ORIGINAL ARTICLE

The Administration *Centella asiatica* Ethanol Extract Increases AMPAR-GluR1 Expression in CA1 Region Hippocampus Male Wistar Rat

Auliyani Andam Suri[1], Siti Farida[2], Sri Redjeki[3]

[1] Department of Physiology, Faculty of Medicine Universitas Islam Negeri Syarif Hidayatullah, Jalan Kertamukti No. 5, Banten, Indonesia, 15412
[2] Department of Medical Pharmacy, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta, Indonesia, 10430
[3] Department of Physiology, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta, Indonesia, 10430

ABSTRACT

Introduction: Memory decline can occur in early adulthood and could be related to synaptic plasticity impairment that involves some synaptic protein, such as ionotropic glutamate receptor AMPAR-GluR1. Preventive treatment may be conducted to avoid memory decline by consuming some foods or supplements to enhance memory. *Centella asiatica* (CeA) is an alternative herb known as good for the brain and easy to find in Indonesia. This study is aimed to investigate the effect of CeA ethanol extract in AMPAR-GluR1 expression on the CA1 region of hippocampus normal adult male Wistar rats. **Methods:** Eighteen male Wistar aged 6 months were divided into three groups randomly. Each group consisted of six rats (n=6); the control group was received aqua dest, and two groups were treated with different doses of CeA: 300 mg/kg BW (CeA300) and 600 mg/kg BW (CeA600). The treatment was administrated orally for twenty-eight consecutive days. The expression of AMPAR-GluR1 in the CA1 hippocampus was assessed using the immunohistochemistry technique. **Result:** CeA600 group had the highest AMPAR-GluR1 expression (1.70±0.28) among the control group (1.478 ± 0.163) and the CeA300 group (1.642±0.25). Statistical analysis using Post-hoc showed a significant difference between the control and CeA600 groups (p=0.02). **Conclusion:** Administration of CeA ethanol extract 600 mg/kg BW was needed for maintaining AMPAR-GluR1 protein expression enhancement on CA1 hippocampus region male Wistar rats.

Keywords: Synaptic plasticity, AMPAR, *Centella asiatica*, Hippocampus

Corresponding Author:
Auliyani Andam Suri, MSc
Email: auliyani.andam@uinjkt.ac.id
Tel: +62 2174716718

INTRODUCTION

Memory loss occurs with aging and is associated with neurodegenerative diseases that can reduce a person's quality of life (1). One of the causes related to the impairment of synaptic plasticity. Synaptic plasticity is defined as the short and long-term modification of effectivity in chemical synapses (2). It can occur functionally (change in neurotransmitters, receptors, ion channels, signalling, and others) and /or structurally; changes in proportions and number of synaptic connections regulated by plasticity gen expression (3). Synaptic modification causes an increase of long-term potentiation (LTP), which is associated with long-term memory stored in the hippocampus, particularly in the CA1 region (2, 4). LTP process involves many kinds of protein and receptors.

A-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor or AMPAR is the main glutamate ionotropic receptor that mediates excitatory synaptic transmission, located on post-synaptic density (PSD); electron-dense areas seen on electron micrographs of the postsynaptic membrane (5). AMPAR is a tetrameric heteromeric complex, consisting of four homologous subunits, namely GluR1 to GluR4 (6). GluR1 is the main mediator in hippocampal neuroplasticity is important for fast induction and forms short but persistent synaptic potentiation (7). AMPAR is regulated by BDNF (Brain-Derived Neurotrophic Factor) (8). Its role in increasing the expression of GluR1 and GluR2 subunits (9). BDNF induction causes AMPAR-GluR1 phosphorylation in hippocampal synapses (10, 11). Deletion of GluR1 in rats causes spatial working memory deficiency (12, 13).

