The Effect of 3-months Trigona Honey Supplementation and Associated Factors on the Progression of Mild Post-Ischaemic Stroke Cognitive Impairment at Hospital Universiti Sains Malaysia: A Unicentral Randomised Controlled Trial

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by

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List of Abbreviations

- 8-OHdG: 8-Hydroxydeoxyguanosine
- AD: Alzheimer's Disease
- AIS: Acute Ischaemic Stroke
- ANOVA: Analysis of Variance
- APG: Apigenin
- BBB: Blood-Brain Barrier
- BDNF: Brain Derived Neurotrophic Factor
- BM: Bahasa Malaysia
- CA: Cinnamic acid
- CA1: Cornuammonis 1
- CA2: Cornuammonis 2
- CA3: Cornuammonis 3
- CAA: Cerebral Amyloid Angiopathy
- CE: Cardioembolism
- CfA: Caffeic Acid
- Chy: Chrysin
- CI: Cognitive Impairment
- CMB: Cerebral Microbleed
- CoQ10: Co-enzyme Q10
- CRP: C-Reactive Protein
- Ct: Catechins
- CT: Computed Tomography
- CVL: Cerebrovascular Lesions

- DAMPs: Damage-Associated Molecular Patterns
- Df: Degrees of Freedom
- DN: Diastase Number
- DTI: Diffusion Tensor Imaging
- DWI: Diffusion-Weighted Imaging
- ERK: Extracellular Signal-Regulated Kinases
- FAB: Frontal Assessment Battery
- FBG: Fasting Blood Glucose
- fMRI: Functional Magnetic Resonance Imaging
- g/100 g: Grams per 100 Grams
- $GABA_AR$: γ-AminoButyric Acid A Receptors
- GA: Gallic Acid
- GH: Gelam Honey
- HbA_{1C}: Haemoglobin A_{1C}
- HDL-C: High-Density Lipoprotein Cholesterol
- HMF: Hydroxymethylfurfural
- HPA: Hypothalamic–Pituitary–Adrenal axis
- HUSM: Hospital Universiti Sains Malaysia
- ICC: Intraclass Correlation Coefficient
- ICH: Intracerebral Haemorrhage
- IFN: Interferon
- IHD/CDH: Ischaemic Heart Disease/ Coronary Heart Disease
- IL: Interleukin
- ITPR1: Inositol 1,4,5-Triphosphate Receptor Type 1

- Kcal: Kilo Calorie
- KF: Kaempferol
- KH: Kelulut Honey
- LAA: Large-Artery Atherosclerosis
- LACI: Lacunar Infarcts
- LADIS: Leukoaraiosis And Disability Study
- LDL: Low-Density Lipoprotein
- LDL-C: Low-Density Lipoprotein Cholesterol
- LTD: Long-Term Depression
- LTP: Long-Term Potentiation
- M: Mean
- MCI: Mild Cognitive Impairment
- MDA: Malondialdehyde
- mg/Kg: Milligram per Kilogram
- MH: Manuka Honey
- mmHg: Millimetres Mercury
- mmol/l: Millimoles per Litre
- MMSE: Mini-Mental State Examination
- MoCA: Montreal Cognitive Assessment
- MoCA-BM: Montreal Cognitive Assessment (Bahasa Malaysia)
- mPFC: Medial Pre-Frontal Cortex
- MRI: Magnetic Resonance Imaging
- mS/cm: Millisecond per Centimetre
- NIHSS: National Institutes of Health Stroke Scale
- NMDA: N-Methyl D-Aspartate

- nmol/l: Nanomole per Litre
- OCSP: Oxfordshire Community Stroke Project
- ODA: Stroke of Other Determined Aetiology
- PACI: Partial Anterior Circulation Infarct
- POCI: Posterior Circulation Infarct
- PSCI: Post-Stroke Cognitive Impairment
- Qc: Quercetin
- RF: Rheumatoid Factor
- ROS: Reactive Oxygen Species
- r-TMS: Repetitive Transcranial Magnetic Stimulation
- SAH: Subarachnoid Haemorrhage
- SBH: Stingless Bee Honey
- SD: Standard Deviation
- SPSS: Statistical Package for Social Sciences
- SVO: Small-Vessel Occlusion
- T2DM: Type 2 Diabetes Mellitus
- T3: Triiodothyronine
- TC: Total Cholesterol
- TG: Triglycerides
- TH: Tualang Honey
- Th1: T-helper cell 1
- Th2: T helper cell 2
- TNF: Tumour Necrosis Factor
- TOAST: Trial of Org 101072 in Acute Stroke Treatment

- UDA: Stroke of Undetermined Aetiology
- VCI: Vascular Cognitive Impairment
- WML: White Matter Lesions

List of Symbols

α: Alpha

β: Beta

γ: Gamma

ε: Epsilon

λ: Lambda (Small)

Λ: Lambda (Capital)

η: Eta

κ: Kappa

r : Rho

n: Sample Size/ Frequency

N: Population Size

n_1: Adjusted Sample Size

d: Expected Drop-out Ratio

>: Greater Than

≥: Greater Than or Equal To

< : Smaller Than

≤: Smaller Than or Equal To

=: Equal To

≈: Almost Equal To

± : Plus or Minus

g: Grams

Abstract

Background: Affecting up to 80% of ischaemic stroke patients, post-stroke cognitive impairment (PSCI) is a complication that predicts poor quality of life and restricted activity and has also been associated with decreased independence and substantial caretaker and economic burden.

