Chapter

Green Tea with Its Active Compound EGCG for Acute Ischemic Stroke Treatment

Abdulloh Machin and Shafira Putri Widiawan

Abstract

The current standard of treatment for acute ischemic stroke is thrombolysis. However, only less than 2% of the world undergo thrombolysis. Recent studies have shown that Citicholin, one of the popular neuroprotectants, is less effective as stroke therapy, so it is necessary to develop a new approach to protective therapy for ischemic stroke patients. Green tea (Camellia sinensis) is the most consumed beverage in the world and is a source of polyphenols known as catechins, including epigallocatechin-3-gallate (EGCG), which is 63% of total catechins. Many studies explain that green tea consumption will decrease stroke risk, but not many studies explain its benefit in treating acute stroke. This chapter will discuss the benefit of green tea in acute stroke. C. sinensis with the active ingredient EGCG inhibits neuronal cell death through apoptosis and necroptosis in acute ischemic stroke as in the Rattus norvegicus model of Middle Cerebral Artery Occlusion (MCAO), it also can decrease necroptosis and increase M2 type microglia. The study on the benefit of green tea should be conducted in the clinical setting to know the benefit of green tea in acute ischemic stroke. Its potential benefit can be an adjunct treatment for acute ischemic stroke besides standard treatment.

Keywords: green tea, Camellia sinensis, EGCG, acute stroke, stroke treatment

1. Introduction

According to WHO, stroke is a focal or global neurological deficit that more than 24 hours or dies before 24 hours, caused by vascular disorders in the brain [1–3]. There are three types of strokes: ischemic stroke, intracerebral hemorrhage, and spontaneous subarachnoid hemorrhage. Ischemic stroke is the most common type in about 70–80% of stroke cases [4]. Stroke is the second leading cause of death in the world and caused the death of 5.7 million people in 2005 [2, 5, 6]. Around 69% of stroke cases occur in low- and middle-income countries, about 71% of the 5.9 million stroke cases [7–9]. Most stroke patients will have a residual disability, although about 50–70% return to functional independence [10]. Based on this, it is necessary to understand the pathogenesis so that an acute ischemic stroke therapy approach can be carried out [6, 11].

The current standard of treatment for acute ischemic stroke is thrombolysis. However, only 2–8.5% of stroke patients can undergo thrombolysis in America, and less than 2% in the world undergo thrombolysis [2, 3, 12]. During 1995–2015, 430 candidates for stroke therapy were divided into two categories, namely thrombolytic agents and neuroprotectants [13–15]. One of the popular neuroprotectants used in stroke therapy is Citicholin. However, recent studies have shown that Citicholin is less effective as stroke therapy, so it is necessary to develop a new approach to protective therapy for ischemic stroke patients [16–20].

Acute stroke is caused by decrease blood flow, a decrease in the amount of Adenosine triphosphate (ATP). This event will cause lactic acidosis and loss of ion homeostasis in neuronal cells [21]. In addition, disruption of ion homeostasis will cause high levels of calcium and Adenosine diphosphate (ADP) in the cells, which will stimulate mitochondrial reactive oxygen species (ROS) and other sources of free radicals [22–24].

Green tea (*C. sinensis*) is the most consumed beverage in the world and is a source of polyphenols known as catechins, including epigallocatechin-3-gallate (EGCG), which is 63% of total catechins [25–31]. A meta-analysis showed that individuals who consumed 3 cups a day had a 21% lower risk of stroke than those who consumed <1 cup of tea daily [26, 32]. Many studies in animal models have shown that administering EGCG to ischemia–reperfusion brain tissue will reduce the expansion of ischemia [33, 34]. EGCG is also a potent free radical scavenger and can protect neuronal cells from oxidative damage induced by prooxidants [35]. Green tea with the active ingredient EGCG has a role in preventing neuronal cell death in ischemic conditions by inhibiting oxidative stress and improving mitochondrial function [25, 27, 28, 36]. Many studies explain that green tea consumption will decrease stroke risk, but not many studies explain the benefit of green tea in the treatment of acute stroke. This chapter will discuss the benefit of green tea in acute stroke [33, 37].

