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The Effect of *Acalypha indica* L. Root's Ethanolic Extract on Old Male Sprague-Dawley Rats' Malondialdehyde Levels and Cognitive Function

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Abtract. Aging is physiological process characterized by degeneration of body functions and increased oxidative stress level. Malondialdehyde (MDA) is an oxidative stress marker which concentration will rise along with age. Increased level of MDA is associated with tissue aging and degeneration. In other hands, herbal plants are often used as an alternative medication, believed to contain many useful substances. *Acalypha indica* (AI) is one of the herbal plants that can be found in many regions of Indonesia, therefore AI can be used as a strategic alternative medication considering its availability. As of now, the relationship between AI extract consumption with aging and cognitive function is still unknown. The aim of this study is to assess the effect of ethanolic AI root extract related to aging by measuring MDA level in brain tissue and long-term memory level using Y-maze. Total of 18 old male *Sprague-Dawley* rats (age 20 – 24 weeks) were divided into 3 groups, which are control group, AI group, and vitamin E group. An additional group consisted of 6 young *Sprague-Dawley* (age 8 – 12 weeks) rats were used as negative control. Treatment was given to respective group every day for 4 weeks. On the last day of the experiment, the rats were terminat, and their brains were collected to measure MDA level. The results of MDA level analysis were 3,776+1,083 nmol/L for control group, 3,695+0,3947 for AI group, 3,118+0,7845 nmol/L for vitamin E group, and 3,704+0,4624 nmol/L for young rats' group without any significant difference (p=0,5220). The analysis result for weekly data (p=0,3692 for day 7 and p=0,8943 for day 28) and intergroup data (p=0,5000 for control group; p>0,9999 for AI group, vitamin E group, and young rats group) for long term memory also didn't show any significant difference. From the results, it can be concluded that AI extract consumption is not able to decrease MDA levels and slow down cognitive decline.

INTRODUCTION

Aging is a process of decreasing bodily functions. It is a physiological process that happens to all living being along with its age. In molecular biology perspective, aging happens mainly caused by cumulative damage in a cell's genetic materials. The damage will accumulate and eventually disturb the cell's homeostasis, which in turn will affect the respective cell's function and metabolism. This results in decreased function of many system organ in the body, including cognitive function. Decreased cognitive function is caused by aging process that happens on neurons [1].

There are many theories that try to explain the pathophysiological process of aging. One of the most well-established theory is free radical theory. The theory argues that oxidative stress is the main cause of degenerative process of aging. Oxidative stress is defined as a destructive process inside a cell caused by a group of chemicals called free radicals. Free radicals are chemicals that have an unpaired electron in its atomic framework, which is very reactive in nature. The unpaired electron will try to bind itself to electron in a cell organelle, which results in

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the respective organelle structural instability. The theory also states that the amounts of free radicals will rise along with age, and this will cause a bigger disturbance in cell function [2,3].

There are many ways to measure the extent of oxidative stress damage, mainly by measuring the level of certain biochemical substance level in body. One of the most prominent markers to measure oxidative stress is malondialdehyde (MDA). MDA is an end-product formed by fatty acid peroxidation. In many neurodegenerative diseases, MDA level is found to be raised, which implies an association between MDA level and cognitive degeneration [2,3].

Herbal medicine is more in demand these days because it is associated with lesser side effects compared with conventional drug. Herbal medicine with anti-aging is especially popular since it is considered as an alternative to obtain longevity. *Acalypha indica* Linn (AI) is an herbal plant that can be found in many regions of Indonesia, and the plant was known to hold many medicinal effects, including antioxidative, anti-inflammatory and hepatoprotective effects. According to previous studies, AI can decrease many degenerative diseases progression, including arthritis, diabetes, and hyperlipidemia [4]. The aim of this study is to know whether the application of AI root's ethanolic extract can help decrease oxidative stress in Sprague-Dawley rats, which measured by looking at MDA levels inside the rat's tissue.