Despite the wider attention directed towards stroke sequelae, PSCI remains a major post-stroke complication with limited therapeutic options. Based on current understanding of the pathogenesis of PSCI, Trigona honey makes an attractive therapeutic option, as it can act on numerous neuropathological substrates implicated in PSCI's pathogenesis via the action of multiple polyphenols.

Objectives: To investigate possible benefits of Trigona honey supplements in halting progression of mild PSCI in acute ischaemic stroke patients admitted to Hospital Universiti Sains Malaysia (HUSM). The study also investigated the effect of demographic and clinical characteristics, stroke severity, and pathophysiological subtype and vascular territory on the progression of PSCI.

Methods: The study was conducted at Neurology Ward and Specialist Clinic at HUSM, following a unicentral, randomised, concurrent active treatment controlled, open-label superiority trial with two parallel groups model. The interventional group included participants who were administered supplemental honey in addition to standard care regiments, while those in the control group solely continued the standard therapeutic protocol.

48 acute ischaemic stroke patients between the ages of 20 and 70 years old and suffering from mild PSCI were recruited and equally randomised between the two groups. Patients with impeded ability to answer the neuropsychological assessments

or are suffering from compromised cognitive functions prior to the stroke were excluded.

Two honey sachets were administered for daily oral consumption for the duration of 12 weeks, with a total daily dose of 20 g. Demographic data and clinical characteristics questionnaire, MoCA, and NIHSS were used for data collection. Data collected was analysed using SPSS software.

Results and Discussion: Difference in mean gain in MoCA score between the two groups after three months was statistically significant ($p = 0.007$), with the interventional group having greater mean gain by 3 points. No statistically significant relationship between demographic and clinical characteristics, stroke parameters, and 3-months progression of PSCI was detected, possibly due to small sample size. The only exception was an improved cognitive function in current smokers in the interventional group ($p = 0.01, \beta = 0.514$), possibly due to smoking cessation.

Conclusion: Trigona honey supplements were effective in halting progression of PSCI, and smoking can affect 3-months progression of PSCI. However, stroke severity, TOAST and OCSP classifications had no effect on 3-months progression of PSCI.

While conclusions from this study can be helpful in guiding hypotheses about the employment of Trigona honey in the therapy for PSCI, as well as the different factors and predictors influencing the trajectory of PSCI, they must first be confirmed in a larger population and in homogenous groups.

Key Words: Post-Stroke Cognitive Impairment, Honey, Stingless Bee, MoCA, NIHSS, TOAST, OCSP

Chapter 1: Introduction

1.1 Research Background

A stroke occurs when cerebral blood supply is disturbed, whether due to vessel blockage (ischaemic strokes) or rupture (haemorrhagic strokes), leading to focal deficits in neurological function (Hui et al.; 2022; Tadi & Lui, 2022; Unnithan & Mehta, 2020). While the majority of the approximately 13.7 million stroke cases recorded annually around the world are of ischaemic origin (Khaku & Tadi, 2020; Kuriakose & Xiao, 2020), both ischaemic and haemorrhagic incidents contribute to stroke's status as the global second-leading cause of mortality, and third-leading of combined death and disability, as per Feigin *et al.* (2022).

Various physical and cognitive sequela are associated with cerebrovascular accidents, including mobility issues, pain and fatigue, and depression and anxiety (Xu et al., 2021). Improvements in stroke survival rates have shifted more focus to such complications, including ones that are often secondary in attention to the more apparent physical debilities, such as post-stroke cognitive impairment (PSCI) (Obaid et al., 2020b).

PSCI is a member of a spectrum of cognitive disorders of cerebrovascular aetiology known collectively under the blanket term of vascular cognitive impairments (VCI) (Rundek et al., 2021). Disorders on this spectrum can range from mild cognitive impairment to frank dementia, and they can impact one or more of numerous cognitive faculties, such as language, attention, memory, orientation, or executive function faculties (Al-Qazzaz et al., 2022; Rundek et al., 2021).

PSCI can be defined as a novel impairment in cognitive faculties that is observed in the first 90 days post-stroke, with a minimum duration of six months, and cannot be attributed to another disease or condition (Hashim et al., 2022). While the severity of PSCI exists along a continuum among stroke patients (Lo et al., 2019), it is possible to establish a classification based on the number and type of cognitive domains impaired, as per Corbo *et al.,* (2023) and Dong *et al.,* (2012)

Accounting for variations in diagnostic tools and criteria and ethnic and sociocultural differences, studies from different countries report that PSCI can impact between 20% to 80% of ischaemic stroke patients (Baraka et al., 2023; Kim et al., 2022).

The diagnosis of PSCI relies on the use of neuropsychological tests (Rost et al., 2022), however, there is a lack of consensus on the best approach to assess cognitive profile post-stroke (He et al., 2023). The two most commonly employed screening tools of PSCI are the Montreal Cognitive Assessment (MoCA) and the Mini-Mental State Examination (MMSE), which are favoured for their broad scope of assessment and ease and swiftness of administration (Dautzenberg et al., 2020; Gallegos et al., 2022; Khaw et al., 2021). However, their usage in stroke settings have been criticised due to their vulnerability to stroke-intrinsic impairments, as well as the influence of different sociocultural parameters on diagnostic cut-off scores and interpretation of the tests (Khaw et al., 2021; Spencer et al., 2022). Neuroimaging techniques such as magnetic resonance imaging (MRI), computed tomography (CT), and functional magnetic resonance imaging (fMRI) have also been investigated as a diagnostic tool for PSCI (Ball et al., 2021; Kim et al., 2022; Rost et al., 2022), and several biomarkers are being studied as potential diagnostic targets (Kim et al., 2022; Rost et al., 2022).

