: Behavioural analysis and an electron microscopic study. *Malays J Med Health Sci* **16**(suppl 1). 106-11.

Di Meo., S., Reed, T.T., Venditi, P., Victor, V.M. 2016. Role of ROS and RNS sources in physiological and pathological conditions. *Oxid Med Cell Longev* **2016**: 1245049. https://doi.org/10.1155/2016/1245049

Faul, F., Erdfelder, E., Buchner, A., Lang, A.G. 2009. Statistical power analyses using G* Power 3.1: Tests for correlation and regression analyses. *Behav Res Methods* 41(4): 1149-60. https://doi.org/10.3758/BRM.41.4.1149

Hou, Y., Wang, K., Wan, W., Cheng, Y., Pu, X., Ye, X. 2018. Resveratrol provides neuroprotection by regulating the JAK2/STAT3/PI3K/AKT/mTOR pathway after stroke in rats. *Genes Dis* **5**(3): 245-55. https://doi.org/10.1016/j.gendis.2018.06.001

Ja'afar, N.L., Mustapha, M., Mohamed, M., Hashim, S.A. 2024. A review of post-stroke cognitive impairment and the potential benefits of stingless bee honey supplementation. *Malays J Med Sci* **31**(3): 75-91. https://doi.org/10.21315/mjms2024.31.3.5

Jones, T.A. 2017. Motor compensation and its effects on neural reorganization after stroke. *Nat Rev Neurosci* **18**(5): 267-80. https://doi.org/10.1038/nrn.2017.26

Kamal, F.Z., Lefter, R., Jaber, H., Balmus, I.M., Ciobica, A., Iordache, A.C. 2023. The role of potential oxidative biomarkers in the prognosis of acute ischemic stroke and the exploration of antioxidants as possible preventive and treatment options. *Int J Mol Sci* **24**(7): 6389. https://doi.org/10.3390/ijms24076389

Kiprono, S.J., Mengich, G., Kosgei, J., Mutai, C.., Kimoloi, S. 2022. Ethnomedicinal uses of stingless bee honey among native communities of Baringo County, Kenya. *Scientific African* **17**: e01297. https://doi.org/10.1016/j.sciaf.2022.e01297

Koppula, S., Hemant, K., More, S.V., Byung W.K., In S.K., Dong K.C. 2012. Recent advances on the neuroprotective potential of antioxidants in experimental models of parkinson's disease. *Int J Mol Sci* **13**(8): 10608-29. https://doi.org/10.3390/ijms130810608

Li, J., Dai, X., Zhou, L., Li, X., Pan, D. 2021a. Edaravone plays protective effects on LPS-Induced microglia by switching M1/M2 phenotypes and regulating NLRP3 inflammasome activation. *Front Pharmacol* **12**: 691773. https://doi.org/10.3389/fphar.2021.691773

Li, Z., Wang, H., Xiao, G., Du, H., He, S., Feng, Y., Zhang, B., Zhu, Y. 2021b. Recovery of post-stroke cognitive and motor deficiencies by Shuxuening injection via regulating hippocampal BDNF-mediated Neurotrophin/Trk Signaling. *Biomed Pharmacother* **141**: 111828. https://doi.org/10.1016/j.biopha.2021.111828

Liu, X., Zhang, M., Liu, H., Zhu, R., He, H., Zhou, Y., Zhang, Y., Li, C., Liang, D., Zeng, Q., Huang, G. 2021. Bone marrow mesenchymal stem cell-derived exosomes attenuate cerebral ischemia-reperfusion injury-induced neuroinflammation and pyroptosis by modulating microglia M1/M2 phenotypes. *Exp Neurol* **341**: 113700. https://doi.org/ 10.1016/j.expneurol.2021.113700

Long, X., Yao, X., Jiang, Q., Yang, Y., He, X., Tian, W., Zhao, K., Zhang, H. 2020. Astrocyte-derived exosomes enriched with miR-873a-5p inhibit neuroinflammation via microglia phenotype modulation after traumatic brain injury. *J Neuroinflammation* **17**(1): 89. https://doi.org/10.1186/s12974-020-01761-0

Luo, L., Li, C., Du, X., Shi, Q., Huang, Q., Xu, X., Wang, Q. 2019. Effect of aerobic exercise on BDNF/proBDNF expression in the ischemic hippocampus and depression recovery of rats after stroke. *Behav Brain Res* **362:** 323-31. https://doi.org/10.1016/j.bbr.2018.11.037

Mitjavila, M.T., Moreno, J.J. 2012. The effects of polyphenols on oxidative stress and the arachidonic acid cascade. Implications for the prevention/treatment of high prevalence diseases. *Biochem Pharmacol* **84**(9): 1113-22. https://doi.org/10.1016/j.bcp.2012.07.017

Morris, G.P., Wright, A.L., Tan, R.P., Gladbach, A., Ittner, L.M., Vissel, B. 2016. A comparative study of variables influencing ischemic injury in the longa and koizumi methods of intraluminal filament middle cerebral artery occlusion in

mice. *PLoS One* **11**(2): e0148503. https://doi.org/10.1371/journal.pone.0148503

Ooi, T.C., Yaacob, M., Rajab, N.F., Shahar, S., Sharif, R. 2021. The stingless bee honey protects against hydrogen peroxide-induced oxidative damage and lipopolysaccharide-induced inflammation in vitro. *Saudi J Biol Sci* **28**(5): 2987-94. https://doi.org/10.1016/j.sjbs.2021.02.039

Pakaprot, N., Khampaya, T., Kwankaew, P., Ninsuwan, S., Laisunthad, S., Thonoi, K., Kuraeiad, S. 2024. Neuroprotective effects of paederia foetida Linn. on scopolamine-induced cognitive impairment in rats. *Vet World* **17**(9): 1972-82. https://doi.org/10.14202/vetworld.2024.1972-1982

