Effect of Thai-Eastern Indigenous *Curcuma longa* L. Extract on Brain-derived neurotrophic factor and Oxidative Stress Markers in Cerebral Ischemia Rat

Pratchaya Kaewkaen 1, 2, *

[1] College of Research Methodology and Cognitive Science, Burapha University, Thailand 20131

[2] Animal Cognitive Neuroscience Laboratory (ACoN), Burapha University, Thailand 20131

* Corresponding author: pkkinformation@gmail.com; Tel.: +66-087-215-3255

Abstract

The cerebral ischemia is resulting from occlusion the blood flow to the brain and cause of activated the neuronal death. The searching novel neuroprotective agent against this condition is still require. Evidence-based medicine demonstrate the crucial role of oxidative stress on the pathological of cerebral ischemia cascade. Based on the the antioxidant effect of *Curcuma longa* L., this study was designed to determine the neuroprotective effect and oxidative stress markers in animal model of cerebral ischemia. Male adult Wistar rats, weighing 250-280 g, were orally given *Curcuma longa* L. extract at various doses ranging from 100, 200 and 400 mg/Kg BW at a period of 2 weeks before and 2 weeks after middle cerebral artery occlusion surgery (MCAO). Then, all rats were determined the neuroprotective mechanisms in Brain-derived Neurotrophic Factors (BDNF) immunopositive neuron and Survival neuron. In addition, the extract were also determined via the alteration of Malondialdehyde (MDA) or lipid peroxidation product and via the activities of scavenger enzymes including Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxides (GSH-Px). The results showed that *Curcuma longa* L. extract significantly increased survival neuron ,BDNF positive neuron. For oxidative markers, the *Curcuma longa* L. decreased MDA. but increased the activities of SOD, CAT and GSH-Px at dose 200 and 400 mg/KgBW The possible underlying mechanism associated with its effect to decrease oxidative stress and increase BDNF. Therefore, the current results suggest that *Curcuma longa* L. extract is the potential neuroprotective agent. However, further researches are required in others cascades of the cerebral ischemia.

Keywords: Cerebral Ischemia, Curcuma longa L., BDNF**,** Oxidative Stress, Curcumin

Introduction

Cerebral ischemia caused by occlusions of arteries vasculature in the brain accounts for more than 80% of all stroke cases.(Fernandes M.*et al*., 2016) The pathophysiology of stroke is complex and involves with a number of biological mechanism, including inflammation, oxidative stress, apoptosis and excitotoxicity.(Woodruff TM., *et al*.,2011) Especially, cerebral ischemia leads to increases of intracellular Ca^{2+} and mitochondrial dysfunction, which in turn results in neuronal cell death or apotosis due to the activation of enzymes that generate reactive oxygen species (ROS). The intracellular Ca^{2+} overload triggers cell death programs, whereas inhibitors of intracellular Ca^{2+} influx attenuate mitochondrial damage and preserve neuronal cells from ischemic injury. Thus, intracellular Ca^{2+} overloading is an essential event in ischemic brain injury. (Nicholls DG. 2009). Experimental studies have shown that limiting the amount of intracellular calcium accumulation in neurons, by blocking entry through either voltage-gated or glutamate-activated channels, decreases neuronal injury and infarct size. Neurons also possess endogenous means by which intracellular calcium levels are tightly controlled. Recent findings showed that oxidative stress played an important role on the pathogenesis of ischemic neuronal injury. Overproduction of reactive oxygen species during ischemia could cause an imbalance between oxidative and antioxidative processes. Reactive oxygen species can damage lipids, proteins, and nucleic acids, thereby inducing apoptosis or necrosis. Moreover, oxidative stress also plays an important role in the post-ischemic neuronal progressive cell death in the penumbra area of the brain. (Talley WL., *et al*, 2013)

Neurotrophic factors have been known to promote survival and function of distinct neuronal populations during the development of nervous system.(Cohen-Cory S, *et al*., 2010) Presently, the family of Neurotrophic factors could be group into distinct family. There are four neurotrophins characterized in mammals. NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4) are derived from a common ancestral gene, are similar in sequence and structure, and are therefore collectively named neurotrophins.(Krüttgen A, *et al*., 1998) Although members of other families of proteins, most notably the glial cell–derived neurotrophic factor (GDNF) family and the neuropoietic cytokines, have been shown to also regulate survival, development, and function in the nervous system. It is believed that the presence of a specific polymorphism of BDNF is a factor determining the model of neurological damage and the possibility of neurological improvement after mechanical injury and ischemic damage, and in autoimmune diseases.(Lasek-Bal A, *et al*. (2015) BDNF concentration correlates

with the degree of vasogenic damage to white matter of the brain. According to recent studies, BDNF genotype plays a role in development of cerebral ischemia and is significant for the prognosis of improved mobility after stroke.(Pikula A., *et al.*, 2013)