PSCI can be caused by all subcategories of ischaemic strokes, including large vessel occlusion and small vessel disease, as well as by intracerebral and subarachnoid haemorrhages (Rost et al., 2022). However, the exact mechanism by which PSCI occurs remains to be elucidated, and several pathways and processes have been implicated in the pathogenesis of the disease (Aam et al., 2020; Hashim et al., 2022). These include lesions and injury to the white matter (Rost et al., 2022), neuroinflammation, oxidative damage (Zhang & Bi, 2020), apoptosis and cerebral atrophy (Chi et al., 2023; Rost et al., 2022), reduced neurotrophic factors (Sui et al., 2021; Mojtabavi et al., 2022), and disturbance of major neurotransmitter systems, the blood-brain barrier (BBB), and synaptic plasticity (Chi et al., 2023; Geranmayeh, 2022; Gupta et al., 2022; Rost et al., 2022; Wang et al., 2022).

Research also suggests that preclinical presence of Alzheimer's disease (AD) predisposes stroke patients to a higher cognitive impairment and dementia risk (Rost et al., 2022). Indeed, multiple pathological elements have been detected in both disorders, including cerebral amyloid angiopathy (Rost et al., 2022), exacerbated plaques' aggregation (Rost et al., 2022), cerebrovascular lesions (Gupta et al., 2022; Rost et al., 2022), BBB dysfunction (Hussain et al., 2021), and impaired amyloid clearance and cholinergic transmission (Battle et al., 2021; Rost et al., 2022). This mechanistic intersection of PSCI and AD could have inspired the use of AD therapies, such as cholinesterase inhibitors, in the treatment of PSCI (Battle et al., 2021; Chi et al., 2023), but they remain hindered by their modest and inconsistent effect on global function and daily living activities (Battle et al., 2021; Du et al., 2020), and the side-effects associated with their long-term use (Liu et al., 2023). Additionally, non-invasive methods such as cognitive rehabilitation and repetitive administration of transcranial magnetic stimulation (r-TMS) have also been

employed as potential therapies, but they are limited by various shortcomings (Liu et al., 2023).

As such, preventative measures for PSCI are a top area of interest and research (Obaid et al., 2020a). This approach is encouraged by the better understanding and greater clinical efficacy of primary preventative techniques compared to tertiary therapeutic regimens, which are difficult to establish in patients with severe cognitive impairment (Hashim et al., 2021; Ritter & Pillai, 2015).

Multiple strokes, number of infarcts, and lesion volume have also been correlated to a higher risk for dementia and exacerbated cognitive decline (Rost et al., 2022). Additionally, several stroke characteristics, such as severity and pathophysiological subtype, are other parameters capable of influencing cognitive dysfunction following a stroke (Aam et al., 2020; Boutros et al., 2022; Drozdowska et al., 2020; Hashim et al., 2022). Given this well-established correlation between strokes and progression of cognitive decline (Hachinski et al., 2019; Rost et al., 2022), secondary prevention of strokes and amelioration of associated risk factors, particularly modifiable ones, is a key step in the management of PSCI and prevention of dementia (Drozdowska et al., 2020; Du et al., 2020; He et al., 2023).

In addition to vascular risk factors, such as Diabetes Mellitus, smoking, and hypertension (Boutros et al., 2022; Lo et al., 2019), demographic factors such as age and gender are other additional factors that can affect the progression of PSCI (Boutros et al., 2022). Proper management of vascular risk factors following a stroke has also been suggested to offer a long-term protection of cognitive functions (Mellon et al., 2015). According to Ministry of Health (Malaysia) (2020a), ischaemic heart disease, hypertension, Diabetes Mellitus, hyperlipidaemia, and smoking are

among the most common modifiable ischaemic stroke risk factors among Malaysians (Ministry of Health (Malaysia), 2020a).

Preventative measures for ischaemic stroke include lifestyle changes, such as diet modifications and cessation of smoking habits, although these are undermined by the lack of controlled trials on a large scale (Chi et al., 2023). Pharmacological interventions employed in stroke prevention, including anti-coagulants and anti-hypertensive agents, can also be useful (Hachinski et al., 2019; He et al., 2023).

Possible benefits of herbal supplements such as Ginkgo Biloba have also been investigated, with promising potential for the prevention and amelioration of PSCI (Chi et al., 2023). This encourages the investigation of other compounds, such as honey, in the same capacity (Ranneh et al., 2021).

Since ancient times, honey has been incorporated into both medicinal and beauty products (Kowalczuk et al., 2023; Zammit Young & Blundell, 2023). Modern laboratory techniques have revealed the broad-spectrum antibacterial, antiviral, and antifungal behaviour of honey (Al-Kafaween et al., 2023), as well as its potential in the preservation of cardiovascular health and as an anticancer agent, among other benefits (Kowalczuk et al., 2023; Zammit Young & Blundell, 2023).

It has also been demonstrated that honey dietary supplements have numerous desirable neurological and neuroprotective effects, including memory-enhancing and learning-promoting qualities (Zamri et al., 2023), pain combating effects (Zamri et al., 2023), antidepressant and anti-anxiety behaviour (Zamri et al., 2023), as well as anti-apoptotic, antioxidant, and anti-inflammatory effects (Che Mohd Nassir et al., 2022; Zamri et al., 2023).

