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Potential effect of green tea extract for adjuvant treatment of acute ischemic stroke by S100 β upregulation in non-thrombolysis patient

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ABSTRACT

Introduction: In ischemic stroke, the cerebral cortex suffers from hypoxia-ischemia, leading to inflammation and oxidative stress. Green tea extract has an anti-inflammation effect and antioxidant. This study aimed to determine the efficacy of green tea extract for adjuvant treatment of acute ischemic stroke in non-thrombolysis patients.

Methods: A double-blind randomised controlled trial was conducted in November 2020-November 2021. The subjects were all acute ischemic stroke patients who presented to the Emergency Room during recruitment, randomised into control (n=13) and intervention groups (n=18); the intervention groups were given green tea extract 350 mg. Treatment was for 30 days. National Institutes of Health Stroke Scale (NIHSS), modified Rankin Scale (mRS), Montreal Cognitive Assessment - Indonesia (MoCAIna), IL-10 and S100 β were analysed.

Results: Data were compared with a significance level of p<0.05. The differences in NIHSS from day 0 to 7, day 0 to 14 and day 0 to 30 were statistically significant in the intervention group (p=0.019, p=0.002 and p=0.000, respectively). The mRS score was statistically significant in the intervention group on day 30 (p=0.46). The differences in mRS score from day 0 to 14 and day 0 to 30 were statistically significant (p=0.042 and p=0.001, respectively) The S100 β were statistically significant in day 7 (p=0.006). The difference in S100 β from day 0 to 7 was statistically significant (p=0.001).

Conclusions: The green tea extract, through up-regulation S100 β , can improve the clinical outcomes of acute ischemic stroke.

Keywords: acute ischemic stroke, EGCG, green tea extract, S100 β

Introduction

Stroke is the second leading cause of death and the first cause of disability worldwide (Caplan and Caplan, 2016; Powers et al., 2019). Thrombolysis using Intravenous Recombinant Tissue Plasminogen Activator (iv-rTPA) in 3-4,5 hours after stroke attack is the only

treatment approved by the Food and Drug Administration (FDA) (Che et al., 2019). Another drug class for acute stroke is neuroprotectant, but it has not been mentioned in AHA stroke guidelines since 2007 because of a lack of evidence. Searching for alternative treatment for acute stroke is ongoing. There are many



drug candidates for acute stroke, about 430 drug candidates for stroke treatment, and many of them have failed to show benefit in acute stroke patients.

Stroke is a complex event; it begins with decreased blood flow and causes energy depletion that will cause cell membrane impairment. Ischemic brain tissue can cause neuronal cells to secrete some Danger Associated Molecular Patterns (DAMPs); one of the DAMPs is S100 β (Michetti et al., [2012](#)). S100 β is normally very low, and the event is not detectable; its level will be only detected during certain pathological conditions. Serum S100 β represent infarct volume in stroke patients (Einav et al., [2012](#)). It suggests that the blood-brain barrier is leaked during ischemic stroke, so its level will increase in the serum (Nash, Bellolio and Stead, [2008](#)). Ischemic brain tissue can also induce inflammatory pathways. IL-10 is an anti-inflammatory cytokine (Arponen et al., [2015](#)). Increased IL-10 concentration may have a neuroprotective effect, according to some research. Spera et al. ([1998](#)) said that administration of IL-10 in the MCAO model significantly reduces infarct volume and percent hemisphere infarct.

Green tea is the second most common drink in the world. It has polyphenols that have some benefits for health. One of its polyphenols is Epigallocatechin-3-gallate (EGCG) which has a potent antioxidant effect. EGCG has an anti-inflammation effect and prevents cell death during ischemic events (Singh, Mandal and Khan, [2016](#); Zhang et al., [2017](#)). An epidemiological study in 2019 showed that green tea consumption of > 1 cup daily can prevent a cerebrovascular event, and in a patient who has had a cerebrovascular event, those who routinely consume green tea have better outcomes compared to those who do not (Lee and Kim, [2019](#)). Previous animal model studies show that either EGCG or green tea extract can prevent necroptosis and apoptosis. It can decrease RIP3 expression in the MCAO model compared to the control MCAO. It also decreases inflammatory markers. EGCG and green tea extract can inhibit Caspase-3, a proapoptotic protein, and increase the expression of the anti-apoptotic protein, BCL-2 (Machin, Susilo and Purwanto, 2021).

