Effect of Centella asiatica Ethanol Extract in Spatial Working Memory on Adult Male Rats

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Background: Cognitive decline can be started at early adult. It may be prevented with administration of Centella asiatica (CeA). CeA already known has some medicinal values for the brain such as to increase dendritic growth, to improve cognitive function and memory performance in rats after chronic stress. Objective: This study is aimed to investigate effect CeA ethanol extract on spatial working memory of normal adult male rats. Method: Eighteen normal adult male Wistar rats were divided into three groups: control/aquadest group and two groups treated with different doses (mg/kg) of CeA: 300 (CeA300) and 600 (CeA600). Ethanol extract of CeA were administrated orally for 28 consecutive days with weekly weight-adjusted dose. Memory performance was tested using Y-Maze before, on 14th days of treatment and after treatment. Data were analyzed using Kruskal-Wallis test and continued with Mann-Whitney test. Result: Treatment groups showed a better spatial working memory performance than control group, but there were no significant result between CeA300 and CeA600 groups (p < 0.05). Conclusion: Ethanol extract of CeA prevents spatial working memory decline on normal male adult Wistar rats. The optimum dosage of CeA might be 300 mg/kg of body weight.

Keywords: Centella asiatica, Spatial-Working Memory, Y-Maze.

1. INTRODUCTION
Learning and memory are important for doing daily activities. Those are cognitive aspects that essential for the full functioning and independent survival.1 Cognitive decline may begin at young adult about 18–20 years old.2 It could lead to some neurodegenerative disease such as dementia.3 Some preventive treatment may be conducted to avoid it such as doing exercise,4 getting various stimuli from environmental enrichment5 or consuming some foods or supplements that could enhance memory.6 Numerous of herbs are widely used as supplementary to enhance memory. Among of them is Centella asiatica (CeA).

CeA is a herbaceous annual plant that growing in moist plase in most Asian countries.7 In Indonesia, CeA is known as 'pegagan.' CeA has some neuroprotective and neurothropic factors that contribute to improve memory function.7,8 Some active compounds that have important role on memory function are asiatic acid9 and asiaticoside which are parts of triterpenoids properties,10 and flavonoid.11 Fresh leaves juice of CeA increased dendritic arborizations in neonatus rats which is a neuronal basic in memory enhancement.12,13 Another study also showed that treatment with CeA powder improved retention of memory.14 Ethanol extract of CeA is better than aqueous and methanol extract.15 Administration of CeA ethanol extract in 28 days increased serum BDNF level and better memory performance in young rats after chronic stress.16

Previous study showed the role of CeA to memory performance in stress-induced and growth-age rats but there was no study to investigate the effect of CeA ethanol extract in normal adult rat. In the present study, we aimed to show the effect of CeA ethanol extract on spatial-working memory in male adult Wistar rats which was assessed with Y-Maze tool.

2. METHODS
2.1. Experimental Design
This study was an in vivo experimental study using eighteen male Wistar rats aged 6 months that were randomly divided into three groups: (1) aquadest/vehicle group (Control); (2) CeA 300 mg/kg of body weights (CeA300); (3) CeA 600 mg/kg of body weights (CeA600). This study design and methods were approved by the Medical and Health Ethics Committee Faculty of Medicine Universitas Indonesia number B24/UN2.F1/ETIK/2016.

2.2. Animals
Male Wistar, weighing 300–350 grams were acquired from PT. Biofarma, Bandung. The rats were maintained under standard laboratory conditions: 25 °C temperature, 12 h dark/light cycle and allowed to have adequate food and water that were supplied ad libitum.
2.3. Administration of CeA Ethanol Extract

Simplicia of CeA plants were acquired from Pusat Studi Biofarmaka of Bogor Agricultural Institute. Ethanol extract of CeA was obtained using maceration methods from Balai Penelitian Tanaman Rempah dan Obat (Balitro), Bogor. Formulation and preparation of CeA ethanol extract were conducted in Medical Pharmacy Department, Faculty of Medicine, Universitas Indonesia. In order to prepare the various dose-dependent preparations: 300 mg/kg and 600 mg/kg, ethanol extract was freshly diluted with sterile aquadest. CeA ethanol extract were administrated orally for 28 consecutive days with weekly weight-adjusted dose.

2.4. Spatial-Working Memory Assessment Using Y-Maze Tool

Y-Maze tool (Fig. 1) consists of three arms which all of length are 30 cm, wide are 12 and diverging at 120° angle. Each animal was given two sessions during the memory assessment, training and test session. Both sessions were conducted in dark room and two days before it each animal were food deprived to 50% of daily intake.

Y-Maze was cleaned after each rat with alcohol 70% and dried. Memory test score was obtained from percentage of correct answer that was calculated by dividing the number of correct arm visits by the total number of arm visit and multiplied by 100. Spatial-working memory was assessed before the treatment (day 0), during the treatment (day 14) and after the treatment (day 29).