2. Pathophysiology of ischemic stroke

There is a decrease in blood supply which causes a reduction in the amount of ATP in acute ischemic stroke. This condition leads to anaerobic metabolism with the result of lactic acid. The decrease in blood flow also causes an imbalance in ion homeostasis in neuronal cells and leads ischemic cascade that will be followed by multimodal and multicellular mechanisms that cause neuronal cell death [6, 21].

Severe cerebral ischemia also causes loss of energy stores, ion imbalance, the release of excitatory neurotransmitters, and inhibition of glutamate re-uptake [6, 38]. In addition, glutamate will bind to NMDA and AMPA receptors which will cause calcium influx [1, 39]. This calcium overload will cause the stimulation of phospholipases and proteases that will degrade membranes and proteins [38]. Glutamate receptors also cause sodium and water influx and cause cell swelling, edema, and shrinkage of the extracellular space [6, 40]. In addition, the influx of excessive calcium causes the activation of catabolic processes that will activate proteases, lipases, and nucleases [1, 6, 39].

High calcium, sodium, and ADP levels in ischemic cells will stimulate the production of oxygen radicals in the mitochondria, accompanied by the production of free radicals from other sources such as prostaglandins and the degradation of hypoxanthine [39, 41]. These reactive oxygen species (ROS) will damage membrane lipids, proteins, nucleic acids, and carbohydrates [23, 42, 43]. Furthermore, these

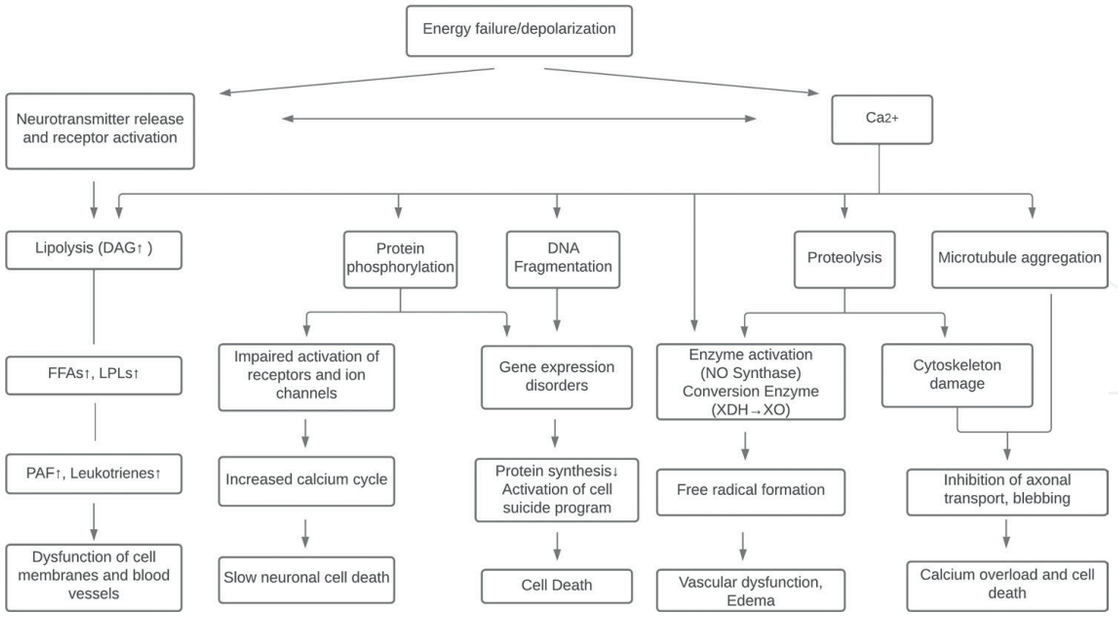

Figure 1.
Ischemic cascade in stroke [47].