Based on previous research, there is need to conduct clinical research to determine the efficacy of green tea extract for adjuvant treatment of acute ischemic stroke in non-thrombolysis patients.

Materials and Methods

The study has received permission from The Research Ethics Committee of Universitas Airlangga

Hospital which examined and approved study procedure with the certificate number 176/KEP/2022 on 8 September, 2020. Anwar Medika General Hospital and Siti Khodijah Islamic Hospital have agreed and given permission to include the study ethic process from Airlangga Hospital.

Study design

This study is a double-blind, randomised controlled trial to know the effect of green tea extract on acute ischemic stroke. Study participants were recruited from November 2020 to November 2021 and included 31 acute ischemic stroke patients. The study was conducted at Universitas Airlangga Hospital, Anwar Medika General Hospital, and Siti Khodijah Islamic Hospital. Since the research was conducted during the Covid-19 pandemic and the same doctor handled the patients, we chose these three hospitals since it was simpler to collect samples there.

Selection of patients

Total sampling is used in the sampling method. The sample size was determined based on the total sample acquired from November 2020 to November 2021. The study participants were divided into two groups. The first group consisted of acute ischemic stroke patients who received a placebo, while the second group consisted of acute ischemic stroke patients who received green tea extract.

To be included in this study, the subject must have a first-time stroke, the onset of the stroke is less than 24 hours, age 18-70 years, and the first NIHSS score is 4-18. NIHSS score of less than 4 is considered a mild stroke, whereas a score of more than 18 is considered a severe stroke. There may be an ethical issue with research when the score is higher than 18, which has a significant mortality risk. Because of this, we adopt NIHSS 4-18, which is considered a moderate stroke. The exclusion criteria are seizure at stroke onset, sepsis, blood sugar at ER <70 mg/dl or >450 mg/dl, patient with dysphagia, and patient Covid-19 positive. The subject will be dropped from this research if they get sepsis or has bad compliance during research.

Randomisation and treatment

Each eligible subject was given informed consent and information for consent forms at the time of admission to the emergency room. Participation in this study was completely voluntary, and the subject was allowed to withdraw from it at any time. Each patient received a randomly generated computer ticket assigned to one of the two groups. This ticket will be exchanged in the pharmacy for either a placebo or green tea sachet for 30

Table 1. Characteristics of the subjects

Characteristic	n (%)	Mean	Intervention(n=18)		Control (n=13)		P value
			n (%)	Mean	n (%)	Mean	
Gender							0.291
Male	20 (64.5)		13 (72.2%)		7 (53.8%)		
Female	11 (35.5)		5 (27.8%)		6 (46.2%)		
Age group (years)		56.48		56.50		56.46	
Education							0.635
Elementary school	14 (45.2)		7 (38.9%)		7 (53.8%)		
Junior high school	4 (12.9)		3 (16.7%)		1 (7.7%)		
Senior high school	13 (41.9)		8 (44.4%)		5 (38.5%)		
Profession							0.653
Does not work	10 (30.3)		6 (33.3%)		4 (30.8%)		
Housewife	8 (24.2)		4 (22.2%)		4 (30.8%)		
Labourer	5 (15.2)		2 (11.1%)		3 (23.1%)		
Merchant	2 (6.1)		1 (5.6%)		1 (7.7%)		
Had Retired / Pensionary	2 (6.1)		2 (11.1%)		0		
Taxi bike	1 (3.0)		0		1 (7.7%)		
Security	1 (3.0)		1 (5.6%)		0		
Farmer	1 (3.0)		1 (5.6%)		0		
Driver	1 (3.0)		1 (5.6%)		0		
Race							0.388
Javanese	30 (96.8)		17 (94.4%)		13 (100%)		
Madurese	1 (3.2)		1 (5.6%)		0		
History of diseases							
Hypertension	29 (93.5)		18 (100%)		11 (84.6%)		0.085
Diabetes Mellitus type 2	6 (19.4)		3 (23.1%)		3 (16.7%)		0.656
Cardiac disease	2 (6.5)		2 (11.1%)		0		0.214
Hyper-cholesterol	1 (3.0)		1 (5.6%)		0		0.388
Atrial fibrilysis	0 (0)		0		0		-
Malignancy	0 (0)		0		0		-
Depression	0 (0)		0		0		-
Dementia	0 (0)		0		0		-
Risk factors							0.440
Smoking history	12 (38.7)		8 (44.4%)		4 (30.8%)		
Alcohol	1 (3.2)		1 (5.6%)		0		

days. On the first and seventh days of this study, blood was drawn. On the first, seventh, fourteenth, and thirty days of this research, the NIHSS, mRS score, and MoCAIna score were conducted.

