Conference Paper

Impact of Centella Asiatica Extract on Memory and Adrenal Weight after Chronic Stress on *Spraque Dawley rats*

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Abstract

Chronic stress is a common daily problem which may lead to the disruption of brain function including memory. One of the treatments for stress-related problems is the administration of medicinal plants such as *Centella asiatica* (CA). The objective of this research is to investigate the effects of the extract of *Centella asiatica* on adrenal weight and memory after chronic stress. Thirty adult male *Spraque-Dawley* rats (*Rattus norvegicus)*, were divided into 6 groups; each group consisted of 5 rats (n=5). The control group (C) was as normal control without stress and Treatment groups were Stress, CA150, CA300, CA600 and Fluo10 that receiving chronic immobilization stress. Stress group only received chronic stress treatment while other groups were also treated with CA ethanolic extract at 150, 300, and 600 mg/kgBW and 10 mg/kgBW of fluoxetine respectively. Chronic stress was triggered by immobilizing the rats in an acrylic tube for 6 hours per day for 21 days. Following this, the Moris Water Maze test was performed for 6 days to test the memory. One day after the test, the rats were terminated; the adrenal glands were evacuated and weighed. Acquisition trial showed improvement in memory performance from day to day ($p<0.05$) but there was no difference between groups ($p > 0.05$). Probe trial revealed the same result ($p>0.05$). The average percentage of adrenal glands relative to body weight in group Control, Stress, Stress, CA150, CA300, CA600 and Fluo10 were: 0.1592, 0.1838, 0.1942, 0.167, 0.1774 and 0.2024%, respectively ($p>0.05$). We conclude that the CA extract might influence memory performance and adrenal weight after chronic stress exposure although it was not statistically significant.

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1. Introduction

Stress is a common daily problem. Chronic stress exposure has been associated with various health problems. The stressful event was also a predisposing factor for the progression of several mental problems; for example, anxiety and depressive disorders. Stress affects brain function such as cognition and memory function [1, 2]. The previous

study revealed that stress exposure impaired cognitive function such as learning and memory [3].

Stress can affect several brain areas including the prefrontal cortex, hippocampus, and amygdala [4]. In particular, the hippocampus which serves as the center for memory processing is vulnerable to stress. Stress-induced alteration in the structure and function of certain brain areas, in particular, hippocampus, has been well understood [5]. Several mechanisms by which stress can cause hippocampal damage include neuronal remodeling, suppression of synaptic plasticity and decreased neurogenesis [6].

Several plants have been widely used as traditional medicine. Among these plants is *Centella asiatica* (CA) which, in Indonesia, is locally known as *pegagan*. CA was reported to increase learning and memory capacity and prevent cognitive deficit [7]. Other studies also revealed that CA stimulated axon regeneration [8] and increased dendrite arborization in the hippocampus and amygdala [9]. Furthermore, triterpenoids found in CA could modulate neurotransmitter in the brain and improved HPA axis function [10]. Considering these facts, we hypothesize that the administration of CA extracts can ameliorate memory deficit and adrenal weight changes caused by stress exposure.

2. Materials and Methods

2.1. Animals

Thirty adults (aged about 4 months, weighing about 250 gram) male rats (*Rattus norvegicus, Spraque Dawley* strain) were used in this study. These rats were purchased from the National Agency of Drug and Food Control, the Republic of Indonesia. The rats were allowed to acclimatize themselves for at least 7 days prior to the experiment. The rats were kept in groups, 2-3 rats/cage under standard housing conditions with the room temperature of 24-26 °C, humidity of 60–65%, and a 12/12 h natural light/dark cycle. Food and water were available *ad libitum*. The procedures of the experiment were approved by Research and Ethics Committee of Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia (number: 24/UN27.1.17.1/ERC/2012).

2.2. Purchasing of plant material and preparation of a standardized extract

Air dried whole plants were purchased from a commercial market in Yogyakarta. Air dried whole plants of CA were used for ethanolic extraction by maceration.

2.3. Experimental procedure

The rats were divided into 6 groups including Control group as the normal control without stress and Treatment groups were Stress, CA150, CA300, CA600 and Fluo10 that receiving chronic immobilization stress. Stress group only received chronic stress treatment while other groups were also treated with CA ethanolic extract at 150, 300, and 600 mg/kgBW and 10 mg/kgBW of fluoxetine respectively.

2.4. Drug administration

The extracts of CA at graded doses (150, 300 and 600 mg/kg/day and fluoxetine 10 x/kg/day were suspended using PGA (as a surfactant to make emulsion having uniform particle distribution). The extract and fluoxetine were administered orally 30 minutes before restraint stress for 21 days.

2.5. Chronic stress procedure

After adaptation for 7 days, the experiment began. The stress groups were given chronic stress by restraining the animals inside an acrylic cylindrical plastic tube (5,5 cm diameter, 15 cm long) for a period 6 hours from 09.00-15.00 for 21 consecutive days. This situation made the rat could not move freely, causing physical and psychological stress. The control group was housed at the other room while the stress groups were restraining.