ROS are toxic because their basal levels are related to the upregulation of antioxidant enzymes such as SOD, catalase, and glutathione and the scavenger mechanism (α-tocopherol, vitamin C), which is too slow to respond to the production of these ROS [6, 39]. Along with the above mechanism, there will also be a process known as Cortical spreading depression (CSD) which is the depolarization of neurons and glial cells that will spread to surrounding cells at a speed of 2–6 mm/minute [44]. CSD is characterized by an almost complete breakdown of the ion gradient associated with volume shrinkage, loss of electrical activity, swelling of neuron cells, and distortion of dendrites [43, 45]. CSD occurs when the extracellular K+ level exceeds a critical threshold [43]. This CSD wave in ischemic conditions will reach the peri-ischemic area and expand the infarct area (**Figure 1**) [6, 46, 47].

3. Ischemic stroke and oxidative stress

Uncontrolled oxidative stress, an imbalance between pro-oxidant and antioxidant levels that support pro-oxidants, can cause cell, tissue, and organ injury [22]. High Reactive Oxygen Species (ROS) are known to cause direct damage to lipids [43, 48]. The primary sources of endogenous ROS production are mitochondria, plasma membranes, endoplasmic reticulum, and peroxisomes through various mechanisms, including enzymatic reactions and/or autoxidation of several compounds, such as catecholamines and hydroquinones. In addition, different exogenous stimuli, such as ionizing radiation, ultraviolet light, tobacco smoke, pathogenic infections, environmental toxins, and exposure to herbicides/insecticides, are sources of ROS production in vivo [49].

The two most common ROS affecting lipids are hydroxyl radicals (HO) and hydroperoxyl (HO_2). The hydroxyl radical (HO) is a small, active, water-soluble, and chemically reactive oxygen species. This short-lived molecule can be produced from O_2 in cellular metabolism under various stress conditions [48, 50]. These radicals can be neutralized or even attack other biomolecules in the cell. Hydroxyl radicals cause

oxidative damage to cells because they are not determined by how much they attack biomolecules and are involved in cellular disorders such as neurodegeneration, cardiovascular disease, and cancer. It is generally assumed that HO in biological systems is formed through a redox reaction by the Fenton reaction; in this reaction, iron (Fe^{2+}) reacts with hydrogen peroxide (H_2O_2), and the Haber–Weiss reaction results in the production of Fe^{2+} when superoxide reacts with ferrous iron (Fe^{3+}). In addition to the iron redox cycle described above, several other transition metals, including Cu, Ni, and Co, can be responsible for forming HO in living cells [22, 48].

Heme oxygenase (HO) is a crucial enzyme of Heme metabolism. The HO-1 isoform is expressed mainly in vascular structures but is very low in normal CNS and can be induced after brain tissue injury. HO-1 is strongly induced after ischemia and will be overexpressed and play a protective role against ischemia after permanent vascular occlusion [6, 51–55].

An initial study to determine the role of HO-1 in ischemic conditions found that HO-1 will significantly reduce infarct volume. Mice that do not have HO-1 will have a larger infarct volume than the wild type [56–58]. Several materials induce HO-1 with promising results in preclinical studies. Some natural ingredients that activate the Nrf-HO-1 pathway, such as dimethyl fumarate, ginkgo biloba, curcumin, polyphenols, and terpenoids, will increase the neuroprotective effect on stroke models [56, 59, 60].

4. Green tea and antioxidant

Green tea is a traditional drink made from the Camellia sinensis tree, which is widely consumed in various countries, especially in Asia. Polyphenols from green tea, especially its active component, namely EGCG (epigallocatechin-3-gallate), have received more attention because they have potential therapeutic agents to prevent neurodegeneration, inflammatory diseases, and cancer [25, 61, 62]. The ability of green tea is mainly due to its antioxidant, free radical scavenger, metal chelation, anticancer, anti-apoptotic, and anti-inflammatory properties [26].