We use a green tea extract from Meditea (BPOM 192233901), Agaricus Sido Makmur Sentosa, Malang, Indonesia, which comes in sachet powder form. Each sachet included 2 grams of maltodextrin and 50 milligrams of EGCG. We also provide a placebo regimen with the same components but only 2 grams of maltodextrin. For 30 days, each group was instructed to consume a sachet of powder diluted with 50 mL of water three times a day, two sachets in the morning, two sachets in the afternoon, and three sachets in the evening.

Outcome

The outcome of this research is the change of NIHSS, mRS score, and MoCAIna score from baseline at day 0, 7, 14, and 30t. The IL-10 and S100 β change from day 0 to 7th days.

Statistical analysis

We perform descriptive statistics for each variable and Kolmogorov-Smirnov for each variable to describe the normality of data. We perform an independent t-test if the distribution is normal, and we perform the Mann-Whitney test if the data is abnormal. We compare control and intervention groups for delta NIHSS between NIHSS score on day 0 and day 7, day 0 and day 14, day 0 and day 30. We also conduct a chi-square analysis for the comparing of NIHSS score of more than 2 on the observation day.

Results

Result should be presented continuously start from main result until supporting results. Our study was conducted from January to November 2021. Of the 31 patients, 20 were male, and 11 were female. The mean of patient's ages was 56.48. The patient's last education was 14 (45%) elementary school, four (12.9%) junior high school, and 13 (41.9%) senior high school. Ten patients (30.3%) did not work. The patients were divided into two races, 30 (96.8%) Javanese and 1 (3.2%) Madurese. Twenty-nine patients (93.5%) presented with hypertension, six patients (19.4%) suffered from diabetes mellitus type 2, two patients (6.5%) had cardiac disease, and one patient (3%) had hyper-cholesterol.

Table 2. NIHSS differences between the control group and the interventional group

Group		Median (Min-Max)	Kolmogorov-Smirnov	P-value
NIHSS Day 0	Control (n = 13)	6 (4-10)	0.005	0.186
	Intervention (n = 18)	8 (3-16)		
NIHSS Day 7	Control (n = 13)	4 (2-10)	0.021	0.984
	Intervention (n = 18)	4.5 (0-16)		
NIHSS Day 14	Control (n = 13)	4 (0-10)	0.010	0.650
	Intervention (n = 18)	3 (0-14)		
NIHSS Day 30	Control (n = 13)	3 (0-9)	0.013	0.242
	Intervention (n = 18)	2 (0-14)		
Delta NIHSS Day 0 to 7	Control (n = 13)	0 (0-2)	0.000	0.019
	Intervention (n = 18)	2.5 (0-8)		
Delta NIHSS Day 0 to 14	Control (n = 13)	1 (0-4)	0.012	0.002
	Intervention (n = 18)	4 (1-9)		
Delta NIHSS Day 0 to 30	Control (n = 13)	2 (0-4)	0.029	0.000
	Intervention (n = 18)	4.5 (1-9)		

Twelve patients (38.7%) had smoking history, and one patient (2%) consumed alcohol (Table 1).

NIHSS differences between the control group and the interventional group

Table 2 presents the NIHSS differences between the control and interventional groups. The NIHSS control groups were lower than the interventional group, except on days 14 and 30. The NIHSS days 14 and 30 seemed to be higher in the control group (4 (0-10) and 3 (0-9), respectively) than in the interventional group (3 (0-9) and 2 (0-14), respectively). There was no significant difference in NIHSS between groups for days 0,7,14, and 30 (p=0.186, p=0.984, p=0.650, p=0.242, respectively).

All the data of delta-NIHSS showed that the control group's median was lower than the interventional group's. All the delta-NIHSS (day 0 to 7, day 0 to 14, day 0 to 30) were found to be statistically significant (p=0.019, p=0.002 and p<0.001, respectively).