2.6. Morris water maze (MWM) test

The device comprised of a round tank with a distance across of 1.5 m and a stature of 0.4 cm. The tank was divided into four similarly nonexistent quadrants, which were assigned as A, B, C and D. A round stage made of a white tin holder (width 13 cm, tallness 16.5 cm) was put amidst one haphazardly chosen quadrant. The situation of the stage was kept consistent for each rodent all through the test. Four uniformly dispersed beginning stages were set apart around the internal mass of the tank. A few distinctive shading pictures were additionally appended at the perimeter of the tank. The pool was topped with water off to a tallness of 18 cm (for example 1.5 cm over the stage). The water temperature was around 25 °C and made dark by adding coconut milk to shroud the stage. A camcorder was set over the focal point of the pool and transferred the

picture of the tank and development of the rodents to neighboring PC [11]. The rats were prepared in 5 days comprising of four trials for every day. On every trial for 60 s, the rats were set in the water at various begin areas (A, B, C, and D) and time for coming to on the stage (escape latency) were recorded. On the 6th day, the hidden stage was expelled and rodents were scored amid a 60 s probe test for intersections over the past stage area [12].

2.7. Adrenal weight

One day after the experiments all rats were terminated, adrenal glands were evacuated. Relative adrenal weight was determined as a proportion to body weight and communicated in rate (mg adrenal weight/body weight x 100%).

2.8. Data analysis

Data were analyzed using ANOVA (analysis of variance) followed by Post Hoc Test with SPSS for Windows version 22.0. The significance level was set at $P< 0.05$.

3. Results

3.1. Memory performance

We assessed the spatial memory by looking at their performance during the Morris Water Maze test which consisting of the acquisition and the probe trial. We looked at the spatial learning during the acquisition trial. In this test, the rats have to learn to find a hidden stage by using external cues. The duration of time required to find the stage was counted. The spatial acquisition of each rat improved from day to day ($p<0.05$) showing that they used their memory and learned how to find the stage effectively is shown in Figure 1. However, we found no significant difference among groups in the acquisition trial ($p>0.05$). We also did the probe trial to assess reference memory at the end of the learning process. In this test, we removed the stage and counted the time spent in the quadrant where the stage was used to be placed. Similarly, we found no significant difference among groups in the probe trial.

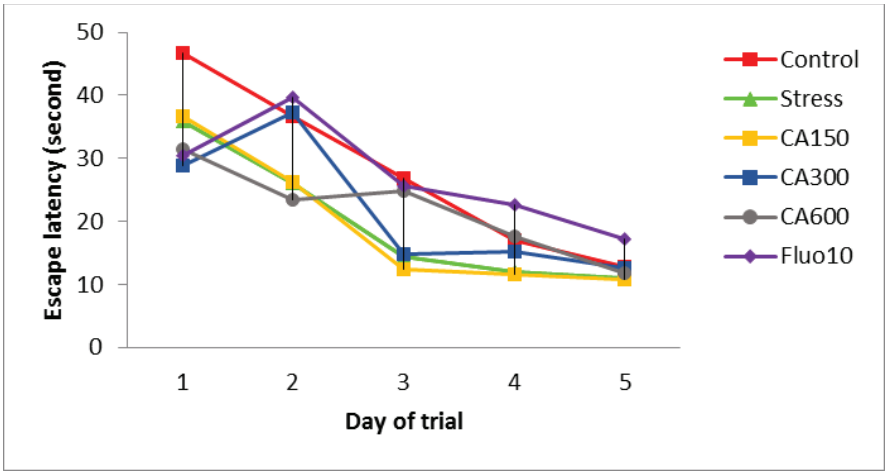

Figure 1: The results of the spatial learning assessment in the acquisition trial. Improvement in memory performance was found in all groups from day to day but there was no difference among groups.

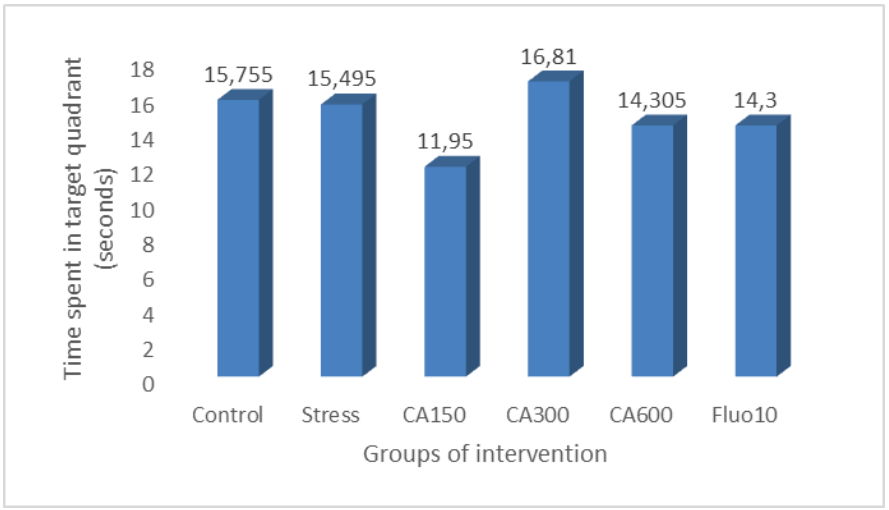

Figure 2: The results of the probe trial showing the total time spent in the quadrant where the stage was located.