Such health benefits are attributed to the action of bioactive molecules, mainly phenolic acids and flavonoids, which belong to the polyphenol family (Al-Kafaween et al., 2023; Iftikhar et al., 2022). These bioactive constituents can be found in varying percentages in different honey types (Martinez-Armenta et al., 2021).

Stingless Bee Honey (SBH) is the name given to the variety of honey that is produced by any of the more than 600 species of the *Meliponini* tribe of the *Apidae* bee taxonomical family (Toledo-Hernández et al., 2022; Zulkifli et al., 2023). One of the largest genera of stingless bees (Grüter, 2020), and in fact the most common in Southeast Asia (Hashim et al., 2021), is that of Trigona, with over 30 of its species found in Malaysia (Hashim et al., 2022; Zulkifli et al., 2023). As such, SBH and Trigona honey, in addition to Kelulut honey, Meliponine honey, and pot-honey, are terms that can be used interchangeably in reference to the same compound (Che Mohd Nassir et al., 2022; Hashim et al., 2022; Zulkifli et al., 2023).

In comparison to honeybee honey, SBH is characterised by greater moisture content, higher invertase activity, higher free acidity, and a lower pH value and diastase activity, in addition to an abundance of potassium cations (Hashim et al., 2022; Zulkifli et al., 2023). It also offers a higher and more varied polyphenolic content, and thus greater antioxidant activity, since the two have been statistically correlated (Hashim et al., 2022; Zulkifli et al., 2023).

1.2 Problem Statement

Despite the wider attention directed towards sequelae in the wake of improved stroke survival rates, PSCI remains one of the major post-stroke complications that still suffer from limited therapeutic options, with the currently available measures

hindered by numerous deficiencies. Thus, the absence of an effective therapy for PSCI necessitates further research.

1.3 Rationale and Study Significance

With approximately 13.7 million cases annually, strokes are a leading cause of deaths worldwide (Kuriakose & Xiao, 2020). While improved access to therapy and the implementation of preventative strategies and mitigation of risk factors may have contributed to the observed decrease in global stroke mortality and the improvement of functional outcomes (Ilic & Ilic, 2022; Tian et al., 2023), strokes remain a major cause of physical and cognitive disability, where it is estimated that 75% of survivors suffer from a dysfunction following a stroke, and that up to 30% become severely disabled (She et al., 2022; Yao et al., 2021). Among the most common complications, occurring in up to 80% of patients, is PSCI (Bao et al., 2023; Liu et al., 2023).

The risk for cognitive impairment is reported to be heightened at least five to eight times following a cerebrovascular accident (Zhang & Bi, 2020), and that 20% to 30% of patients go on to develop dementia (Bao et al., 2023). Furthermore, it has been demonstrated that PSCI is a frequent presence, even in patients who have successfully recovered clinically (Lin et al., 2022). Obaid *et al.* (2020b) also noticed that PSCI forecasts a higher risk of dependency, compromised mental health, institutionalisation, death, and overall poor long-term outcomes, including diminished ability of affected individuals to resume their professional and daily lives (Koren et al., 2023), as well as a heightened risk for stroke recurrence and post-stroke morbidity (Cramer et al., 2022; Tian et al., 2023). PSCI was also linked to a triple increase in healthcare costs of stroke patients and a substantial burden on the

caregivers (Liu et al., 2023), as well as poor life quality and restricted activity (Chi et al., 2023).

Despite the wider attention directed towards sequelae in the wake of improved stroke survival rates (Obaid et al., 2020b), and a 10% reversibility rate in stroke patients after one year (Kim et al., 2020), PSCI remains one of the major post-stroke complications that still suffer from limited therapeutic options, with the currently available measures hindered by numerous deficiencies, such as long term side-effects or variance of effectiveness among patients (Liu et al., 2023). Thus, the absence of an effective therapy for PSCI necessitates further research.

Based on the current understanding of the major injury mechanisms involved in the pathogenesis of PSCI (Hashim et al., 2022), honey makes an attractive therapeutic option to halt the progression of the cognitive impairment.

A review of literature (Zamri et al., 2023) strongly supports honey's therapeutic potential, with evidence of its cognitive effects observed in studies of various human and animal populations, including postmenopausal women, ovariectomised and stressed rats, schizophrenic patients, elderly subjects with mild cognitive impairment or depression, and patients suffering from major neurocognitive disorders (Azman & Zakaria, 2019; Zamri et al., 2023). More pertinently, at least two animal studies- both in rats suffering from chronic cerebral hypoperfusion- demonstrate honey's potential for neuroprotective action in ischaemic brain injuries (Che Mohd Nassir et al., 2022). Additionally, an early study investigating the neuroprotective potential of honey illustrated its benefits against the development of dementia, as reflected by the small

percentage of dementia cases among patients administered honey supplements (Che Mohd Nassir et al., 2022).

The cognitive benefits associated with honey, including memory enhancing effects and neuroprotective actions (Zamri et al., 2023), have been attributed to the numerous bioactive constituents of honey, mainly the multiple flavonoids and phenolic acids (Al-Kafaween et al., 2023; Zamri et al., 2023; Zulkifli et al., 2023), which possess notable antioxidant and anti-inflammatory effects (Zulkifli et al., 2023).