EGCG is roughly composed of four derivatives based on structural variations, including Epicatechin (EC), Epigallocatechin (EGC), Epicatechin gallate (ECG), and epigallocatechin-3-gallate (EGCG). EGCG consists of 10% dry green tea extract and about 50–80% or 200–300 mg of one cup of brewed green tea (**Figure 2**) [26, 63].

The metabolism of green tea polyphenols in the body has been widely studied. It is reported that green tea polyphenols are absorbed, distributed, metabolized, and excreted within 24 hours. In humans, when given 1.2 g of green tea that has been decaffeinated, within 1 hour, it will increase plasma levels by 46–268 ng/ml and excreted in the first 24 hours in the range of 1.6–3.2 mg. Therefore, drinking 6 cups of green tea a day will increase the concentration of green tea polyphenols by 12 times and will be sufficient for antioxidant activity against oxidative damage. These data are then supported by animal studies, where giving green tea 35 mg/kg/day will prevent oxidative damage and memory regression and can delay aging [6, 25, 52, 59, 63].

Free radicals, including ROS and nitrogen species such as NO, superoxide, and hydroxyl free radicals, are naturally produced to support the host defense system against oxidative stress and inflammation stimulated by pathogens and infections. Still, these species have two natural faces, namely, in the event of free radicals that the host produces. Excessive amounts in the body will cause destructive processes that cause DNA, protein, and lipid damage, leading to apoptosis and cell death [65, 66].

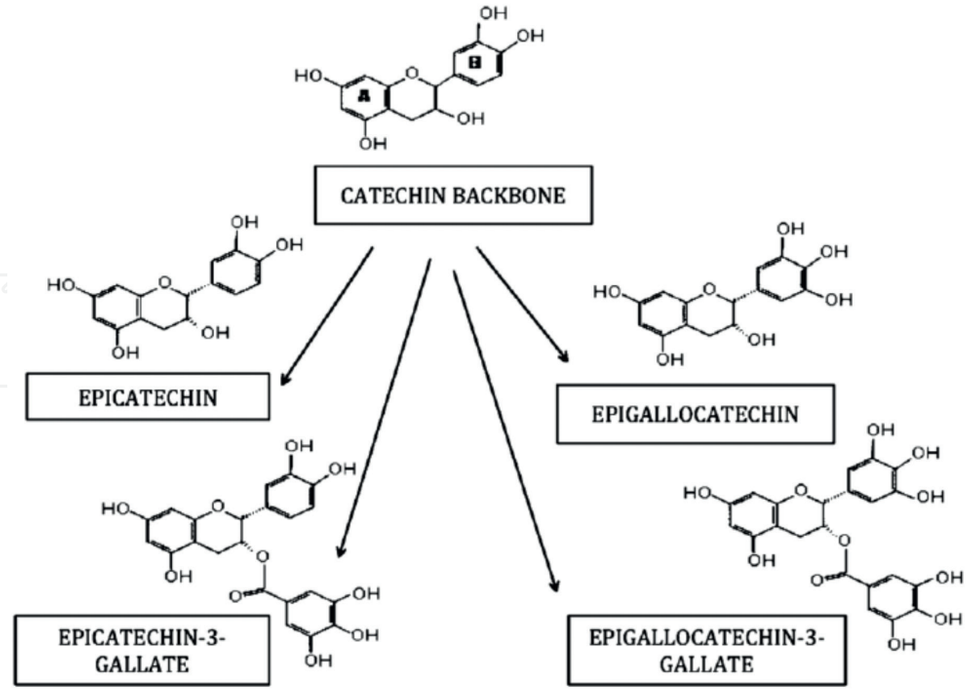

Figure 2.
The structure of green tea catechins and their four derivatives have antioxidant effects, namely Epicatechin, Epigallocatechin, Epicatechin-3-gallate, and epigallocatechin-3-gallate [26, 64].