3.2. Adrenal gland weight

After the Morris Water maze procedure was completed, all rats were terminated and the adrenal glands were evacuated. We then weighed the gland and compared the results among groups. The average percentage of adrenal glands relative to body weight in group Control, Stress, Stress, CA150, CA300, CA600 and Fluo10 were: 0.1592, 0.1838, 0.1942, 0.167, 0.1774 and 0.2024%, respectively ($p>0.05$).

4. Discussion

In this study, we investigated whether the ethanolic extract of CA can modulate memory performance in the rat after chronic stress exposure. We also looked at the weight of

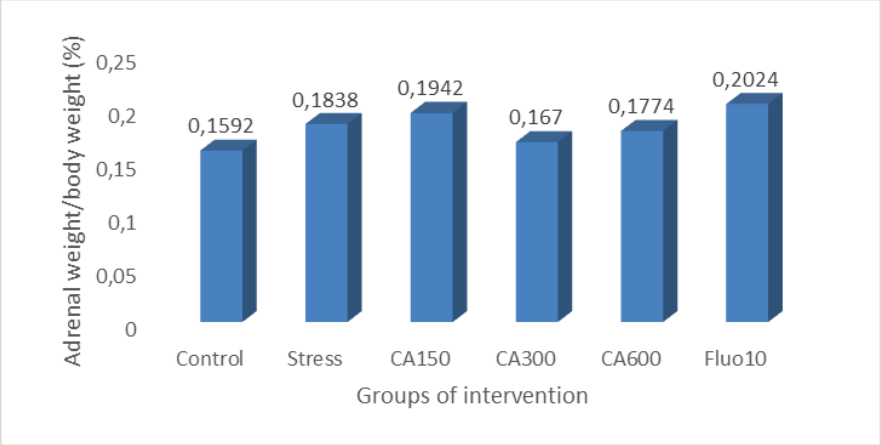

Figure 3: The average weight of adrenal gland per body weight in each group.

the adrenal gland and assessed whether any significant changes in adrenal gland size can be identified due to stress exposure. In our study, we used chronic immobilization stress to induce memory impairment. Since this method uses the same kind of stressor every day thus raising the possibility that the rats had adapted to the stress so that the effects of stress were not so prominent.

The primary finding in this research is that the memory performances in all groups were enhanced from day to day but there was no significant difference between groups. But there is a tendency that CA can improve memory especially in dose 150 and 300 mg/kg/day. This result is consistent with the previous finding that the administration of CA could significantly improve memory performance in the Morris Water Maze test after chronic stress exposure [6]. The brain responds to various kinds of stressor by releasing transmitters and hormones in order to cope with the stressful condition and brings back the organism into homeostasis or balance condition [13]. Two main systems omit are activated as a response to stress including the sympathetic division of the nervous system and the hypothalamus-pituitary-adrenal (HPA) axis system. The sympathetic nervous system provides a fast response known as the 'fight-or-flight' response by inducing the release of adrenaline and noradrenaline from the medulla of the adrenal gland giving rise to increased heart rate and blood flow to skeletal muscle. On the other hand, the HPA axis mediates a slow but more long-lasting response to stress by stimulating the adrenal cortex to release glucocorticoids [14] and stimulate increasing of adrenal weight [15].

Glucocorticoids can directly enter the brain due to their ability to cross the blood-brain barrier. Subsequently, they will bind to glucocorticoid receptors and mineralocorticoid

receptors in the limbic area [14]. In contrast, catecholamine such as adrenaline and noradrenaline cannot pass the blood-brain barrier but they can activate the adrenoceptors on vagal nerves in nucleus tractus solitaries of the brainstem which later stimulate amygdala either directly or through locus coeruleus. Then, amygdala modulates memory processing in certain brain areas such as the prefrontal cortex and hippocampus [16].

Hippocampus is the main target of glucocorticoid [17] because the highest concentration of the glucocorticoid receptor was found in this structure [18, 19]. Prolonged exposure to glucocorticoid can induce neuronal atrophy and decrease neurogenesis in the hippocampus leading to memory impairment [20, 21].

CA with the main content of flavonoids and triterpenoids can reduce the adverse effects of chronic stress. The administration of CA showed no significant differences but there was a tendency to improve the appearance of memory after chronic stress. One of these mechanisms may be through modulation HPA axis, marked by decreased of adrenal weight. The other mechanism of this effect may be attributed to anti-oxidants, anti-inflammatory and neurotrophic factors of CA [22, 23]. In conclusion, CA extratc might influence memory performance and adrenal weight after chronic stress exposure althought it was not statistically significant.

Acknowledgment

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