Pimentel, T.C., Rosset, M., Sousa, J.M.B., Oliveira, L.I.G., Mafaldo, I.M., Pintado, M.M.E., Souza, E.L., Magnani, M. 2022. Stingless bee honey: An overview of health benefits and main market challenges. *J Food Biochem* **46**(3): e13883. https://doi.org/10.1111/jfbc.13883

Sardooi, A.R., Reisi, P., Yazdi, A. 2020. Protective effect of honey on learning and memory impairment, depression and neurodegeneration induced by chronic unpredictable mild stress. *Physiol Pharmacol* **25**(1): 21-35. http://dx.doi.org/10.32598/ppj.25.1.90

Shaikh, A., Ahmad, F., Teoh, S.L., Kumar, J., Yahaya, M.F. 2024. Unveiling the therapeutic potential of kelulut (stingless bee) honey in Alzheimer's disease: Findings from a rat model study. *Antioxidants* **13**(8): 926. https://doi.org/10.3390/antiox13080926

Shen, H., Wu, X., Zhu, Y., Sun, H. 2013. Intravenous administration of achyranthes bidentata polypeptides supports recovery from experimental ischemic stroke in vivo. *PLoS One* **8**(2): e57055. https://doi.org/10.1371/journal.pone.0057055

Shi, G., Feng, J., Jian, L.Y., Fan, X.Y. 2023. DNA hypomethylation promotes learning and memory recovery in a rat model of cerebral ischemia/reperfusion injury. *Neural Regen Res* **18**(4): 863-8. https://doi.org/10.4103/1673-5374.353494

Shi, X., Bai, H., Wang, J., Wang, J., Huang, L., He, M., Zheng, X., Duan, Z., Chen, D., Zhang, J., Chen, X., Wang, J. 2021. Behavioral assessment of sensory, motor, emotion, and cognition in rodent models of intracerebral hemorrhage. *Front Neurol* 12: 667511. https://doi.org/10.3389/fneur.2021.667511

Tao, T., Liu, M., Chen, M., Luo, Y., Wang, C., Xu, T., Jiang, Y., Guo, Y., Zhang, J.H. 2020. Natural medicine in neuroprotection for ischemic stroke: Challenges and prospective. *Pharmacol Ther* **216:** 107695. https://doi.org/10.1016/j.pharmthera.2020.107695

Umbar, G.S.A., Mohamed Kasturi, F.E., Li, A.R., Zainul Abidin, N.H.K., Muhammad, H., Jazuli, I., Kumari, Y., Zulkapli, A., Che Ramli, M.D. 2024. Therapeutic potential of alkaloid-rich mitragyna speciosa extract in mptp-induced zebrafish parkinson's disease model. *Med Health* **20**(2): 510-24. https://doi.org/10.17576/MH.2025.2002.06

Xu, L., Gao, Y., Hu, M., Dong, Y., Xu, J., Zhang, J., Lv, P. 2022. Edaravone dexborneol protects cerebral ischemia reperfusion injury through activating Nrf2/HO-1 signaling pathway in mice. *Fundam Clin Pharmacol* **36**(5): 790-800. https://doi.org/10.1111/fcp.12782

Yao, X., Yang, W., Ren, Z., Zhang, H., Shi, D., Li, Y., Yu, Z., Guo, Q., Yang, G., Gu, Y., Zhao, H., Ren, K. 2021. Neuroprotective and angiogenesis effects of levetiracetam following ischemic stroke in rats. *Front Pharmacol* **12:** 638209. https://doi.org/10.3389/fphar.2021.638209

Yu, L., Zhang, Y., Chen, Q., He, Y., Zhou, H., Wan, H., Yang, J. 2022. Formononetin protects against inflammation associated with cerebral ischemia-reperfusion injury in rats by targeting the JAK2/STAT3 signaling pathway. *Biomed Pharmacother* **149:** 112836. https://doi.org/10.1016/j.biopha.2022.112836

Zhang, L., Li, X., Wolfe, C.D., O'Connell, M.D., Wang, Y. 2021. Diabetes as an independent risk factor for stroke recurrence in ischemic stroke patients: an updated meta-analysis. *Neuroepidemiology* **55**(6): 427-35. https://doi.org/10.1159/000519327

Zhou, R, Yang, Z., Tang, X., Tan, Y., Wu, X. Liu, F. 2013. Propofol protects against focal cerebral ischemia via inhibition of microglia-mediated proinflammatory cytokines in a rat model of experimental stroke. *PLoS One* **8**(12): e82729. https://doi.org/10.1371/journal.pone.0082729

Zhu, T., Wang, L., Feng, Y., Sun, G., Sun, X. 2021. Classical active ingredients and extracts of Chinese herbal medicines: Pharmacokinetics, pharmacodynamics, and molecular mechanisms for ischemic stroke. *Oxid Med Cell Longev* 2021: 8868941. https://doi.org/10.1155/2021/8868941

Zlatanova, H.I., Georgieva-Kotetarova, M.T., Vilmosh, N.B., Kandilarov, I.K. 2022. Evaluation of the effect of cariprazine on memory and cognition in experimental rodent models. *Int J Environ Res Public Health* **19**(22): 14748. https://doi.org/10.3390/ijerph192214748

Zulkifli, N.A., Hassan, Z., Mustafa, M. Z., Azman, W. N.W., Hadie, S.N.H., Ghani, N., Mat Zin, A.A. 2023. The potential neuroprotective effects of stingless bee honey. *Front Aging Neurosci* **14**: 1048028. https://doi.org/10.3389/fnagi.2022.1048028