Brain Derived Neurotrophin Factor (BDNF) Level in Aged Sprague Dawley Rats Brain after the Treatment of Centella Asiatica Leaf Extracts

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Abstract: Functional decrease in learning and memory is one of the characteristics of the aging process. It has been known that a lower concentration of Brain Derived Neurotrophin Factor (BDNF) found on the brain, plays a role in the phenomenon. This study was designed to determine whether a herbal plant, Centella asiatica (CA), would increase the BDNF level on the aging brain tissue. 27 Male Sprague-Dawley rats aged 20-24 months and 4 weeks which were used in the study were divided into: negative control (were given aquadest), positive control (supplementation of Vitamin E), young rats as a comparison (4 weeks old), and treatment groups, which were given ethanol extract of CA leaves administered orally (300 mg/kg BW/week) for 28 days. At the end of the treatment, the rats were terminated and the brain BDNF levels were assessed. The data were analyzed by using a one-way ANOVA. The results showed a mean concentration of BDNF for negative control, positive control group, young and treatment groups were 44.09±3.854, 43.09±11.99, 65.88±13.46, and 30.2±12.33 mmol/ml, respectively ($p<0.05$ vs control group). The treatment group showed a higher BDNF level compared to all the groups. Interestingly, the BDNF level showed in the positive control group were found to be lower than the treatment group. This result showed that the supplementation of CA was effective in increasing BDNF brain level, thus raising the potential of having a neuroprotective effect. These results implied the need of further research to find out the mechanism of neuroprotective function exerted by CA.

1 INTRODUCTION

The global population is currently going through a phenomenon called epidemiological transition. This transition showed a pattern of population shifting from high mortality and fertility pattern of population to low mortality and fertility.The life expectancy of population aged 65 years old and above will continue to increase. In 2020, for the first time in history, the population aged 60 years or more would be higher than the children population aged below 5 years. This shows that in the future, our population would be constructed with more elderly than before. (United Nations, 2017; WHO, 2011) However, living longer does not mean living healthier. Almost a quarter (23%) of the global burden on morbidity and mortality of disease are from the age group of people aged 60 years and more. Elders are also more prone to many communicable and non-communicable diseases. Therefore, with the predicament of the increase of this particular group, the global healthcare system should answer accordingly. Beside of the increasing age-span, *ageing well* should also be the goal of future global healthcare system.(WHO, 2011)

One of the most aging-influenced aspect of human life is cognitive health. Cognitive health concerns about the brain function in ensuring the independence of one individual, including the ability of learning, intuition, judgment, language, and remembering. A decline in cognitive function could mean a loss of independence in older individuals, causing burden to self and others. This decline results from impaired neuronal plasticity (Tapia A et al., 2008)

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The pathogenesis of such neurodegenerative condition still has not been established definitely and involves multiple factors that influences several systems, however it was known to be related to the declining level of BDNF responsible for the loss of the neuron function and structure.(Erickson et al., 2012) BDNF has been known to have an important roles in proliferation, differentiation, target innervation, and survival of neurons of the central and peripheral neuron system.(Tapia-Arancibia et al., 2008). Lower levels of BDNF were also associated with poorer memory. (Cunha, 2010)

Centella asiatica (CA), a small annual herb from the family Apiaceae and native to Indonesia, India, and many part of Asia has always been used widely as a traditional medicines such as Aryuvedic medicine, Chinese medicine, and many Southeast Asian countries traditional medicine. (Lokanathan et al., 2016). The CA, also known as pegagan in Indonesia or gotu kola in India, have a significant number of reviews on their medicinal uses along with their supportive evidences (Gohil, Patel, & Gajjar, 2010; Lokanathan et al., 2016; Orhan, 2012; Rajakumari, 2010). Indicating the strong potential of the plant in medicinal sector.

The primary active components of CA are saponins (also called triterpenoid). (Singh & Rastogi, 1969) Saponins include asiaticoside, a trisaccharide with aglycone asiatic acid, madecosside, and madasiatic acid. These components are responsible for some of the CA medicinal effects such as wound healing and vascular effects. (Gohil et al., 2010)

CNS, cognitive, and antioxidant actions of CA has been studied in many research and maybe due to its brahmoside and brahminoside components, but are yet to be confirmed by clinical studies. (Gohil et al., 2010; Khotimah, Sumitro, Ali, & Widodo, 2015). Khotimah et al also found that methanolic extract of CA could increase the BDNF level in neuronal tissue of Rattus noevegicus strain Wistar that were exposed to lipopolysaccharide. (Khotimah, Riawan, & Kalsum, 2009)

However, there has been a gap in the current research, in which study of the neuroprotective effects of the CA has never been demonstrated on a subject with aging. Using 20-24 months old Sprague Dawley rats, we hypothesized that treatment with CA extract could effectively induce a neuroprotective effect on old age rats, hence this study aims to expand the knowledge in CA specifically its effectiveness to the level of BDNF found on the brain tissue of aged rats.