Green tea polyphenol compounds are biological antioxidants that have a radical scavenger effect. Green tea contains two ingredients that have potent antioxidant properties, namely EGCG and ECG. This antioxidant ability is caused by the presence of ortho-trihydroxy groups in the B chain, 4-keto and 5-hydroxyl in the C chain, and galloyl moiety in the A chain. The difference in antioxidant activity in EGCG and ECG is very slight, which is related to each group's hydroxyl group. Therefore, these molecules can generally clean the radical group 1,1-diphenyl-3-picrylhydrazyl, as well as peroxyl radicals, NO, free fatty radicals, singlet oxygen, peroxynitrite, hydroxyl free radicals, and superoxide anions through three mechanisms, namely, by chelating the metal ion into an inactive form, direct interaction between catechin and peroxyl radicals through electron transfer to prevent DNA damage, and prevent free radical deamination by forming semi-quinone stable radicals [36, 61, 67].

EGCG is reported to be more effective as a radical scavenger when compared to vitamin E and vitamin C. When compared between green tea derivatives, EGCG (epigallocatechin-3-gallate) > ECG (Epicatechin gallate) > EGC (Epigallocatechin) > EC (Epicatechin) has a positive effect. Antioxidant, while EGC > EGCG>EC > EGC has a protective effect in vitro. The ability as a scavenger radical is due to the presence of ortho-3′,4′-dihydroxy moiety groups, or ortho-trihydroxy groups and is not based on steric structure. An increase in the number of hydroxyl groups will increase the strength of EGCG as a radical scavenger because of the presence of three hydroxyl groups in the B chain group and also consisting of galloyl moiety with three hydroxyl groups in the C chain [6, 26, 36, 68].

Oral administration of EGCG in vivo research showed a decrease in lipid peroxide levels by increasing levels of enzymatic and non-enzymatic antioxidants. EGCG can also completely reverse the effect of AlCl3 through its superoxide dismutase activity and by increasing glutathione peroxidase, Cyt-C oxidase, and acetylcholine esterase. The study aimed to see the impact of improving EGCG in rats and found

an improvement in the levels of enzymatic and non-enzymatic antioxidants in about 50% of malondialdehyde levels and a 39% decrease in protein carbonyl in both groups of rats. This effect was also obtained by reducing the dose from 100 to 2 mg/KgBW [26].

Consumption of green tea in humans also shows an increase in antioxidant levels in the body. Long-term consumption of green tea as much as 2–3 cups a day is reported to increase antioxidant activity and total polyphenols, accompanied by a decrease in lipid peroxide, glutathione and hydroperoxide levels. This shows that green tea polyphenols such as EGCG can directly or indirectly affect antioxidant levels to reduce oxidative stress [26, 31, 69].

Besides functioning as a radical scavenger, EGCG also has a chelating effect on heavy metals. Two structures give rise to this chelating effect, including ortho-3′,4′-dihydroxy moiety and 4-keto, 3-hydroxyl or 4-keto and 5-hydroxyl moiety groups. This structure serves as a binding point for heavy metal transitions. It neutralizes their activity by converting from the active form to an inactive redox complex and preventing oxidative damage to cells. In vitro studies using astrocyte cultures have demonstrated the ability of many flavonoids to diffuse, which is also supported by in vivo studies. Administration of EGCG orally for 5 to 10 days indicates the presence of these molecules in brain tissue; this shows the ability of EGCG to penetrate the blood–brain barrier [33, 36, 66, 70].

5. Green tea for stroke prevention

As already mentioned, polyphenols from green tea, especially its active component, namely EGCG, have received more attention because of their potential therapeutic agents for preventing neurodegeneration, inflammatory diseases, and cancer [63, 71]. The ability of green tea is mainly due to its antioxidant, free radical scavenger, metal chelation, anti-cancer, anti-apoptotic, and anti-inflammatory properties. In addition, research on EGCG has provided hope about its potential to improve health in old age by enhancing the morphological and functional disorders that occur in normal aging and its ability to suppress cognitive impairment [34].