Improvement of NIHSS between the control group and the intervention group

Table 3 presents the improvement of NIHSS between the control and intervention groups. From day 0 to day 7, there was no NIHSS improvement in the placebo group, while the NIHSS of five patients (27.78%) improved in the intervention group. The relative risk was 8.105, which means the intervention group will improve 8.105 times compared to the placebo group. There is no significance for the intervention (p=0.058).

From day 0 until day 14, there were two patients (15.38%) with NIHSS improvement in the placebo group. While the NIHSS of 10 patients (55.56%) improved in the intervention group. The relative risk was 3.611, which means the intervention group will improve 3.611 times compared to the placebo group. There is no significance for the intervention (p=0.058).

From day 0 until day, w While the NIHSS score of 95% 12 patients (66.67%) improved in the intervention group. The relative risk was 4.333, which means the intervention group will improve 4.333 times compared to the placebo group. There is a significant result for the intervention (p=0.014).

Differences in mRS score between the control group and intervention group

Table 4 shows the differences in mRS scores between the control and intervention groups. The control group's average mRS score on day 0 was lower than the intervention group. These three groups (day 0, 7 and 14) had non-significant results (p=0.341, p=0.869, p=0.447, respectively). There was a significant difference in mRS day 30 between control and intervention groups (p=0.046).

Of the delta-mRS day 0 to 7, the control group's median was 0 (-1-0), and the intervention group's median was 0 (-1-3). There was no significance for the intervention (p=0.134). The mean delta-mRS for day 0 to 14 was -1 (-1-0) for the control group and -1 (-1-(-4)) for the intervention group (p=0.042). For day 0 to 30, the mean is -1 (-1 - 0) for the control group and 2 (-4-0) for the intervention group (p=0.001). The intervention for these two groups is found to be statistically significant

Differences in MoCAIna score between the control group and the intervention group

Table 5 shows the differences in MoCAIna scores between the control and intervention groups. The

Table 3. Improvement of NIHSS between the control group and the intervention group

		Group		RR (CI 95%)	P
		Control	Green Tea Extract		
NIHSS Improvement	Day 0 to 7	0 (0,00%)	5 (27,78%)	8,105 (0,487 – 134,843)	0,058
	Day 0 to 14	2 (15,38%)	10 (55,56%)	3,611 (0,945 – 13,793)	0,058
	Day 0 to 30	2 (15,38%)	12 (66,67%)	4,333 (1,162 – 16,157)	0,014*

Note : *p<0.05; **p<0.01; ***p<0.001

Table 4. Differences in mRS score between the control group and the intervention group

Group	Median (Min-Max)	Kolmogorov-Smirnov	p-value
mRS Day 0	Control (n = 13) 3 (2-4) Intervention (n = 18) 4 (2-4)	0.000	0.341
mRS Day 7	Control (n = 13) 2 (1-4) Intervention (n = 18) 2 (0-4)	0.003	0.869
mRS Day 14	Control (n = 13) 2 (1-4) Intervention (n = 18) 2 (0-4)	0.018	0.447
mRS Day 30	Control (n = 13) 2 (1-4) Intervention (n = 18) 1 (0-4)	0.001	0.046
Delta mRS Day 0 to 7	Control (n = 13) 0 (-1-0) Intervention (n = 18) 0 (-1-3)	0.000	0.134
Delta mRS Day 0 to 14	Control (n = 13) -1 (-1-0) Intervention (n = 18) -1 (-1-(-4))	0.000	0.042
Delta mRS Day 0 to 30	Control (n = 13) -1 (-1-0) Intervention (n = 18) -2 (-4-0)	0.001	0.001

median MoCAIna scores on days 7, 14 and 30 in the control group were 17 (9–26), 27 (13–30), and 18 (13–30), respectively. The median MoCAIna scores on day 7, 14 and 30 in the intervention group were 20 (7–27), 22.5 (7–28), and 23.5 (7–28), respectively. These three groups (days 7, 14 and 30) had non-significant results ($p=0.984$, $p=0.643$, $p=0.587$, respectively).

The median delta-MoCAIna day 7 to 14 in the control group was 1 (-1–7), and the intervention group was 1 (0–7) with no significant result ($p=0.933$). The median delta-MoCAIna day 7 to 30 in the control group was 1 (0–7) and the intervention group was 2 (0–9) with no significant result ($p=0.373$).