MATERIALS AND METHODS

Animals Preparation for Experiment

The study was done on old male Sprague Dawley rats aged 20 – 24 months. The rats were obtained from National Institute of Health Research and Development, Ministry of Health Republic of Indonesia. Rats were divided into 4 different groups which consist of control group, AI extract group, vitamin E group, and an additional group consisting of young male test were done using Y-maze to measure long term memory. The amount of needed sample was counted using Federer formula. Because there were 4 different groups, the minimum amounts of samples needed in each group were 6 rats. In this study, a total of 28 rats were used.

Animals Treatment : Y-Maze Test

The Y-maze test was carried in 2 phase, training phase and test phase. In the training phase, each rat was put in one axis of the Y-maze. One another axis was closed and in the remaining axis, some food was put inside. The rat was able to roam freely in the Y-maze. Every time the rat managed to get inside the axis with food, the rat was pulled to its starting point again. The training was complete if 5 minutes has lapsed or if the rat managed to get inside the axis containing food 5 times. The test phase was done on the next day for each rat. The closed axis in the training phase is now opened and rat food were put inside. The axis that contained food before was emptied. The testing phase was done for 5 minutes, and the frequency of the rat entering the axis with food was recorded.

Animals Treatment : MDA Level Measurement

Cognitive received AI extract for 250mg/kgBW twice daily. Rats in positive control group were treated with 6 IU of vitamin E twice a day and negative control group was treated with water. The young rat group also only receive water as a treatment. Treatment last for 28 days. On the last day of treatment, the rats were terminated, and their blood was collected to measure MDA level using spectrophotometer.

Statistical Analysis

Laboratory results from blood samples of Sprague-Dawley rats was analyzed using analysis of variance (ANOVA). Statistical analysis performed with GraphPad Prism 7 for Microsoft Windows and visualized into graphs.

Ethical Consent

Ethical consent regarding the usage of rats as experimental subjects in this study was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia-Cipto Mangunkusumo Hospital in 2017, with extended approvement in 2018.

RESULTS AND DISCUSSION

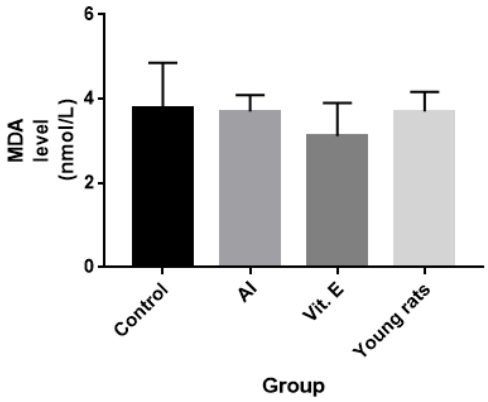

FIGURE 1. MDA concentration of different treatment groups.

The mean MDA concentration were 3,776+1,083, 3,695+0,3947,3,118+0,7845, and 3,704+0,4624 nmol/L for control group, treatment group, vitamin E group, and young rats' group respectively (FIG 1).

Among the groups, the negative control group consisted of old rats had the highest MDA concentration compared to another group. However, the difference of MDA concentration between the control group and the young rat's group was not significant. AI group showed very little difference of MDA level compared with the control group ($p = 0,998$). The lowest MDA concentration was found in the positive control group treated with vitamin E. Intriguingly, even the difference between control group and vitamin E group is not significant statistically ($p = 0,512$).

In this study, we tried to prove Acalypha indica's anti-aging potency by measuring MDA level in brain tissue. We used vitamin E as positive control because we knew that vitamin E is a potent antioxidant, so it was projected to decrease MDA level in aging rats. Hence, we used young rats as a negative control because the production of MDA in young rats, as a sign for aging, should be very low. By using these control groups, we could assure that the reseach was conducted well.

In this research, we found that administration of AI extract only slightly decreased MDA level. Even though the result we obtained was not significant statistically, it remains an interest for further studies since administration of AI can decrease MDA level to that of younger rats' group. Our study is coherent with another study, where AI can decrease MDA level in streptozotocin-induced diabetic Ba1b/C rats, but not significant statistically [5]. In another hand, a study found that combination between AI and Centella asiatica can decrease MDA level significantly in hypoxic rat liver [6]. However, another study found that administration of AI extract combined with *Centella asiatica* cannot decrease MDA level significantly in heart tissue of hypoxic Sprague-Dawley rats [7].

