

ORIGINAL ARTICLE

The Use of *Camellia sinensis* to Lower Neuron Specific Enolase Level in Acute Ischemic Stroke

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ABSTRACT

Introduction: Stroke is one of the most commonly encountered non-communicable diseases. EGCG in *Camellia sinensis* demonstrates angiogenesis and neuroprotection effect if consumed on a daily basis. This study aim to determine the efficacy of *Camellia sinensis* in acute ischemic stroke. **Materials and methods:** A double-blind, randomized, placebo-controlled trial was conducted in November 2020 – August 2021. Subjects were all acute ischemic stroke patients presented to the emergency room during the recruitment period. Patients were then randomized into placebo or experimental group and followed up to 7 days of treatment. Both the NSE level and NIHSS score was measured on the first and seventh day of admission. **Results:** A total of 32 subjects (13 control, 19 experimental) is included in this study. In day-7, NSE level is significantly lower in the experimental group compared to the control group (17.05±21.72 vs 62.02±39.20 ng/mL; p<0.001). Odds ratio analysis reveals that the experimental group is 2.19 times more likely to have a normal NSE level in day-7 compared to the control group. The decrease of day-7 NIHSS score is also greater in the experimental group compared to the control group (-2.42±2.22 vs -0.62±0.77; p=0.012). In addition, the experimental group is 1.58 times more likely to have neurological improvement on day-7 compared to the control group. **Conclusion:** This study confirms the efficacy of *Camellia sinensis* to lower NSE level and improve neurological outcome in acute ischemic stroke patients. However, further studies with similar intervention are needed to confirm this finding in a wider population.

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Keywords: Acute ischemic stroke, *Camellia sinensis*, EGCG, Neuron specific enolase, Randomized controlled trial

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INTRODUCTION

Stroke is one of the most commonly encountered non-communicable diseases with a devastating impact in public health. It is estimated that someone has a stroke every 40 seconds, with 85.5% of the mortality occurs in developing nations (1). Cerebral ischemia constitutes 85% of all stroke, which is correlated with high morbidity and high treatment cost (2). A cerebral ischemia occurs when the nutrient and oxygen to the brain is disrupted either through thrombus formation or embolus blockage in the cerebral blood vessels. This may progress into increased apoptotic gene expression which leads to an irreversible neuronal cell death (3).

Inflammatory reaction may occur in the ischemic core and penumbra due to activation of microglial cells and circulating immune cells (4).

Neuron specific enolase (NSE) is one of the most common inflammatory biomarkers which is commonly found during events of neuronal cell death (5). Previous studies have demonstrated the correlation of NSE with several pathological diseases in the brain, such as traumatic brain injuries, neurodegenerative disorders, and cerebral ischemia (6,7). A persistently high level of NSE after 72 hours of cerebral ischemia onset correlates with increasing severity and poor neurological outcomes (8).

Green tea leaf (*Camellia sinensis*) is one of the most popular tea drinks among Asian population. It has high concentration of epigallocatechin-3-gallate (EGCG) with antiinflammation, antioxidant, anticarcinogenic,

and antimicrobial potency (9,10). *Camellia sinensis* also demonstrates angiogenesis and neuroprotection effect if consumed on a daily basis (11,12).

Stroke is a big part of public’s health burden, especially in non-communicable diseases sector. Researches are done to better known how to prevent, treat, and rehabilitate stroke patients. Currently, there is no published study that correlates *Camellia sinensis* consumption and NSE level in ischemic stroke patients. Therefore, this research was carried out to determine the efficacy of *Camellia sinensis* in ischemic stroke patients. Consumption of *Camellia sinensis* is expected to improve the neurological outcome of ischemic stroke patients.

MATERIALS AND METHODS

Study Material

We use *Camellia Sinensis* extract in the form of sachet powder (Meditea, Agaricus Sido Makmur Sentosa, Malang, Indonesia). The *camellia sinensis* extract sachet contained 50 mg of EGCG and 2 grams of maltodextrin. The placebo sachet consisted of only 2 grams of maltodextrin. Both groups, control and experimental, were instructed to mix a sachet of powder with 50 mL of water and drink it before meal three times a day; two sachets in the morning, two sachets in the afternoon, and three sachets in the evening, for 7 days.

Study Design

This study was a randomized, double-blinded, placebo-controlled trial to evaluate the efficacy of *Camellia Sinensis* to lower NSE level in acute ischemic stroke patient. The sample recruitment period was conducted in November 2020 – August 2021. This study followed the Principles of the Declaration of Helsinki and had obtained ethical clearance from the Ethical Research Board of Airlangga University Hospital (number 176/KEP/2020). An independent team was established to monitor the safety data during this study and had regular meetings before the start of the trial, 50% enrollment milestone, and after the trial was completed.