Differences in IL-10 level between the control group and the intervention group

Table 6 presents the differences in IL-10 level between the control group and the intervention group. The mean IL-10 day 0 in the control group was 0.339 (0.250002), and in the intervention group was 0.255 (0.160689) with no significant result ($p=0.264$). The median IL-10 day 7 in the control group was 0.235 (0.084–2.235), and in the intervention group was 0.318 (0.136–0.696) with no significant result ($p=0.123$). The delta-IL-10 day7-0 in the control and intervention groups were -0.040 (-0.450–1.150) and 0.143 (-0.400–0.600). The delta IL-10 had no significant result ($p = 0.157$).

Table 5. Differences in MoCAIna score between the control group and the intervention group

Group	Median (Min-Max)	Kolmogorov-Smirnov	p-value
MoCAIna Day 7	Control (n = 13) 17 (9-26) Intervention (n = 18) 20 (7-27)	0.012	0.984
MoCAIna Day 14	Control (n = 13) 27 (13-30) Intervention (n = 18) 22.5 (7-28)	0.016	0.643
MoCAIna Day 30	Control (n = 13) 18 (13-30) Intervention (n = 18) 23.5 (7-28)	0.029	0.587
Delta MoCAIna Day 7 to 14	Control (n = 13) 1 (-1-7) Intervention (n = 18) 1 (0-7)	0.000	0.933
Delta MoCAIna Day 7 to 30	Control (n = 13) 1 (0-7) Intervention (n = 18) 2 (0-9)	0.000	0.373

Differences in S100 β level between the control group and the intervention group

Table 7 presents the differences in S100 β level between the control and intervention groups. The mean S100 β day 0 in the control group was 2.49554 (2.0033259), and in the intervention group was 1.30733 (1.396559). There was no significance for the intervention ($p=0.084$). The mean S100 β day 7 in the control group was 1.11015 (0.706374), and in the intervention group was 2.67072 (2.031395). There was a significant difference in S100 β day 7 between the control and intervention groups ($p=0.006$). The delta-S100 β day7-0 in the control and intervention groups were -1.3854 (1.95609) and 1.3634 (2.04562). There was a significant difference in delta S100 β day 0 to 7 between the control and intervention groups ($p=0.001$).

Discussions

Green tea (*Camellia Sinensis*) has polyphenols that act as antioxidants to counteract oxidative stress, which is known to cause various neurodegenerative disorders and neuronal injuries. Polyphenols have an anti-inflammation effect and prevent cell death during ischemic events (Singh, Mandal and Khan, 2016; Zhang et al., 2017). Epigallocatechin-3-gallate (EGCG), epicatechin (EC), epigallocatechin (EGC), and (-) epicatechin-gallate are the four primary types of monomers found in each polyphenol (ECG). EGCG has the strongest biological activity. Tea has the largest EGCG concentration. An epidemiological study shows that green tea consumption can prevent a cerebrovascular event, and for patients who have a

Table 6. Differences in IL-10 level between the control group and the intervention group

Group		Kolmogorov-Smirnov	p-value
IL-10 Day 0 (mean±SD)	Control (n = 13)	0.339_(0.250002)	0.067
	Intervention (n =18)	0.255_(0.160689)	
IL-10 Day 7 (Median [Min-Max])	Control (n = 13)	0.235(0.084 – 2.235)	0.000
	Intervention (n =18)	0.318 (0.136 – 0.696)	
Delta IL-10 Day 0 to 7 (Median [Min-Max])	Control (n = 13)	-0.04 (-0.45 – 1.15)	0.025
	Intervention (n =18)	0.143 (-0.4 – 0.6)	

cerebrovascular event, the outcome in those who routinely consume green tea has better outcomes compared to those who do not. Nan et al. (2018) found that EGCG protects MCAO animal models by regulating the PI3K/AKT/eNOS signalling pathway. As a free-radical scavenger, EGCG can prevent oxidative damage to brain cells by pro-oxidant agents. According to several animal researches, EGCG improves mitochondrial function while reducing oxidative stress (Machin et al., 2021).

Stroke is a complex event that causes ischemic brain tissue. Thrombolysis is the first choice for treating acute ischemic stroke. However, it has narrow therapeutic windows. Because of that, not all patients can receive thrombolysis (Che et al., 2019). Another drug class for acute stroke is neuroprotectants, but it has not been mentioned in AHA stroke guidelines since 2007 because of a lack of evidence. Searching for alternative treatment for acute stroke is ongoing. There are many drug candidates for acute stroke, about 430 for stroke treatment, and many of them have failed to show benefit in acute stroke patients.