In addition to decreasing oxidative stress and the production of reactive oxygen species (ROS), antioxidant activity can also contribute to the amelioration of inflammatory responses through the inhibition of the activity of several pro-inflammatory enzymes (Zulkifli et al., 2023). This is augmented by the anti-neuroinflammation qualities of the flavonoids, which lowers the generation of free radicals as well as pro-inflammatory cytokines (Zamri et al., 2023; Zulkifli et al., 2023). Further neuroprotective qualities of honey include its potential to minimise apoptotic signals through the action of multiple polyphenolic compounds, enhancement of cholinergic transmission, protecting neuronal cells from free radicals-mediated damage, as well as its contribution to synaptic plasticity and neurogenesis via its acting on Brain Derived Neurotrophic Factor (BDNF) pathways (Hashim et al., 2022; Zamri et al., 2023; Zulkifli et al., 2023).

Oxidative stress, neuroinflammation, cerebral atrophy, and dysfunction of synaptic connectivity are among the numerous processes implicated in the pathogenesis of PSCI (Chi et al., 2023; Hashim et al., 2022; Rost et al., 2022; Zhang & Bi, 2020), and as such, it could be hypothesised that honey can help halt the progression of

cognitive impairment by targeting multiple neuropathological substrates along the PSCI pathological cascade, as well as by promoting learning and memory (Hashim et al., 2022).

Since such benefits are due to the actions of the bioactive polyphenols, their higher concentrations and variety in Trigona honey makes this variety of honey a strong candidate in potential therapy for PSCI (Hashim et al., 2021, Hashim et al., 2022; Zulkifli et al., 2023). Utilisation of SBH is further encouraged by its ease of access and cultivation, owing to the bees' stingless nature (Zulkifli et al., 2023), resistance to disease (Pimentel et al., 2021), and their amenability towards indiscriminate colony locations and artificial hives (Pimentel et al., 2021), which further encourages wider investigation of the honey's quality standard profile, bioactive constituents, and therapeutic potential, as well as its possible role in the context of PSCI (Hashim et al., 2021; Zamri et al., 2023; Zulkifli et al., 2023).

However, there is a distinct lack of studies on the potential of SBH in ischaemic brain injuries and associated cognitive decline in human subjects (Che Mohd Nassir et al., 2022; Zulkifli et al., 2023), and thus a lack of knowledge on the potential benefits of honey in promoting functional recovery of cognitive function post-stroke or the halting of PSCI.

As such, this interventional study aimed to investigate the possible benefits of Trigona honey supplements in halting the progression of mild PSCI in acute ischaemic stroke patients admitted to Hospital Universiti Sains Malaysia (HUSM) by comparing MoCA scores prior to and following a three-months supplementation period. The study also investigated the correlation between the progression of PSCI

and multiple demographic parameters, clinical factors, and stroke characteristics, due to their influence the progression of cognitive impairment (Boutros et al., 2022).

The study's focus on mild PSCI, as well as the specification of the intervention period as three months, was due to the evidence in literature to the benefits of early cognitive rehabilitative intervention on improving cognition post-stroke (Xuefang et al., 2021), as well as the difficulty of establishing tertiary therapeutic regiments in patients with severe cognitive impairment (Hashim et al., 2021). Moreover, it has also been demonstrated that the greatest improvement in cognitive function occur in the first 90-days post-stroke (Yap et al., 2021). Recovery of neurological function after a stroke has also been shown to plateau after three months (Grefkes & Fink, 2020; He et al., 2023), and that there is a limited time window of heightened neuroplasticity following a cerebrovascular event (He et al., 2023).

While PSCI can be caused by both ischaemic and haemorrhagic strokes (Rost et al., 2022), this study recruited participants only from the former category to allow for more accurate analysis, due to reports of variance in the cognitive domains affected by ischaemic and haemorrhagic PSCI (Elrewainy et al., 2023). Furthermore, focusing only on ischaemic patients allows the generalisation of the study's findings to 85% of stroke patients, compared to the 15% contributed by haemorrhagic strokes (Barzegar et al., 2021).

Findings from this study can help encourage the inclusion of Trigona honey in therapeutic approaches for PSCI, as well as further research on a larger scale. Additionally, understanding the influence of different clinicodemographic and stroke parameters on the development of PSCI can help in understanding the effects of the different contributors, which can be useful in the creation of prognostic models for PSCI with higher precision (Drozdowska et al., 2020), and thus the development of

more effective care and management protocols. Increased interest in SBH in countries with considerable stingless bee population, such as Malaysia, can also raise its commercial profile, thus promoting SBH related industries and employment opportunities (Hashim et al., 2021).

1.4 Operational Definitions

1.4.1 Acute Ischaemic Stroke (AIS)

As per the Stroke Council of the American Heart Association/American Stroke Association consensus statement (Sacco et al., 2013), an ischaemic stroke is an episode of neurological dysfunction induced by focal cellular death in the brain, spinal cord, or retina. Evidence of ischaemic injury in a specific vascular distribution- as obtained through imaging, pathological, or other objective methods, diagnoses the cellular death as the result of the injury. Persistence of the symptoms that cannot be attributed to another cause for at least 24 hours or until death is another clinical proof of ischaemic injury.

1.4.2 Ischaemic Heart Disease (IHD)

IHD, also known as coronary heart disease (CHD), is insufficient myocardial perfusion, mainly due to atherosclerotic blockage of the coronary arteries, leading to myocardial ischaemia (Severino et al., 2020). According to the Ministry of Health of Malaysia, IHD is among the top modifiable stroke risk factors among the Malaysian population (Ministry of Health (Malaysia), 2020a).