2.5. Statistical Analysis

Result was expressed as mean ± SD. Difference between group were analyzed by Kruskal-Wallis test continued with Mann-Whitney post-hoc test and considered statistically significant at a p value <0.05.

![Fig. 1. Assessment of spatial-working memory function using Y-Maze. (A) During on training session, one of the arms was blocked randomly and other arm was placed by daily food as a reward. Rat would enter the open arm voluntarily to find the reward, but was not allowed to eat the food by picking up it back to the start arm before ate the food. The training session was conducted in five times. (B) The test session was performed after delay 20 seconds and was conducted in 5 minutes. In this session, the blocked was removed so all the arms were opened and rat allowed a free choice between the two arms. The food was placed previous-blocked arm (1) and there was a food powder around the walls and floor of previous-opened arm (2) as a smell-camouflage. In this session, the animal was considered “correct” if it had entered the previous-blocked arm and would then be allowed eating the food reward before being returned to the start arm. If the rat entered to previous-opened arm, it was recorded “incorrect.”](image)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of correct (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kontrol</td>
<td>68 ± 0.09</td>
</tr>
<tr>
<td>CeA300</td>
<td>63 ± 0.13</td>
</tr>
<tr>
<td>CeA600</td>
<td>54 ± 0.16</td>
</tr>
</tbody>
</table>

Fig. 2. Percentage of correct responses in each group during Y-Maze test in 5 minutes before the treatment (day-0), middle of treatment (day-14) and after the treatment (day-29). Each value represented as mean (n = 6). Significant differences were indicated by ∗p < 0.05 versus control group.

3. RESULT

The mean of correct responses on each group before the treatment (day-0) are shown in Table I. Normality test of Shapiro-Wilk and homogeneity test showed that the data are normally distributed and homogenous. There was no difference between each group.

The result of spatial-working memory performance using Y-Maze test are shown on Figure 1.

Figure 2 showed the spatial-working memory performances were increased in all groups from day-0 to day-14. On day-29 the spatial-working memory performance in control group were decreased by 25% whereas both CeA300 and CeA600 groups were increased from the previous test. There was significant difference between each group on day-29 (p = 0.041), continued with Mann-Whitney test there was significant difference between control group and CeA300 group (p = 0.037); control group and CeA600 group (p = 0.021).

4. DISCUSSION

In this study Y-Maze tool was used to assess spatial-memory performance in rat. It is a simple memory test that has been known to observe spatial memory accurately and stress less in rodent. Memory function in each individual may be influenced by various factors, such as nutrition, disease, gene and behavioral pattern. Statistical test were used to analyze mean of spatial-working memory performance before the treatment (day-0) to ensure that each rat had same baseline on memory function. Based on the result (Table I) there was no difference in correct responses between all groups, so the result can be used as a baseline to determine whether the CeA ethanol extract has an effect to spatial-working memory on rat.
The result was showing increased of spatial working memory performance from day-0 to day-14 in all groups. It may indicate that the rat experienced learning process from previous test. On the day-29 or after the treatment, the spatial-working memory performances were decreased only on control group and were increased on all treatment groups. Memory decline might be related to the presence of age in rats, and it has been known may begin in young adult, but it may be prevented when treated with CeA. We assumed that treatment with CeA ethanol extract may maintain spatial-working memory on rats that may prevents the impairment of cognitive function.

This study shows that on day-29 treatment group with CeA ethanol extract had a better spatial-memory performance than control group. Some active substances in CeA were asiatic acid, asiaticoside and flavonoid, may have neurostimulant and neuroprotective effect that contribute to memory improvement. CeA may have compound that role as neurostimulant which increase dendritic growth, neurogenesis and induce hippocampal to produce corticortropin-releasing factor that may stimulate synaptic efficacy to promote memory formation.

Asiatic acid is an active compound in CeA that might contribute to antioxidant, neurogenesis and neuroprotective activities. Asiatic acid is an active property of triterpenoid that could penetrate blood brain barrier. Some studies reported asiatic acid could improve learning and memory, prevent spatial-working memory decline and increase hippocampal neurogenesis. Flavonoid increased BDNF expression in rat hippocampal continued with better spatial memory. BDNF has been known as essential factor that contribute on memory formation.

There is no significant difference on spatial-working memory performance between CeA300 group and CeA600 group. We suggest that the optimum dosage of CeA ethanol extract might be 300 mg/kg of body weights.

5. CONCLUSION

Ethanol extract of CeA prevents spatial working memory decline on normal male adult Wistar rats. The optimum dosage of CeA might be 300 mg/kg of body weight.

Acknowledgments: This work was supported by Beasiswa Tesis dan Disertasi Lembaga Pengelola Dana Pendidikan (LPDP) 2017, Ministry of Finance Republic of Indonesia.

References and Notes

Received: 20 November 2017. Accepted: 30 December 2017.