Patients

All patients with acute ischemic stroke presented on the emergency room of two private hospital during the recruitment period were eligible for this study. The inclusion criteria of this study were 1) Age 18-70 years old, 2) Having first attack of acute ischemic stroke as demonstrated by imaging or clinical criteria, 3) Onset of stroke less than 48 hours, 4) National Institutes of Health Stroke Scale (NIHSS) of 4-18, and 5) Have a preserved swallowing function. Patients presenting with seizure, sepsis, dysphagia, COVID-19 infection, pregnancy, hypo- or hyperglycemia, had received intravenous thrombolytic agent, or had regular history of green tea consumption in the last 3 months were excluded from this study. During the study period, all patients who had

sepsis or did not adhere to the intervention protocol were also excluded from this study.

Upon admission to the emergency room, informed consent and information for consent form were obtained for each eligible subject. Participation in this study was voluntarily and the subject was allowed to withdraw from the study at any point of time. Each patient was given a random computer-generated ticket to be assigned into one of the two groups. This ticket will be exchanged in the pharmacy to receive either the placebo or green tea sachet for 7 days. The blood serum NSE level and NIHSS examination were conducted in the first and seventh day of this trial.

Data Analysis

The data obtained were validated and analyzed using SPSS Statistics for Windows version 23 (IBM Corp, Armonk, USA). Data were expressed either in frequency table of n (%) or mean±standard deviation. The NSE level and NIHSS score between the two groups were compared using Mann-Whitney U-Test or independent t-test. Risk estimates were presented as Odds Ratio (95% CI). A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 34 patients are enrolled in this study; 20 patients in experimental group and 14 patients in control group. During the study period, 2 patients—one from the control group and one from the experimental group—are excluded due to not completing the study protocol. The final sample in this study is 32 patients (19 experimental, 13 control) (Figure 1).

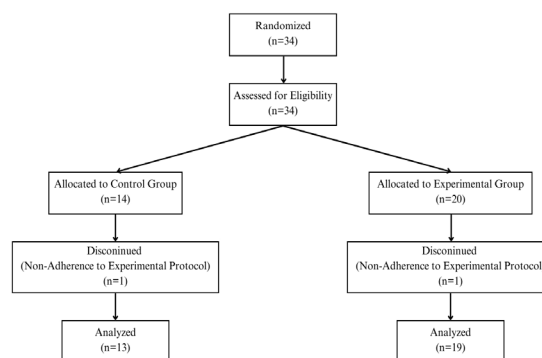


Figure 1: CONSORT 2010 Flow Diagram for Randomized Controlled Trial.

Demographic Data

The patients’ demographic data in this study is presented on Table II. The distribution of patients on the control and experimental group is considered homogenous. There is no significant difference in demographic data between the two groups. The mean age in this study is 56.66 years old. In addition, the most common comorbidities observed in this study is hypertension.

Table I: Demographic Characteristics Between Groups

Parameter	Total	Control	Experimental	p-value*
Male, n(%)	20 (62.5)	7 (53.8)	13 (68.4)	0.473 ^a
Age (Mean±SD)	56.66±9.55	56.46±10.61	56.79±9.05	0.926 ^b
Age (Range)	35-77	40-77	35-71	
Hypertension, n(%)	30 (93.8)	11 (84.6)	19 (100)	0.157 ^a
Diabetes Mellitus, n(%)	8 (25)	3 (23.1)	5 (26.3)	>0.999 ^a
Smoking, n(%)	12 (37.5)	4 (30.8)	8 (42.1)	0.713 ^a
Alcohol Use, n(%)	1 (3.1)	0 (0)	1 (5.3)	>0.999 ^a

*The p-value is calculated for control vs experimental. Using ^aFisher-Exact Test, ^bIndependent t-Test

Comparison of NSE Level between groups

There is no significant difference of baseline NSE level between the control and experimental group (52.15±48.58 ng/mL vs 48.65±65.28 ng/mL; Z=-0.921, p=0.362), which is considered ideal for comparison after intervention. However, we found that the day-7 NSE level is significantly lower in the experimental group compared to the control group (17.05±21.72 vs 62.02±39.20 ng/mL; Z=-3.703, p <0.001). Regarding to the NSE level change, we observe a decrease of 31.61±57.83 ng/mL on the experimental group and an increase of 9.88±55.31 ng/mL in the control group. This difference is considered statistically significant (Z=-3.703; p<0.001) with odds ratio 2.19 (CI 95% 1.27-3.80) (Table 2). The reference value of normal NSE level is ≤ 12 pg/mL.