Our previous study in animal models shows that either EGCG or green tea extract can prevent necroptosis and apoptosis. It can decrease RIP3 expression in the MCAO model compared to the control MCAO. It also reduces inflammatory markers. EGCG and green tea extract can inhibit Caspase-3, a proapoptotic protein, and increase the expression of the anti-apoptotic protein, BCL-2 (Machin, Susilo and Purwanto, 2021).

Table 7. Differences in S100β level between the control group and the intervention group

Group	Mean (SD)	Kolmogorov-Smirnov	p-value
S100β Day 0	Control (n = 13) (2.0033259)	0.165	0.084
	Intervention (n =18) (1.396559)		
S100β Day 7	Control (n = 13) (0.706374)	0.148	0.006
	Intervention (n =18) (2.031395)		
Delta S100β Day 0 to 7	Control (n = 13) (1.95609)	0.133	0.001
	Intervention (n =18) (2.04562)		

This study was conducted from November 2020 until November 2021. A total of 31 stroke patients were enrolled in the study, considering this study was conducted during the COVID-19 pandemic. Of 31 patients, 20 patients (64.5%) were female, with a mean age of 56.48. Most of them have the risk factor of stroke, such as hypertension (93.5%), diabetes mellitus type 2 (19.4%), cardiac disease (6.5%), hyper cholesterol (3%) and smoking history (38.7%).

The National Institutes of Health Stroke Scale (NIHSS) measures the neurological deficit in stroke patients. In previous study, the NIHSS score significantly related to the clinical outcomes at three months after stroke attack (Sari Aslani, Rezaeian and Safari, 2020). In 1995, the National Institute of Neurological Disorders and Stroke (NINDS) study group reported that patients with acute ischemic stroke who received r-TPA within three hours after onset had no significant difference in neurological improvement at 24 hours as assessed by the NIHSS, compared with the placebo group. But the group given r-TPA had a favourable outcome as assessed by the NIHSS on three months follow-up ('Tissue Plasminogen Activator for Acute Ischemic Stroke', 1995). Aoki et al. (2013) and Sari Aslani, Rezaeian and Safari (2020) reported that patients with r-TPA treatment improved NIHSS score at three months after stroke onset 2020. Our present study shows the difference in NIHSS score between the control and intervention groups taking green tea extract in 30 days. The delta NIHSS on day 0-7, 0-14, and 0-30 were statistically significant, especially in the difference on day 0-30. The difference was more prominent in the intervention group than in the control group. The intervention group had an NIHSS improvement and was statistically significant on day 30 (CI 95% = 4.333; p-value = 0.014). Lim et al. (2010) found that treatment with EGCG improved forelimb function in the MCAO rat model at two weeks after stroke onset. The forelimb function is one of the categories in the NIHSS score.

The modified Rankin Scale (mRS) score is used to measure the disability outcome of stroke patients. The mRS score has been valuable in clinical outcomes when evaluated three months after stroke onset (Chalos et al.,

2020; Sari Aslani, Rezaeian, and Safari, 2020; ElHabr et al., 2021). Our study shows the intervention group had a lower mRS score, especially on day 30 and was more statistically significant than mRS score in the control group. The delta mRS scores on day 0-14 and 0-30 were found to be statistically significant. Elhabr et al. (2021) found the mRS score between 30 days and 90 days after stroke onset had changed significantly in two-thirds of patients ($\frac{1}{3}$ improved, $\frac{1}{3}$ unchanged, and $\frac{1}{3}$ worsened) with following treatment such as r-TPA, EVT, or both). It needs further study to have more follow-up time.

The Montreal Cognitive Assessment - Indonesia (MoCA-Iنا) score is an assessment instrument to determine the cognitive impairment in neurological patients (Abzhandadze et al., 2019). Our study found differences in MoCA-Iنا scores between both groups, but they were not statistically significant. This condition may be because the patients only had 30 days of follow-up. MoCA-Iنا score can predict post-stroke cognitive impairment progression at 3,6, and 12 months (with accuracy > 90%) (Chiti and Pantoni, 2014; Sitepu, Loebis and Husada, 2022).