2 METHODS

2.1 Study Design & Subjects

The Sprague-Dawley (SD) young (2-3-month-old) and aged (20-24-month-old) male rats, were obtained from Research and Development Department of the Ministry of Health. In total, there were 27 rats that were used in this study. SD is a strain of albino rat which is used in many researches because its calmness and ease of handling.

Before the experiment, the rats were acclimatized for 1 week with lighting of 12 hours (light on from 06:00 p.m. to 06:00 a.m.), constant room temperature of 24^{o}C, were given standard food and ad libitum drink. The rats were divided into 4 groups: the aged rats with no treatment as negative control, the aged rats with treatment of CA extract (300mg/kgBW), the aged rats with Vitamin E treatment of 6 IU and the young rats. The Vitamin E treatment were used as a positive control, whereas the young rats were used to provide comparation between aged and young rats. The animals were kept for 28 days under the same environment where the rats underwent acclimatization. The treatment were given once every 7 days.

2.1.1 Daily Nutrients

The rats were fed with a type of pellets made from a mixture of cornmeal, rice bran powder, fishmeal, soybean, coconut, meat and bone meal, oat, ground nut, canola, skimmed milk, and fish pellet with brand names of SPA-Z and FF999. This standard pellets contained 18.5%-20.5% protein, 4% fat, 6% fiber, 8% ash, 0,9% calcium, 0,7% phosphor and has metabolized energy of 3100-3200 kcal/kg. The rats were given water, ad libitum.

2.2 Extraction of CA

The CA leaves were dried on a drying racks or sundried until all the water content evaporated. After being dried, the CA leaves were then grinded until the leaves become powdery. Then, the grinded CA leaves were extracted/macerated with an ethanol solvent, until all the active constituents were dissoluted into the solvent. This extraction process were performed for 24-48 hours and then proceeded with a separation of the active component from the solvent. This was achieved by evaporation using rotary evaporator. At last, the gravimetry analysis were performed to analyze the water content so that the solute percentage can be determined.

2.3 BDNF Measurement by ELISA

After 28 days of treatment, the rats were sacrificed by an intraperitoneal injection of ketamine and xylacine. Consequently, the rats were dissected and the tissue were obtained. The brain tissues were then isolated and contained in an alcohol solution in separate tubes. The tubes then were stored in a refrigerated storage.

BDNF ELISA kit were used to evaluate the BDNF level in the brain tissue. The ELISA kit that were used were from ELabScience®. This ELISA kit utilizes a Sandwich-ELISA method. The ELISA kit has micro plates precoated with antibodies specific to the rat BDNF. The samples were then added to the micro plate wells and combined with the antibody. Next, added to the well were biotinylated antibodies specific for rat BDNF and Avidin-Horseradish Peroxidase (HRP) conjugates. The microplates were then incubated. After incubation, the free components were washed away. The next step was to add a substrate reagent to each wells, those wells that contained rat BDNF, biotinylated detection antibody, and Avidin-HRP conjugate would appear blue. The termination of the enzyme-substrate reaction was achieved by adding Stop Solution, changing the samples appearance yellow in color. A spectrophotometry were then used to measure the optical density (OD) of the samples at the 450nm ± 2 nm wavelength. The OD value would be proportional to the BDNF level. A comparation of the samples OD to the standard curve would show the level of BDNF in the samples. Here, we measured every each of the subjects brain tissue BDNF level in a grouped manner.

2.4 Statistical Analysis

All the grouped data from the ELISA tests were then analyzed with an ordinary one-way analysis of variance (ANOVA) using the Graphpad Software, Inc statistical analyzer, Prism 7 for Windows. The results obtained from this analysis were presented as mean data ± SD. P values of less than 0.05 were considered indicating statistical significance.

2.3 Ethical Consideration

The Healths Research Ethical Committee, Faculty of Medicine, Universitas Indonesia – Cipto Mangunkusumo Hospital approved the study protocol and the usage of animal sibjects (December 2016). The ethic registration number is 1016/UN2.F1/ETIK/2016 and 402/UN2.F1/ETIK/IV/2018.