The result of our study implied that vitamin E had better antioxidant capacity compared with AI extract, shown with lower MDA level in group receiving vitamin E treatment. It was found that vitamin E supplementation decreases MDA levels in type 2 diabetes mellitus (T2DM), whether given independently or in combination with vitamin C [8,9]. Vitamin E was also found to be able to significantly reduce serum MDA level in type 1 diabetes mellitus (T1DM) patients [10]. Intriguingly, a study found that administration of AI extract could increase vitamin E serum level in streptozotocin-induced diabetic Wistar rats [11].

Even though vitamin E is widely known as a potent antioxidant, in our study administration of vitamin E was not able to decrease MDA level significantly. This result was different compared to other studies done by Sakr et al and Baghcheghi et al, in which vitamin E administration were able to decrease MDA level significantly in Sprague-

Dawley and Wistar rats. The discrepancy was thought to be caused by difference in vitamin E dosage and administration duration.

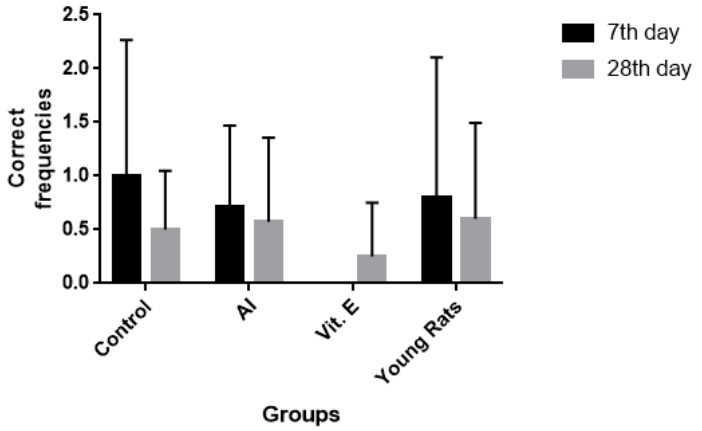

FIGURE 2. Mean frequencies of correct and incorrect attempts in Y-maze test phase.

Fig. 2 illustrated the correct number of rats using the y maze experiment on day 7 and day 28 among the groups. The mean correct frequencies for 7th day are 1,0 for control group, 0,7143 for treatment group, 0 for vitamin E group, and 0,8 for young rats' group. The mean correct frequencies for 28th day were 0,5 for control group, 0,5714 for treatment group, 0,25 for vitamin E group, and 0,6 for young rat group.

Among the groups, the correct attempts frequency average decreased on the 28th day test compared to the 7th day test. The correct attempt frequency only increased in vitamin E group. On 28^{th} day, the correct attempts frequency of the AI group was higher than the control group and vitamin E, closed to the correct number of young rats' group. Unfortunately, there was no significant difference between groups for day 7 ($p = 0.3692$) and day 28 ($p = 0.8943$) and there were no significant differences for inter-week analysis in the control group ($p = 0.500$), Ai group ($p = 0.990$), vitamin E group ($p > 0.999$) and young mice group (> 0.999)

Y-maze is one of the methods often used to measure rat cognitive and spatial capabilities . It was chosen because there was no life-threatening stimulus used in the testing using Y-maze.[12] AI extract was shown unable to increase rats long term memory, significantly. However, rats in the treatment groups has higher mean of correct Y-maze attempt frequencies compared to control group. According to previous research done by Yolanda et al and Ibrahim et al, AI extract administration was capable to induce hippocampal cells proliferation in adult male Sprague-Dawley rats [13,14]. It hypothesized that Ai extract can hinder cognitive decline by enhancing neuronal proliferation, since cognitive capabilites of Sprague-Dawley rats was better in rats with more neuron amount [15].

To our knowledge, this is the first study that directly tests the effect of AI extract supplementation in reducing cognitive aging caused by oxidative stress. The main limitation of this study is the used AI extract dosage of 250 mg/kg body wight, which is relatively small compared to other conducted studies. This can contribute to the insignificant result observed during this study.

CONCLUSION

Ethanolic extract of *Acalypha indica* is not able to decrease MDA levels and slow down cognitive decline in rats.

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