One preventive treatment to avoid memory decline is consuming some foods or supplements to enhance memory. *Centella asiatica* (CeA) or "pegagan" is an alternative herb that is already known as good for the brain and easy to find in Indonesia. CeA leaf is

consumable, tasteless, and odourless, has kidney or fan-shaped, slightly curved up and jagged at its edges (14). The highest concentration of phytochemical CeA was found in the leaves (15). Flavonoids and triterpenoids are their two main components that work as antioxidants and neuroprotective (16–18). Previous research has shown that whole plant of CeA ethanol extract at dose 300 mg/kg of body weight was better to improve memory function in male Wistar rats than aqua dest extract (19). Another study also reported that there was a significant enhancement of BDNF level in stress rats treated with 600 mg/kg of body weight CeA ethanol extract in 28 consecutive days (20).

Some previous studies have shown the effect of CeA extract on memory function. However, still, few show its impact on whether to improve or not in the neuroplasticity protein in CA1 hippocampal, particularly in early adulthood. Our study was aimed to investigate the effect of CeA ethanol extract on AMPAR-GluR1 expression in the CA1 hippocampus of male Wistar rats aged 6 months. It's equal to 18 years converted to human age (21).

MATERIALS AND METHODS

Centella asiatica extract

Simplicia of *Centella asiatica* leaves was obtained from Pusat Studi Biofarmaka Institut Pertanian Bogor (IPB), Indonesia. Extraction was performed at Balai Penelitian Tanaman Rempah dan Obat (Balittro) Bogor, Indonesia, using seventy percent of ethanol through the maceration method. Qualitative analysis was done by thin-layer chromatography (TLC). There were phenolics, alkaloids, tannins, saponins, triterpenoids, and flavonoids on the extract.

Animals

Male Wistar rats aged 6 months with an average body weight of 300-350 grams were obtained from PT. Biofarma (Bandung, Indonesia) and kept at the Pathological Anatomy Laboratory Medical Faculty, Universitas Indonesia using polypropylene cages. The rats were exposed to twelve-hour of light/dark cycles. The ambient temperature was maintained at 24°C and supplied with ad libitum food and water. The experimental protocols have passed the ethical review by the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital (No. 824/ UN.2F1/ETIK/2016).

Groups

Eighteen rats were divided into three groups randomly: the control group (n = 6) was received 2 ml aqua dest; the CeA300 group (n = 6) was received 300 mg/kg of *Centella asiatica* extract, and the CeA600 group (n = 6) was received 600 mg/kg of *Centella asiatica* extract. CeA ethanol extract was freshly diluted with sterile distilled water. It was administered to the rats daily by oral gavage for four consecutive weeks (28 days) with a weekly weight-adjusted dose.

Immunohistochemical analysis

The rats were decapitated at the end of the treatment, then isolated the hippocampal tissue from the brain, then immediately put into a 10% formalin buffer for 24 hours to be processed into a paraffin block. Then the specimens were made into four micrometres of coronal directional slices, floated in a water bath, and put into the surface of coated slides. The dried sliced was then put into a drying oven at 370C for 30 –60 minutes (22). The immunohistochemical staining procedure used was the Neopoly procedure. GluR1 protein expression was assessed using Abcam anti-glutamate receptor 1 AMPA subtype antibody ab 31232 with optimum dilution 1:200. The staining was observed with a binocular light microscope (Leica DM500) using 400x magnification, and the images were taken through a built-in camera (Leica ICC50 HD) on the microscope. There were representing five visual fields (2560x1920 pixels) on each slide. GluR1 protein expression was measured and analysed using the IHC Profiler plugin on the ImageJ program. The program was automatically generated intensity values for each image semi-qualitatively (23). IHC Optical Density Score method was used to do quantitative analysis.

Statistical analysis

The optical density data of expression protein were statistically analysed using a one-way ANOVA, followed by the Post-hoc Bonferroni test.