Presently, Many research pay attention to focus the phytochemical substance such as, Plant in a group of polyphenols are dietary components that possess a variety of biochemical and pharmacological active effects.(Pandey KB & Rizvi SI. 2009)Recently, considerable interest has been focused on polyphenols because of their antioxidant, anti-inflammatory, and antiproliferative activities. (Mhadhebi L., *et al*., 2014) According to the oxidative stress is a key event in the pathogenesis of cerebral ischemia.(Shirley R, *et al*., 2014) *Curcuma longa* L. is a member of the Zingiberaceae (ginger) family, which is used extensively in foods as well as in Ayurvedic and alternative medicine worldwide. (Kumar S, *et al*., 2014) *Curcuma longa* has been used as a common food additive and herbal medicine. Curcumin, the active ingredient in turmeric (*Curcuma longa*), possess multiple pharmacological properties (anti-inflammatory, anti-thrombotic, and anti-oxidative) and these properties further add on to its anti-ischemic property.(Kocaadam B, & Şanlier N., 2017) The anti-ischemic effect of curcumin is believed to be contributed by its free radical scavenging activity which is unique upon having phenolic and diketonic groups present in its structure. (Lee W-H, *et al.,* 2013) However, other natural anti-oxidants lack the presence of two groups together and possess either of these. () These protective effects not only rescue the metabolite alterations but also improve brain edema, Evans Blue leakage and infarct size during ischemic brain injury. (Kalani A, *et al*., 2015)) The beneficiary effect of curcumin is also reported to be executed by lowering lipid peroxidation, when administered orally or intraperitoneally. Hence, the major advantages that lay with curcumin treatment have been explored as its non-toxic effect (even at high doses), ability to cross BBB in aged mice and gerbils, and its cerebro-protective behavior. (Dende C, *et al*., 2017)

According to cerebral ischemic injury leads to neuronal cell death through protein regulation and the modulation of complex mechanisms. However, little information is available regarding BDNF expression and oxidative stress markers after brain ischemia. Thefore, this study investigated BDNF after treatment with *Curcuma longa* extract after middle cerebral artery occlusion (MCAO)-induced cerebral ischemia rat model.

2. Materials and methods

2.1 Animals

Adult male Wistar rats (250-280 g, 8 weeks old) were obtained from National Laboratory Animal Center, Salaya, Nakorn Pathom, Thailand and were housed in standard Polycarbonate cages with temperature at 22±2 ℃ on 12:12 h light-dark cycle. All animals were given access to food and water *ad libitum*. The experiments were performed to minimize animal suffering in accordance with the internationally accepted principles for laboratory use and care of European Community (EEC directive of 1986; 86/609/EEC). The experimental protocols were approved by the Institutional Animal Care and Use Committee.

2.2 Plant preparation

The rhizomes of *Curcuma longa* L. (turmeric) were recruited from Sriracha district, Chon Buri, Thailand. The turmeric rhizome powder (500 g) was macerated twice in 95 % ethanol, followed by filtration. The rhizome residue of this solvent was removed by filtration, and the resulting filtrate was concentrated under reduced pressure at 40 ℃ in a rotary evaporator. The final yield of the turmeric extract was 25.59 % w/w. Then the concentration of curcumin which represented the major active content of the extract was measured using TLC. The concentration of curcumin was 28.08 %. The turmeric rhizome extract was eventually dissolved in the distilled water (DW) solution prior to oral administration to the rats.