1.4.3 Hypertension

As per the 5th edition of the clinical practice guidelines for hypertension, published by the Malaysian Ministry of Health in 2018, hypertension is defined as at least two separate measurements of a persistently elevated systolic blood pressure at 140

mmHg or more and/or elevated diastolic blood pressure at 90 mmHg or more (Ministry of Health (Malaysia), 2018).

1.4.4 Hyperlipidaemia

Hyperlipidaemia, also known as dyslipidaemia, has been diagnosed using the criteria detailed in Table 1.0, as borrowed from the 5th edition of the clinical practice guidelines for dyslipidaemia management, published by the Malaysian Ministry of Health in 2017 (Ministry of Health (Malaysia), 2017). LDL-C values were based on assessment using the Framingham General Cerebrovascular Disease Risk Score.

Table 1.0

Diagnostic Values for Hyperlipidaemia, as per Ministry of Health (Malaysia) (2017)

Parameter	Value (mmol/l)		
Total Cholesterol (TC)	> 5.2		
High Density Lipoprotein Cholesterol (HDL-C)	Male		Female
	< 1.0		< 1.2
Triglycerides (TG)	> 1.7		
Low Density Lipoprotein Cholesterol (LDL-C)	Low & Moderate Cardiovascular Risk	High Cardiovascular Risk	Very High Cardiovascular Risk
	> 3.4	> 2.6	> 1.8

1.4.5 Diabetes Mellitus

Type 2 Diabetes Mellitus (T2DM) was diagnosed using the criteria detailed in Table 1.1, as specified by the Ministry of Health (Malaysia) (2020b).

Table 1.1

Diagnostic Values for T2DM, as per Ministry of Health (Malaysia) (2020b)

Parameter	Value
Fasting Plasma Glucose (FBG)	≥ 7.0 mmol/l
2-hours Oral Glucose Tolerance Test	≥ 11.1 mmol/l
HbA_{1C}	≥ 45 mmol/mol

One abnormal value was used for the diagnosis in symptomatic individuals, while the diagnosis in asymptomatic ones was confirmed using two abnormal values (HbA_{1c}+ glucose measurement), or a repeated blood glucose test on a different day (Ministry of Health (Malaysia), 2020b). Poor control of T2DM have been correlated with poorer cognitive performance (Alkethiri et al., 2021).

1.4.5.1 Hyperglycaemia

According to the Malaysian Endocrine & Metabolic Society (2020), hyperglycaemia can be defined as blood glucose levels higher than 10mmol/L. Hyperglycaemia has been demonstrated to be associated with cognitive decline due to the effect it has on neuronal cells, leading to abnormalities of white and grey matter structures, as well as atrophied cerebral volume and vascular lesions (Gupta et al., 2023).

1.4.6 Smoking Status

Following the classification set by Matsuo *et al.*, (2020), the patients were categorised as either:

- Former smokers: Patients who stopped smoking for more than 6 months before the onset of the stroke.
- Non-smokers: Patients who have never smoked.
- Current smokers: Patients who were active smokers at the time of the stroke onset.

1.4.7 Post-Stroke Therapy

In addition to thrombolysis, antiplatelet medication, and statin, information about the following post-stroke medications was collected using the demographic and clinical characteristics questionnaire (Appendix B):

- Anticoagulants
- Anti-diabetics
- Anti-hypertensive

1.4.8 Stroke Severity

The severity of each patient's stroke was evaluated using the National Institutes of Health Stroke Scale (NIHSS). By assessing deficits in 15 neurological domains, NIHSS allows the quantification of stroke severity out of a total score of 42 (Comer et al., 2023; Dang et al., 2020). A stroke can be categorised as mild (NIHSS score 1-5 points), moderate (5-14 points), severe (15-24 points), or extremely severe (> 24) (Dang et al., 2020). The domains assessed include arm and leg motor function, best language and gaze, level of consciousness, limb ataxia, facial palsy, visual fields, response to sensory stimulus, inattention and extinction, and dysarthria (Dang et al., 2020).

1.4.9 Aetiological/ Pathophysiological Stroke Subtype

Using Trial of Org 101072 in Acute Stroke Treatment (TOAST) classification, the pathophysiology of the ischaemic strokes was categorised as one of five subtypes; large artery atherosclerosis, small vessel occlusion, cardioembolism, of other determined aetiology, or of undetermined aetiology (Simonsen et al., 2022).

1.4.10 Vascular Territory of Stroke/Clinical Stroke Subtype

On the basis of clinical presentation, the cerebral infarctions were subcategorised into four types using the Oxfordshire Community Stroke Project (OCSP) classification system, based on the vascular territories affected (Jose & James, 2022). The subtypes include (Jose & James, 2022):

- Lacunar Infarcts (LACI)
- Partial Anterior Circulation Infarcts (PACI)
- Posterior Circulation Infarcts (POCI)
- Total Anterior Circulation Infarcts (TACI)

1.4.11 Mild Post Stroke Cognitive Impairment (Mild PSCI)

Severity of PSCI was assessed using validated version of MoCA in the Malaysian language (Bahasa Malaysia) (Sahathevan et al., 2014), at both baseline evaluations and post-intervention. MoCA is a 30-point brief assessment of overall cognitive function (Davis et al., 2021), with high reliability and validity, as well as specificity and sensitivity (Gaete et al., 2022). Multiple cognitive domains can be assessed using MoCA, including language and naming, attention and memory, orientation, abstraction, and executive and visuospatial functions (Gaete et al., 2022). 26/30 points was used as a diagnostic cut-off for mild PSCI, following values that are often quoted in literature (Dautzenberg et al., 2019).