IL-10 is an anti-inflammatory cytokine (Arponen et al., 2015). Increased IL-10 concentration may have a neuroprotective effect, according to some research. Spera et al. (1998) found that administration of IL-10 in the MCAO model significantly reduces infarct volume and percent hemisphere infarct. Our study shows no difference in the IL-10 between the control and intervention groups. It shows that green tea extract doesn't influence the level of IL-10 in stroke patients. Previous study showed that green tea extract could improve the clinical outcome of stroke patients but not through the inflammatory pathways (Machin, Susilo and Purwanto, 2021). The mechanism of how ECGC inhibits inflammatory pathways is unclear (Ellis et al., 2011).

S100 β is a calcium-binding protein mainly in the cytosol of glial and Schwann cells (Nash, Bellolio and Stead, 2008; Einav et al., 2012). S100 β is a marker for neurological damage and blood-brain barrier dysfunction. S100 β can be detected at a low level in healthy individuals. Nash, Bellolio and Stead (2008) found that S100 β is a marker of acute brain ischemic and significantly increased after stroke onset. S100 β peak level is 12 to 120 hours after the neural damage (Nash, Bellolio and Stead, 2008). S100 β interacts with RAGE and can release damage-associated molecular pattern molecules (DAMPs) and other endogen molecules that participate in pro-inflammatory pathways (Michetti et al., 2012). Activation of RAGE causes neural death and increased reactive oxygen species (ROS) production (Rodrigues et al., 2013). Our study shows the difference

in S100 β between the intervention and control groups. The S100 β level in the intervention group was higher than the control group, and it was statistically significant on day 7. The delta S100 β day 0-7 was found to be statistically significant. The study result differed from Einav et al. (2012), which said patients with good outcomes had lower S100 β levels than poor outcomes patients after leaving hospital. However, Einav et al. evaluated the S100 β on day 0 and day 3, meanwhile our study did so on days 0 and 7. Other studies found S100 β has neuroprotective and neurotrophic effects in the nanomolecular concentration (Yardan et al., 2011; Rodrigues et al., 2013). The S100 β -induced proliferation and neuron formation of hippocampal progenitor cells can repair brain damage. The neurotrophic and gliotrophic actions of S100 β had essential roles in CNS development and recovery after brain injury (Willoughby et al., 2004; Yardan et al., 2011). Rodrigues et al. (2013) found the long-term increased nanomolar S100 β level did not promote astrogliosis but decreased hippocampal Glial fibrillary acidic protein (GFAP) content. GFAP is a marker of mature astrocytes or astroglial reactivity. The long-term increased nanomolar S100 β level correlated with the proliferation marker such as BrdU and Ki67. BrdU and Ki67 are effective content for measuring neurogenesis. They did not find any long-term increased S100 β level effect on RAGE expression. To interpret this finding, the administration of green tea extract in acute cerebral infarction patients increases the S100 β level in nanomolecular concentrations resulting in ischemic brain repair.

In summary, our study highlights the role of green tea extract in acute cerebral infarction through S100 β upregulation. This present study has improved clinical outcomes in acute cerebral infarction patients with green tea extract as assessed by the NIHSS, mRS, and MoCA-Iنا scores. This finding suggests that green tea extract is a promising stroke therapy.

This is the first study that reports the effect of green tea extract on acute cerebral infarction patients with a double-blind controlled trial in humans. The limitations of our study are the small number of patients and the short duration of the follow-up period, and the administration of green tea extract. Our study does not consider other factors, such as revascularisation conditions and risk factors for stroke. Further study is needed with a multicentre randomised control trial; long-term therapy, and follow-up (3-6 months); also considering the revascularisation condition.

Conclusion

Green tea (*Camellia Sinensis*) has polyphenols that act as antioxidants to counteract oxidative stress, which is known to cause various neurodegenerative disorders and neuronal injuries. This study found that acute ischemic stroke patients with green tea extract treatment have improved clinical outcomes as assessed by the NIHSS, mRS, and MoCA-Ina scores. The green tea extract with epigallocatechin-3-gallate (EGCG) increases S100 β expression. This approach suggests that green tea extract is a promising stroke therapy. Recommendation for further study is needed with multicentre randomised control trial; long-term therapy, and follow-up (3-6 months); also considering the revascularisation condition.

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Conflict of interest

All authors in this article declared no potential conflict of interest.

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