2.3 The experimental design

Rats were randomly divided in to various groups as described following:

(1) Control Vehicle (DW) + MCAO

(2) *Curcuma longa* Extract (100 mg /kgBW) + MCAO

(3) *Curcuma longa* Extract (200 mg /kgBW) + MCAO

(4) *Curcuma longa* Extract (400 mg /kgBW) + MCAO

2.4 Surgical procedure to induce middle cerebral artery occlusion

Focal cerebral ischemia was performed according to modified method of Longa. (Longa EZ, *et al*., 1989) In brief, rats were anesthetized by thiopental sodium at dose of 50 mg kg/ BW. The right common carotid artery and the right external carotid artery were exposed through a

ventral midline neck incision and were ligated proximally. A silicone coated nylon monofilament (4-0) suture (USS DGTM sutures; Tyco Healthcare group LP, Connecticut, USA) with its tip rounded by heating near a flame was inserted through an arteriectomy in the common carotid artery just below the carotid bifurcation and then advanced into the internal carotid artery approximately 17 mm distal to the carotid bifurcation until a mild resistance was felt. Occlusion of the origins of the anterior cerebral artery, the middle cerebral artery and the posterior communicating artery was thereby achieved. Then, the wound was sutured, the rats were returned to their cages with free access to food and water. The incision sites were infiltrated with 10% Povidone- Iodine Solution for anti-septic postoperative care.

2.5 Determination of Survival neurons in hippocampus (Cresyl Violet Staining for Nissl Substance)

Adjacent series of sections containing hippocampus of all treated groups were stained with 0.5% cresyl violet to aid in neuronal death density determination. The neuron density in various regions of hippocampus was observed under light microscope (Olympus light microscope model CX-21; made in Japan) at 40X magnification by blinded observer.

2.6 Determination of Immunohistochemical staining BDNF immunopositive neurons

A series of sections containing hippocampus were reacted in a mouse monoclonal antibodies directed against BDNF (Chemicon Internation, Inc., CA, USA) and a modification of a previously described protocol employing the DAKO Strept ABC Complex/HRP duet kit. (Gong B, *et al*., 2016))

2.7 Determination of malondialdehyde (MDA) levels

A measurement of MDA levels was used to determine lipid peroxidation product. (Gupta YK., *et al.*,2003)) Briefly, the mixture composed of 50 μl of homogenate tissue, 50 μl of 8.1% sodium dodecyl sulphate (SDS), 375 μl of 20% acetic acid, 0.8% of thiobarbituric acid (TBA) and 150 μl of distilled water. Then the mixture was boiled at 100° C for 60 minutes. The mixture was cooled with tap water and added with 250 μl of distilled water and 1,250 μl of n-butanol:pyridine (15:1). Then the mixture was centrifuged at 4000 rpm for 10 minutes. The organic layer was separated to measure the absorbance at 532 nm.

2.8 Scavenging enzymes assay

Superoxide dismutase (SOD) activity was estimated by the method of Sun et al. (Sun Y., *et al*., 1998)) while Catalase and Glutathione peroxidase activities were determined by method of Aebi (Aebi H., 1974)) and Liu et al. (Liu F., *et al.*, 2013) respectively. The activities of all enzyme mentioned above were expressed as U/mg protein.

2.9 Statistical analysis

Data were presented as mean ± standard deviation (SD). The analysis was performed using One-way Analysis Of Variance (ANOVA), A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1 The effects of *Curcuma longa* L. on neuron density and densities of BDNF immunopositive neuron were shown in Figure 1-2. It was found that rats subjected at dose of 200 and 400 mg/kgBW significantly enhanced neuron density in CA2, 3 and Dentate gyrus (DG) of Hippocampus (P-value<.05; compared to vehicle+MCAO)