Polyphenol compounds in green tea are known to have neuroprotective and neurorestorative effects. EGCG has the effect of increasing cell viability, reducing ROS, and increasing levels of stress markers on the endoplasmic reticulum and markers of apoptosis. EGCG also protects against mitochondrial dysfunction, 6-hydroxydopamine (6-OHDA)-induced toxicity, apoptosis induced by oxidative stress in mitochondria, and glutamate excitotoxicity. EGCG also maintains energy in mitochondria and reduces inflammation in brain tissue and damage to neurons. EGCG also has a neurorestorative effect by increasing neurite growth which makes EGCG a potential candidate as a drug that can modify neurological diseases because it has neurorestorative and neuroprotective effects [6, 33, 68].

The active ingredient of green tea, namely EGCG, in addition to reducing and preventing oxidative stress, EGCG can also reduce inflammation. EGCG is a potent leukocyte elastase inhibitor that mediates the activation of MMP-9 and MMP-2, which will trigger inflammation. Oral administration of EGCG will also reduce inflammation in pulmonary fibrosis, block neutrophil-induced angiogenesis, inhibit pro-inflammatory mediators in inflammatory models, and inhibit pro-inflammatory mediators such as dose-dependent myeloperoxidase. This implies that EGCG is an anti-inflammatory agent with therapeutic potential [25, 27, 62].

EGCG was reported to be able to maintain lipid peroxidation and DNA deamination by protecting cells from lipid peroxidation initiators such as t-butylhydroperoxide, 6-hydroxydopamine, iron, ultraviolet radiation, hydrogen peroxide, and 3-hydroxykynurenine. An in vivo study conducted to determine the effect of EGCG on lipid peroxidation showed a significant decrease in lipid peroxidation. This research is done by measuring the levels of Thiobituric reactive substance (TBARS). Simultaneously, with decreased lipid peroxidation levels, several markers of lipid peroxidation, 4-hydroxynonenal and Malonaldehyde, with increased glutathione peroxidase activity and decreased levels of glutathione. This study has implications for the role of EGCG in protecting cells from lipid peroxidation [69, 72–74].

6. Green tea for treatment of acute ischemic stroke

Camellia sinensis with the active ingredient EGCG inhibits neuronal cell death through apoptosis (increased expression of BCL-2 and decreased expression of Caspase-3) and necroptosis (decreased expression of TNFR1 and RIP 3) in the Rattus norvegicus model of Middle Cerebral Artery Occlusion (MCAO) [31].

A study conducted with R. norvegicus model Middle Cerebral Artery Occlusion (MCAO) found the effect of Camelia sinensis with the active ingredient EGCG on HO-1 expression. Inhibition of HO-1 expression started with doses of 1, 20, and 30 mg/kg BW. So that indicates that the antioxidant properties of green tea can inhibit HO-1 expression starting from the lowest dose in this study. The group with 30 mg/KgBW green tea extract intervention also showed a significant difference compared to the control group. Thus, green tea extract and its active ingredient, namely EGCG, showed an inhibitory effect on HO-1 expression. HO-1 protein is an active protein due to oxidative stress through the Nrf-2 pathway; this decrease in HO-1 expression indicates that the administration of green tea extract and its active ingredient (EGCG) can reduce oxidative stress in stroke models [30, 46].

The same study found no difference between the levels of HMGB-1 in control compared to all interventions, and this indicates that neither green tea nor the active ingredient EGCG affects HMGB-1 levels. The results that showed no difference indicated that HMGB1 in this study was secreted passively by stressed cells so that it could not be inhibited by EGCG or green tea extract [30, 46].