Table II: The odds of day-7 normal NSE level and neurological improvement in the experimental group

Parameter	OR (95%CI)	p-value
Day-7 Normal NSE Level	2.19 (1.27-3.80)	0.008*
Day-7 Neurological Improvement	1.58 (1.12-2.23)	0.025*

*Significant for p<0.05

Comparison of NIHSS Score between groups

The NIHSS score is measured in the first and seventh day of admission. We observe no significant difference in the baseline (control 5.77±2.05 vs experimental 7.63±4.11, p=0.143) and day-7 NIHSS score (control 5.15±2.30 vs experimental 5.21±4.52; Z=0.579, p=0.563). However, we found a significant difference in NIHSS score change between control and experimental group (-0.62±0.77 vs -2.42±2.22; Z=-2.504, p=0.012) with odds ratio 1.58 (CI 95% 1.12-2.23) (Table II).

DISCUSSION

NSE is known as the biomarker for acute neuronal injury or inflammation, which is known to be elevated in ischemic stroke and traumatic brain injury (13). NSE is used as a prognostic biomarker in ischemic stroke patients (14-15). Although previous studies have demonstrated the beneficial effect of EGCG in neurodegenerative diseases, traumatic brain injury and intracerebral hemorrhage (16-18), there are no studies that have been conducted to analyze EGCG benefit

in ischemic stroke. This study aims to demonstrate the anti-inflammatory potency of EGCG in *Camellia sinensis* extract. We use the randomized controlled trial experimental design in order to provide more reliable result and minimize the study bias.

The patients' demographic data in this study is presented on Table 1. The distribution of patients on the control and experimental group is considered homogenous. There is no significant difference in demographic data between the two groups. The mean age in this study is 56.66 years old, similar to a previous study that showed increased risk of developing a stroke after the age of 55 (19). In addition, the most common comorbidities observed in this study is hypertension, which is similar to a previous study (20-21).

There is no significant difference of baseline NSE level between the control and experimental group (52.15±48.58 ng/mL vs 48.65±65.28 ng/mL; Z=-0.921, p=0.362), which is considered ideal for comparison after intervention. However, we found that the day-7 NSE level is significantly lower in the experimental group compared to the control group (17.05±21.72 vs 62.02±39.20 ng/mL; Z=-3.703, p <0.001), indicating EGCG beneficial effects to lessen neuronal injury or inflammatory reaction. Regarding to the NSE level change, we observe a decrease of 31.61±57.83 ng/mL on the experimental group and an increase of 9.88±55.31 ng/mL in the control group. This difference is considered statistically significant (Z=-3.703; p<0.001). The reference value of normal NSE level is ≤ 12 pg/mL. We found that the experimental group is 2.19 times more likely to have a normal NSE level in day-7 compared to the control group (Table II). Besides the severity of neuronal injury or inflammation, NSE level may also be affected by other factors such as gender, age, diabetes, and steroid use (6,7). One study hypothesized the role of EGCG in reducing oxidative stress and inflammation of the brain through the activation of Nrf-2 pathway and heme-oxygenase 1 in microglia (22, 23).

The NIHSS score is measured in the first and seventh day of admission. We observe no significant difference in the baseline (control 5.77±2.05 vs experimental 7.63±4.11, p=0.143) and day-7 NIHSS score (control 5.15±2.30 vs experimental 5.21±4.52; Z=0.579, p=0.563). However,

we found a significant difference in NIHSS score change between control and experimental group (-0.62 ± 0.77 vs -2.42 ± 2.22 ; $Z = -2.504$, $p = 0.012$). Neurological improvement is defined as improvement of NIHSS score of 4 or more points or having a zero NIHSS score in the 7th day. We found that the experimental group is 1.58 times more likely to have neurological improvement on day-7 compared to the control group (Table 2). This finding is similar to a previous study where EGCG may exert clinical and functional improvement due to its neuroprotective effect (24-25). However, these effects may be limited as it depends on the patients' initial NIHSS score and disease progression.

CONCLUSION

This study demonstrates the neuroprotective effect of EGCG from *Camellia sinensis* to improve the day-7 serum NSE level and neurological outcome in acute ischemic stroke patients. This finding can be beneficial in public health as a complimentary treatment in managing stroke patients. This study also confirms that acute ischemic stroke may lead to an increased NSE level within the first 24 hours of onset. Further studies using similar intervention, conducted in multiple centers, with additional parameters are needed to confirm the efficacy of *Camellia sinensis* in a wider population.

This study has several limitations. Firstly, the sample collection is only limited in one center and may not fully represent the heterogenous population of Indonesia. Secondly, the NSE level is analyzed from the patients' blood serum, which may have lower accuracy than using CSF analysis. Thirdly, we do not measure the level of EGCG in patient's serum which may result in variability of EGCG level between patients. Further studies with similar intervention are needed to confirm this finding in a wider population

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