3 RESULTS & DISCUSSION

The results showed the BDNF level mean ± SD for the negative control, the treatment groups, the Vit E group, and the young group were 44.09±3.854, 65.88±13.46, 43.09±11.99, and 30.2±12.33 mmol/ml, respectively. In the rats treated with *C. asiatica* (300mg/kgBW), the BDNF level were found to be significantly increased ($p<0.05$ vs control group). However, in the group treated with Vitamin E, there was no significant change in the BDNF level compared to the negative control. There were also no significant difference of the BDNF level found between the young and negative control group. The highest concentration of BDNF was found on the CA treatment group, meanwhile the lowest was found on the Vitamin E group. (Figure 1)

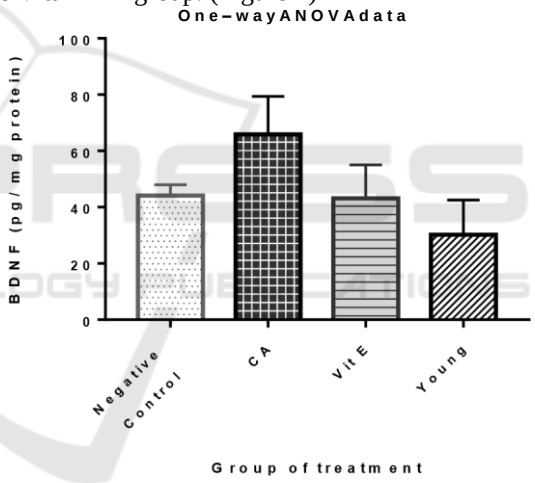

Figure 1: The BDNF levels (pg/mg protein) in 4 groups of Sprague Dawley rats. The data are shown in mean ± SD (P = 0.0014)

BDNF, a neurotrophin that functions in maintaining the integrity of neuron system, have long been thought to play a role in brain aging. Here in this study we tried to demonstrate the effect of CA on the brain BDNF level in an effort to reverse the effect of declining BDNF level on the aging brain. Our findings demonstrated that the ethanol extract of CA succeeded in increasing the BDNF level in the aged rat brain tissue compared to the negative control. This results was consistent with previous study that used LPS-induced rats (Khotimah et al., 2012). Khotimah et al. also stated CA extract

elevated BDNF level through induction on the expression of BDNF in brain tissue. Moreover, another study also showed the antioxidant and antiinflammatory properties of CA play a role in the neuroprotective effect of CA. (Gohil et al., 2010). Studies about CA have showed its remarkable effects on brain aging (Orhan, 2012). This effects have been generally attributed to its tripertene saponosides which covered many substances. However, the main active component varies between studies. Some of the tripertenes components that have been showed responsible for CA neuroprotective effects are asiaticoside, madecassoside, brahmoside, and brahminoside. Unfortunately, the current studies results are limited to answer this question. (Gohil et al., 2010; Orhan, 2012)

In the results, it is apparent that the BDNF level on the CA group was increased significantly but not on the Vitamin E group. This contradicted our former idea of the vitamin E activity as an antioxidant was expected to enhance the BDNF levels. However, study from Sakr et al. conformed with this results (Sakr et al., 2015). According to Sakr, this result might be due to the different between CA and Vitamin E mechanism of action on the BDNF. This previous research showed that CA extract increased the expression of BDNF, while Vitamin E treatment did not exert the same effect. Hence suggestedly the absence of the BDNF level increase in VIt E group. This could be the reason why there was a difference shown by the 2 groups. Still however, CA and Vitamin E both also achieved their neuroprotective effect through antioxidant and anti-inflammatory activities. (Khotimah et al., 2009; Sakr et al., 2015)

Meanwhile, the young rats group had lower level of BDNF than the control group (young: 44.09±3.854, control: 43.09±11.99 pg/mg protein) although it was not significantly different. According to the studies about the BDNF level during aging it was shown that the BDNF expression could also be induced by the process of neuron degeneration to increase the growth of neuron, which happened naturally in aged rats (Katoh-Semba et al., 1998). Moreover, in accordance to this study by Katoh-Semba et al., young and very young rats will have lower BDNF. However, another research by Cunha et al. showed that this phenomenon differs in rats with neurodegenerative condition. In rats with neurodegenerative condition, the BDNF level is expected to be low beacuse of the defect on the BDNF regulation, causing the compensation mechanism to not occur. This would be manifested in the clinical symptoms of the neurodegenerative diseases (Cunha, 2010).

4 CONCLUSIONS

The results demonstrated that the BDNF levels were increased with CA extract treatment. In addition, this study also found evidence that Vitamin E treatment attenuated the oxidative stress thus decreasing the BDNF expression. Whereas, in young rats the BDNF was lower than expected due to less oxidative stress as the BDNF expression trigger. These results call for further studies especially to determine the molecular mechanism of how CA influences BDNF.

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