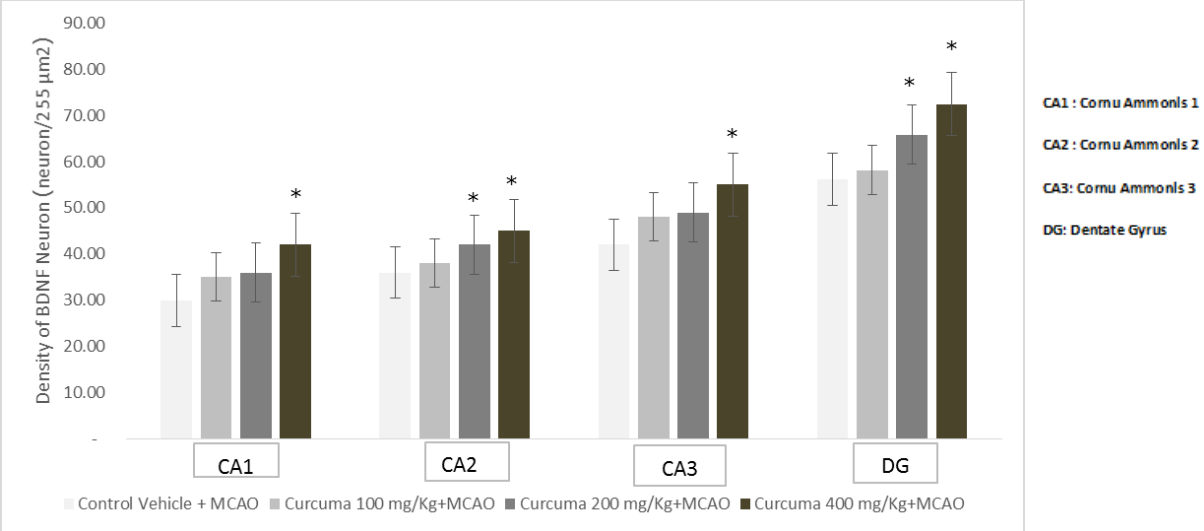

Figure 1: The effects of *Curcuma longa* L. on Survival Neuron of hippocampus

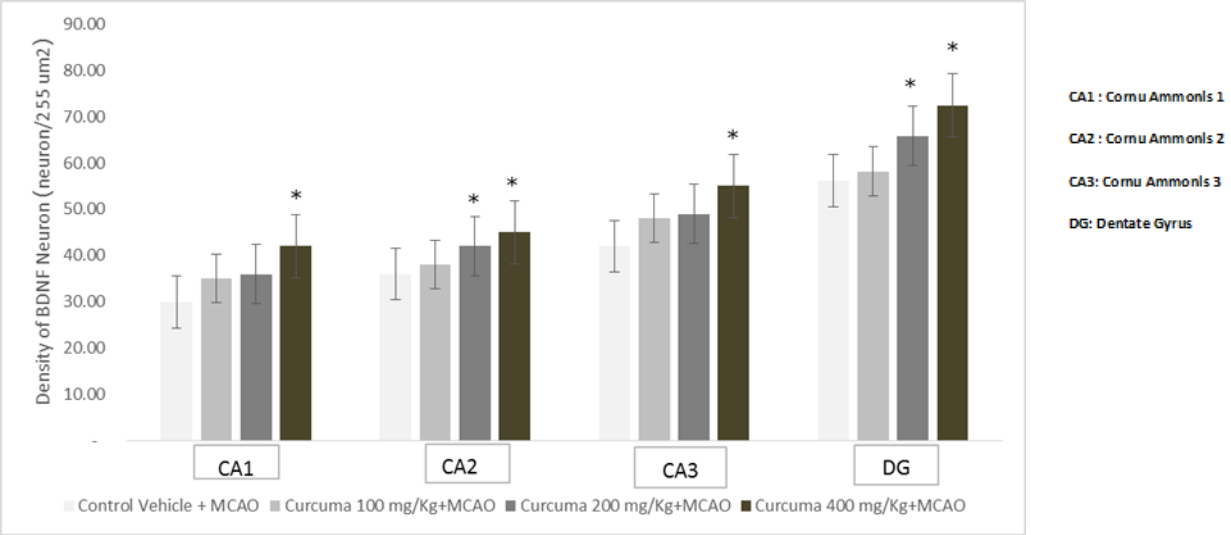

Figure 2: The effects of *Curcuma longa* L. on BDNF immunopositive neuron of hippocampus

3.4 Effect of *Curcuma longa* L. Extract on oxidative stress markers

In this study determined the effect of *Curcuma longa* L. on oxidative stress markers including the level of MDA and the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). The results were shown in table 1. It was found that rats subjected to all doses of extract significantly decreased MDA level in hippocampus P-value<.05; compared to MCAO+vehicle treated group) but increased CAT activity in the mentioned area P-value<.05; compared to MCAO+vehicle treated group). The enhanced activity of SOD in hippocampus was also observed in rats treated with *Curcuma longa* L. extract at doses of 400 mg/kg BW (P-value<.05 compared to MCAO+vehicle treated group). The enhanced GSH-Px activity was also observed in rats treated with *Curcuma longa* Extract at dose of 400 mg/kgBW (P-value<.05; compared to MCAO+vehicle treated group).