1.4.12 Trigona Honey

Trigona honey is a variety of honey obtained from any of the more than 600 species of the *Meliponini* tribe of the *Apidae* bee taxonomical family (Toledo-Hernández et al., 2022; Zulkifli et al., 2023). One of the largest genera of stingless bees (Grüter, 2020), and in fact the most common in Southeast Asia (Hashim et al., 2021) , is that of Trigona, with over 30 of its species found in Malaysia (Hashim et al., 2022; Zulkifli et al., 2023). As such, SBH and Trigona honey, in addition to Kelulut honey, Meliponine honey, and pot-honey, are terms that can be used interchangeably in reference to the same compound (Hashim et al., 2022; Zulkifli et al., 2023). In comparison to honeybee honey, SBH offers a higher and more varied polyphenolic content, and thus greater antioxidant activity, since the two have been statistically correlated (Hashim et al., 2022; Zulkifli et al., 2023), making Trigona honey an attractive therapeutic candidate.

1.4.13 Randomised Controlled Trial (RCT)

RCTs are considered the gold standard for clinically evaluating the efficacy of a novel treatment or interventional procedure. In such clinical trials, also referred to as interventional studies, the participants are randomly assigned to either the control or interventional arms of the trial as a measurement to reduce selection and confounders bias. In a parallel group design, the participants remain allocated to the same group for the entirety of the trial (Nair, 2019; Sil et al., 2019). Block randomisation is one randomisation approach that can help minimise significant inequalities in group allocation (Burger et al., 2020).

Another measure to reduce bias is blinding, whereas any of the various groups (participants, investigators, and data analysts) are made unaware of the treatments received by the participants. Depending on the number of groups blinded, a trial can

be classified as single, double, or triple-blinded. In contrast, open-label studies are designs where both the participants and investigators are aware of the intervention allocation (Sil et al., 2019).

The action of the tested intervention could be compared against multiple control modalities, including placebos, standard treatments and therapies (active treatment concurrent control), or different doses of the same treatment (Nair, 2019; Sil et al., 2019).

Clinical trials can be further categorised on the basis of the expected effect of the intervention when compared to the control modality, whereas a superiority trial predicts a significant difference in outcome between the control and interventional groups (Mahajan & Kishore, 2020). A trial is unicentral if it is fully conducted at a singular location, or multicentral if it involves more than one site (Meinert, 2014).

1.5 Research Questions

The aim of the study was to investigate the potential effects of Trigona honey supplements on the progression of PSCI, in addition to investigating the effects of clinical and demographic parameters and stroke burden on the progression of PSCI:

1. Is there a significant difference in the progression of PSCI between the control and interventional groups?
2. Is there a significant relationship between demographic characteristics, clinical factors, stroke parameters (severity, pathophysiological subtype, vascular territory), and the progression of PSCI?

1.6 Hypotheses

1.6.1 General Hypothesis

There is a change in the progression of PSCI in acute ischaemic stroke patients after 3-months of supplementary Trigona honey therapy.

1.6.2 Null Hypotheses

a. There is no statistically significant difference in the progression of PSCI between the control group and the interventional group after 3-months of Trigona honey supplements.
b. There is no statistically significant relationship between demographic characteristics, clinical factors, stroke parameters (severity, pathophysiological subtype, vascular territory), and the progression of PSCI.

1.6.3 Alternative Hypotheses

c. There is a statistically significant difference in the progression of PSCI between the control group and the interventional group after 3-months of Trigona honey supplements.
d. There is a statistically significant relationship between demographic characteristics, clinical factors, stroke parameters (severity, pathophysiological subtype, vascular territory), and the progression of PSCI.

1.7 Objectives

1.7.1 General Objective

To investigate the effect of 3-months supplementary Trigona Honey therapy, and possible influence of demographic characteristics, clinical factors, and stroke parameters, on the progression of post-ischaemic stroke cognitive impairment.

1.7.2 Specific Objectives

1. To compare the progression of PSCI at baseline and 3-months between the control and interventional groups.
2. To determine the relationship between demographic characteristics, clinical factors, and the 3-months progression of PSCI.
3. To determine the relationship between stroke severity and the progression of PSCI.
4. To compare the effect of stroke pathophysiological subtypes and vascular territories on the 3-months progression of PSCI.

1.8 Summary

PSCI is a common stroke complication that is associated with poor life quality, decreased independence, higher caregiver and economic burden, and a higher risk for mortality. It remains without an effective treatment. Honey has been suggested as a potential therapeutic option, since it contains biologically active molecules, such as flavonoids and phenolic acids, which have antioxidant and anti-inflammatory effects. These compounds are capable on acting on numerous neurological processes implicated in the pathological cascade for PSCI, such as neuroinflammation, oxidative stress and free radical damage, and altered synaptic plasticity. The higher concentrations of these bioactive compounds in the Trigona honey variety, produced by stingless bees, encourages the hypothesis of its therapeutic effects in PSCI (Hashim et al., 2021). Findings from this study can help encourage the inclusion of Trigona honey in therapeutic approaches for PSCI, as well as further research on a larger scale. Additionally, understanding the influence of different clinicodemographic and stroke parameters on the development of PSCI can help in understanding the effects of the different contributors, which can be useful in the

creation of prognostic models for PSCI with higher precision (Drozdowska et al., 2020). Increased interest in SBH in countries with considerable stingless bee population, such as Malaysia, can also raise its commercial profile, thus promoting SBH related industries and employment opportunities (Hashim et al., 2021).