C. sinensis with the active ingredient EGCG also influences the expression of TNFR1 in the MCA model. Significant differences in TNFR1 expression in the intervention group of EGCG 20 mg/kgBW, EGCG 30 mg/kgBW, and green tea extract 30 mg/kgBW compared to the control group. The effect of EGCG and green tea extract on decreasing TNFR1 expression started at a dose of EGCG 20 mg/kgBW, and this shows that both green tea extract and its active ingredient, EGCG, can reduce inflammation that occurs in brain tissue affected by stroke. Inflammation is one of the pathways of cell damage caused by ischemia. TNFR1 protein is the primary receptor of TNF- which will cause the active process of necroptosis and inflammation [30].

Furthermore, a decrease in RIP3 expression was found after green tea intervention, either using the active ingredient in the form of EGCG or green tea extract. A significant difference was also found in the RIP3 expression of the intervention group EGCG 20 mg/kgBW, EGCG 30 mg/kgBW, and green tea extract 30 mg/kgBW compared to the control group. RIP3 protein is an executor protein in the apoptotic process, so the decrease in RIP3 expression indicates that EGCG and green tea extract can prevent necroptosis in the MCAO model. The inhibition of RIP3 expression was

initiated at a dose of EGCG 20 mg/kgBW. Green tea extract 30 mg/kgBW also has a similar effect to EGCG 30 mg/kgBW [30].

Bcl-2 is a protein that is a major regulator of mitochondrial permeability and the release of pro-apoptotic molecules. BCL-2, together with Bcl-xL, are anti-apoptotic proteins located in mitochondria and endoplasmic reticulum. In mitochondria, BCL-2 maintains mitochondrial integrity and prevents apoptogenic molecules' release [75–78]. *C. sinensis* with the active ingredient EGCG affects the expression of BCL-2. Based on reserch results, there were significant differences in the expression of BCL-2 in all intervention groups compared to the control group. In the intervention group, EGCG 20 mg/kgBW, EGCG 30 mg/kgBW and green tea extract 30 mg/kgBW found a very significant difference when compared to the control. These results indicate that both EGCG and green tea extract can increase anti-apoptotic protein so that it will prevent apoptosis [31].

A significant difference was found in the expression of Caspase-3. Caspase-3 expression was lower in the EGCG 30 mg/kgBW group and the green tea extract group. These results indicate that EGCG and green tea extract can prevent apoptosis by inhibiting the apoptotic execution pathway. The inhibition of this execution pathway is very important because there are three apoptotic pathways: the intrinsic or mitochondrial pathway, the extrinsic pathway, and the granzyme pathway. If one of them is inhibited, other pathways will activate apoptosis, but if the executor caspase is inhibited, the apoptotic pathways that are active can be inhibited. Inhibit the apoptotic pathway by increasing the anti-apoptotic protein or down-regulating the pro-apoptotic protein caspase-3 [30, 63, 66].

Our study also shows that green tea can increase anti-inflammatory mediators that will increase recovery after stroke. We also use animal model and immunohistochemistry to measure CD 206, a marker for M2-type mitochondria. According to our research, there is an increase in CD 206 expression in both Green tea and EGCG group [46, 79].

The study on the benefit of green tea should be conducted in the clinical setting to know about the benefit of green tea in acute ischemic stroke. Its potential benefit can be as an adjunct treatment for acute ischemic stroke besides standard treatment.

7. Summary

The current standard of treatment for acute ischemic stroke is thrombolysis. However, only 2–8.5% of stroke patients can undergo thrombolysis in America, and less than 2% in the world undergo thrombolysis.

Green tea (*C. sinensis*) is the most consumed beverage in the world and is a source of polyphenols known as catechins, including epigallocatechin-3-gallate (EGCG), which is 63% of total catechins.

C. sinensis with the active ingredient EGCG inhibits neuronal cell death through apoptosis (increased expression of BCL-2 and decreased expression of Caspase-3) and necroptosis (decreased expression of TNFR1 and RIP 3) in acute ischemic stroke as in the R. norvegicus model of Middle Cerebral Artery Occlusion (MCAO), it also can decrease necroptossis and increase M2 type microglia.

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