Table 1: Effect of Scavenging Enzymes Activity, Data were expressed as mean ± SD for 8 rats in each group. (*p < 0.05 compared to the Control vehicle + MCAO group)

Group	**MDA** ***(u/mg.protein)***	**CAT** ***(u/mg.protein)***	**SOD** ***(u/mg.protein)***	**GSH-Px** ***(u/mg.protein)***
Control Vehicle + MCAO	3.58±0.51	9.32±1.93	1.25±0.81	0.52±0.10
Curcuma longa 100 mg + MCAO	2.88±0.19	11.44±0.18*	1.92±0.21	0.94±0.21*
Curcuma longa 200 mg + MCAO	1.48±0.12*	15.14±0.16*	2.35±0.12*	0.91±0.52*
Curcuma longa 400 mg + MCAO	1.35±0.25*	18.31±2.51*	2.91±0.25*	0.95±0.25*

4. Discussion

In the present study, we demonstrated that *Curcuma longa* extract could increase density of neuron in survival neuron, BDNF positive neuron and reduce oxidative stress in animal model of cerebral ischemia. Curcumin, a polyphenol, has been shown to target multiple signaling molecules while also demonstrating activity at the cellular level, which has helped to support its multiple health benefits. (Gupta SC, *et al*., 2012). It has been shown to benefit inflammatory

conditions. metabolic syndrome, pain and to help in the management of inflammatory and degenerative eye conditions. (Gupta SC., *et al*., 2013)). In addition, it has been shown to benefit the kidneys. (Daily JW, *et al*., 2016).While there appear to be countless therapeutic benefits to curcumin supplementation, most of these benefits are due to its antioxidant and anti-inflammatory effects. Despite its reported benefits via inflammatory and antioxidant mechanisms, one of the major problems with ingesting curcumin by itself is its poor bioavailability. which appears to be primarily due to poor absorption, rapid metabolism, and rapid elimination. Several agents have been tested to improve curcumin's bioavailability by addressing these various mechanisms. (Hewlings SJ. & Kalman DS., 2017).

The main neuropathological cascade of cerebral ischemia. It may involve the following pathophysiologic aspects such as apoptosis, free-radical generation and activation of inflammatory mediators (Chalak LF., *et al*., 2012) and the activating of overproduction in extracellular glutamate excitotoxicity and intracellular accumulation of calcium (Szydlowska K. & Tymianski M., 2010) and depleted energy reserves and loss of high-energy phosphate compounds. Thus, energy deprivation along with increased levels of harmful factors, either intracellular or extracellular, disrupts neuronal homeostasis. This study demonstrated the mechanism of the action of BDNF in protective roles against ischemic brain injury in aspect of BDNF positive neuron.

The various evidence demonstrated that cerebral ischemia enhanced the formation of reactive oxygen species (ROS) in brain tissue. ROS, such as superoxide anions, hydroxyl free radicals, hydrogen peroxide and nitric oxide, were produced as a consequence of metabolic reactions and central nervous system activity. (Phaniendra A, et al., 2015). ROS are directly involved in oxidative damage of cellular macromolecules such as nucleic acids, proteins and lipids in ischemic tissues, which can lead to cell death. ROS are scavenged by superoxide

dismutase (SOD), glutathione peroxidase and catalase. Small antioxidant molecule, glutathione (GSH), is also involved in the detoxification of free radicals (Birben E, *et al*., 2012).

The current data showed that the *Curcuma longa* L. could find that the neuroprotective effect of Curcuma longa were associated with the alteration of MDA level in hippocampus. The neuroprotective effect of *Curcuma longa* extract associated with antioxidant activity. However, other mechanisms might also involve and required further investigation. In addition, we also found that the extract at dose of 200, 400 mg/kg BW enhanced the activities of SOD and GPx and significant reduction of MDA level too. The possible explanation might be related to the effect of curcuminoids on the defense formation of free radicals. (Amalraj A., *et al*., 2017)). Curcumin is a lipophilic compound, which makes it an efficient scavenger of peroxyl radicals, therefore, like vtamin E, curcumin is also considered as a chain-breaking antioxidant. (Gevrek F. & Erdemir F. 2018). The precise underlying mechanism is still required further researches both pre-clinical and clinical phases.

5. Conclusion

Curcuma longa L. Extract is the potential neuroprotective agent and antioxidant agent that may provide benefit for cerebral ischemia. The possible underlying mechanism associated with its effect to decrease oxidative stress and increase BDNF. Therefore, the current results suggest that *Curcuma longa* L. extract is the potential neuroprotective agent. However, further researches are required in others cascades of the cerebral ischemia.

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