Chapter 2: Literature Review

2.1 Stroke

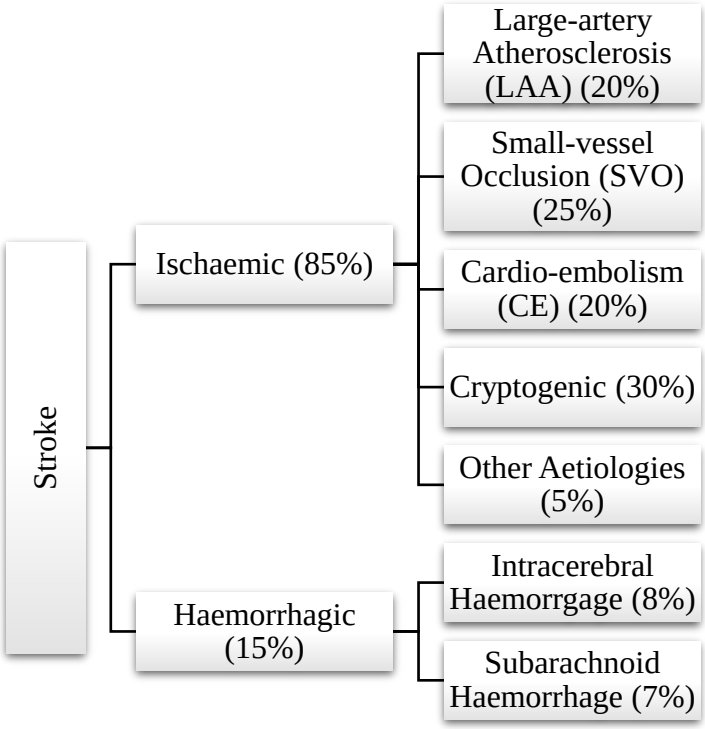
2.1.1 Introduction

As reported by the World Stroke Organization (Feigin et al., 2022), strokes remain recognised worldwide as the second-leading cause of mortality, with an approximated 5.5 million deaths each year (Kuriakose & Xiao, 2020), as well as the third-leading culprit behind combined death and disability. Strokes also exert a substantial economic burden, with an estimated global cost of over US$721 billion (Feigin et al., 2022).

Clinically, strokes can be defined as a vascular injury to the central nervous system that induces a syndrome of focal neurological deficits (Murphy & Werring, 2020). As illustrated in Figure 2.0 (Barzegar et al., 2021), a stroke can be broadly classified as either ischaemic or haemorrhagic, with either type further subclassified on the basis of pathophysiological mechanisms (Barzegar et al., 2021). At a percentage of 85%, ischaemic strokes account for the majority of the 13.7 million cases recorded annually worldwide (Barzegar et al., 2021; Kuriakose & Xiao, 2020), with the remaining percentage contributed by haemorrhagic strokes (Barzegar et al., 2021).

Fig. 2.0

Broad classification of strokes into ischaemic or haemorrhagic events, along with subcategorizations of each type according to pathophysiology, adapted from Barzegar et al., (2021)



Strokes are more prevalent in developing countries, with 75% of deaths and 81% of related disabilities recorded in countries with low and middle income levels (Kalkonde et al., 2020; Kuriakose & Xiao, 2020). In developed and high-income countries, and indeed globally, there has been a noticed decrease in stroke mortality and an improvement of functional outcomes, which have been attributed to improved access to therapy, implementation of preventative strategies, and mitigation of risk factors (Ilic & Ilic, 2022; Tian et al., 2023; Yi et al., 2020).

Risk factors for strokes can be categorised as either modifiable or non-modifiable ones (Nindrea & Hasanuddin, 2023). Age, gender, ethnicity, family history of cerebrovascular accidents, and genetic factors constitute the non-modifiable risk factors, while cardiovascular risk factors, including Diabetes Mellitus, high blood pressure and cholesterol levels, physical inactivity, unhealthy diet and obesity, stress and cardiovascular disease, and smoking, consumption of alcohol and drug use, as well as environmental factors such as air pollution, are considered among the modifiable ones (Cui & Naikoo, 2019; Drozdowska et al., 2020; Kuriakose & Xiao, 2020; Nindrea & Hasanuddin, 2023). The Malaysian Ministry of Health lists hypertension, Diabetes Mellitus, hyperlipidaemia, and smoking as the most common modifiable stroke risk factors among the Malaysian population (Ministry of Health (Malaysia), 2020a). Table 2.1 summarises modifiable and non-modifiable risk factors for both ischaemic and haemorrhagic (Parmar, 2018), while Figure 2.4 depicts a visual overview of the main management strategies for strokes (Kuriakose and Xiao, 2020).

Various physical and cognitive sequela are associated with cerebrovascular accidents, including mobility issues, pain and fatigue, and depression and anxiety (Xu et al., 2021). Improvements in stroke survival rates have shifted more focus to such complications, including ones that are often secondary in attention to the more apparent physical debilities, such as PSCI (Obaid et al., 2020b).