RESULTS

The expression of AMPAR-GluR1 in CA1 rat hippocampus using immunohistochemistry assay is shown in Fig. 1. AMPAR protein is a transmembrane receptor strongly expressed in pyramidal cells (24), so that they appeared on pyramidal neuron cytoplasm. Cells with AMPAR-GluR1 show brownish colour around their nucleus. The expression of AMPAR-GluR1 in the CA1 region rat hippocampus was greater in the CeA600 group (Fig. 1.D.) compared to the control group (Fig. 1.B.) and the CeA300 group (Fig. 1.C.). Quantitative analysis result as the mean of optical density score was shown in Fig. 2. CeA600 group (1.70±0.28) has the highest mean value among control group (1.478 ± 0.163) and CeA300 group (1.642±0.25). Statistical analysis using post-hoc Bonferonni resulted in a significant difference ($p=0.02$) AMPAR-GluR1 expression between the control and CeA600 groups. However, there was no significant difference ($p>0.05$)) between the control group and CeA300 group, also between the CeA300 group and CeA600 group ($p>0.05$).

DISCUSSION

Cognitive decline can occur since young adulthood

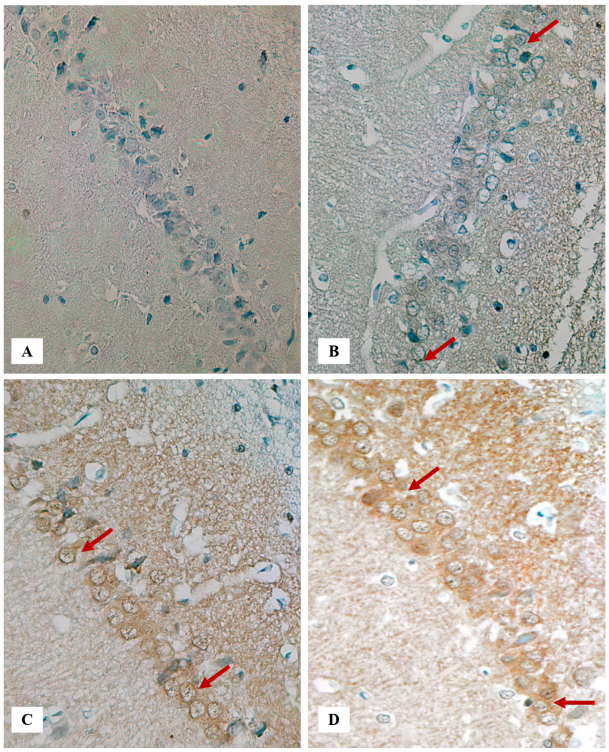

Figure 1: Immunohistochemical staining to analyse qualitative AMPAR-GluR1 expression in CA1 hippocampal tissue. A. negative control (n=6); B. control group (n=6); C. CeA300 group (n=6); D. CeA600 group (n=6). The chromogen 3-3-diaminobenzidine (DAB) was used to labeled the cells and tissue. Red arrows show the expression of AMPAR-GluR1 in pyramidal cells.

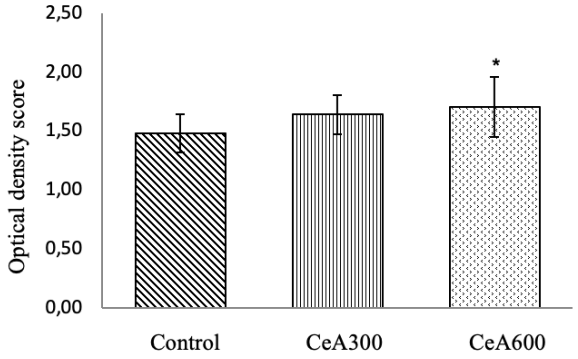

Figure 2: Optical density scores of AMPAR-GluR1 proteins in CA1 hippocampus in each group. Bonferonni Post-hoc Test: *Control vs CeA600 (p=0.020). Significance: p<0.05

(25). Therefore, it is necessary to prevent memory impairment in old age. Herbs consumption has been widely used to improve memory function. Many studies reported that *Centella asiatica* (CeA) is already known to improve memory function through its neuroprotective and neurotrophic factors (16,17,20,26). Administration of fresh juice of CeA leaves in neonate rats increased dendritic arborization, which is a neuronal basis in improving memory function (27). In addition, administration of CeA leaf powder in male Wistar rats played a role in increasing memory retention (28). Memory formation is related to the process of LTP in the hippocampus, which can be associated with AMPA receptor expression in the postsynaptic region (2,29,30). AMPA receptor (AMPAR) is a subtype of glutamate ionotropic receptor that plays a role in rapid nerve transmission and is associated with learning and memory functions in the brain (6). Mechanism the regulation amount of AMPAR to the postsynaptic membrane causes changes in synaptic transmission (31). The process depends on the composition of the AMPAR subunits. AMPAR with the GluR1 subunit plays a role in increasing synaptic strength and LTP (13). In this study, AMPAR-GluR1 protein expression was observed in the hippocampus, which is the site of memory consolidation (32). The transmission of impulses causes LTP at the synapse, resulting in memory formation (2). AMPAR-GluR1 expression was observed in pyramidal cells located in the CA1 region of the hippocampus. The main output from the hippocampus is transmitted via pyramidal cells located in the CA1 region. Information on the CA1 region comes from two pathways (the direct path and the indirect path); therefore, the LTP process is more commonly observed in this area (2).

Our study found that the expression of AMPAR-GluR1 protein in the control group (1.48±0.16) was the lowest when compared to the CeA300 group (1.64 ± 0.25) and the CeA600 group (1.70 ± 0.28). This suggests that the administration of CeA ethanol extract might trigger AMPAR-GluR1 protein expression in the hippocampus. BDNF directly regulates AMPAR-GluR1 protein expression; therefore, a high level of BDNF might cause an increase in the amount of AMPAR-GluR1 in the CA1 area of the hippocampus (10,33). BDNF stimulation will induce AMPAR-GluR1 phosphorylation, resulting in AMPA trafficking to synaptic areas in the hippocampus (10). TrkB activation by BDNF will stimulate the PI3-K pathway that causes Akt phosphorylation and upregulation of mTOR to promote the translation of the GluR1 subunit in the endoplasmic reticulum, which induces the insertion of AMPAR containing GluR1 in the postsynaptic membrane (34). In addition, BDNF-Trkb will cause CaMKK activation to induce CaMKI phosphorylation, thereby triggering an increase in AMPAR-GluR1 in the postsynaptic membrane (34).

Previous research showed that administration of CeA ethanol extract induced serum BDNF levels in rats induced by chronic stress, and it may be stimulated by active compounds present in CeA (35). According to phytochemical analysis results, various active compounds on the extract used in this study, such as flavonoids. Rendeiro found that flavonoids can increase BDNF expression in the rat hippocampus and spatial memory (36). The administration of flavonoid-containing foods to both young and elderly subjects showed an increased serum BDNF followed by better cognitive performance (37). Increasing BDNF by flavonoids correlates with activation of ERK and CREB

(38). Flavonoids are known to modulate the activation of various signalling protein kinases and lipid kinases such as PI3K/Akt, tyrosine kinase, PKC and MAP (39).

The highest expression of AMPAR-GluR1 was found in CeA 600 group. This result was in line with previous studies that state to increase BDNF level might require 600 mg/kg BW dose of CeA ethanol extract is needed to maintain an increase in BDNF levels (35). As it is known that BDNF regulates AMPAR-GluR1 expression, it can be assumed that CeA can increase AMPAR-GluR1 levels through increasing BDNF concentrations.

CONCLUSION

Administration *Centella asiatica* (CeA) ethanol extract dose 600 mg/kg BW was better to increase the expression of AMPAR-GluR1 in the CA1 hippocampus region of male Wistar rats compared to 300 mg/kg BW dose and the control group. To see the correlation, it is necessary to investigate further the effect of *Centella asiatica* on the other factors that involve the formation of LTP, such as proteins associated with increased neurotransmitter release and other proteins regulated by BDNF.

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