

Anti-Oxidative Potential of *Acalypha indica* L. Root Extract on Brain-Derived Neurotrophic Factor Levels in Old Sprague-Dawley Rats

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Abstract: In 2020, life expectancy in Indonesia is estimated to be greater than 70 years. It implies an increasing number of elderly and becomes a major problem in the health sector. Aging is a process that involves oxidative stress; however, it can be anticipated by the presence of brain-derived neurotrophic factor (BDNF). The aim of this study is to investigate the effect of *Acalypha indica* L. (AI) as a notable medicinal plant to BDNF level changes as a marker of anti-aging. This experimental study was conducted in 28 days using male Sprague-Dawley rats. There were four groups identified: negative control, positive control (vitamin E 6 IU), treatment (AI 250 mg/kg BW), and young group (8-12 weeks of age rats). Data are collected from terminating all the rats and the brain tissues are further checked in the biochemistry laboratory. The test used for statistical analysis is one-way ANOVA. Compared with the negative control group, levels of BDNF in brain tissues identified in the treatment group were increased, even though statistically insignificant (p-value = 0,6545). Nevertheless, the BDNF level in the negative control group is still higher than shown in the positive and young group. These results show that AI provides anti-oxidative properties; it also implies that AI can be used to impede the aging process. More studies are still needed to know the molecular mechanisms of action of AI that are particularly involved in increasing BDNF levels of brain tissues.

1 INTRODUCTION

World Health Organization reported that life expectancy has been increasing since 2000. This reflects on numbers of elderly which are getting higher. Studies show mortality due to degenerative disease occurred approximately 2- to 3-fold (World Health Organization, 2018) Aging is a multifactorial process; many factors are involved such as genetics, nutrition, physical activity, exposure to pollutant, radiation, and microorganism (Nigam, Knight et al., 2012). Hence, reactive oxygen species (ROS) are produced and accumulated; later contribute to cellular and DNA damage if the body fails to neutralize them.

Physiologically, there are endogenous substances produced naturally inside human bodies, such as Brain-derived Neurotrophic Factor (BDNF) that

makes neuronal cell able to survive and grow. As a consequence of the aging process, BDNF expression in brain tissues also reduce. It manifests in decreasing neuronal plasticity and cognitive function (Perovic, Tesic et al., 2012).

For this reason, many researchers are striving to find and develop recent medicinal plants that are estimated beneficial to prevent age-related diseases (Ekor, 2014). *Acalypha indica* L., a notable plant whose numerous therapeutic purposes; one of them is its anti-oxidative effect. Role of *Acalypha indica* L. root extract to BDNF level has been performed on a study which used young rats exposed to a hypoxic environment as subjects (Ibrahim, Rahadian et al., 2012). This research was conducted to determine the effect of *Acalypha indica* L. on BDNF expression identified in old Sprague-Dawley rats.

2 MATERIALS AND METHODS

2.1 Study Design and Sample

This experimental study was conducted on the neuronal cell cultures from brain tissues of male Sprague-Dawley rats aged 20 - 24 months with initial weights ranging from 183 to 308 g (young rats), and 333 to 490 g (old rats). It was conducted at the laboratory of National Institute of Health Research and Development (NIHRD), Ministry of Health (Indonesia) in August 2017 – May 2018. Based on the Federer formula, the number of samples was 24, divided into four groups (3 control groups and 1 treatment group). Ethical authorization was obtained from the Committee of the Medical Research Ethics of the Faculty of Medicine, Universitas Indonesia.

2.2 Extraction of Plant

The roots of *Acalypha indica* L. were collected and washed. The materials were dried in the room under sunlight exposure. Then, the dried roots were ground to get the smaller ones. Roots were extracted completely with 200 mL of ethanol (56-60°C). This procedure was done in 24 – 48 hours duration and repeated until all extracts were dissolved within the ethanol. The extract was later evaporated with a rotary evaporator to separate the solute from the solvent. With gravimetric analysis, the water content is also measured to determine the solute percentage.

2.3 Treatment with *Acalypha indica* L. extract

One treatment group was given the ethanol extract of *Acalypha indica* L. with a dose of 250 mg/kg BW. The negative control group was given rat chow

and water *ad libitum*; the positive control group was given vitamin E with a dose of 6 IU; the young group consisted of rats aged 8 - 12 weeks which given the same with the negative control group got.

2.4 Measurement of BDNF Levels

Samples were taken from neuronal culture medium. They were centrifuged, and the supernatant produced was stored in temperature -20°C. Examination of BDNF level was done by using BDNF kit according to the standardized procedures listed in its manual kit. The measurement was done by counting optical density (OD) with spectrophotometry at a wavelength of 450 nm ± 2 nm.

2.5 Statistical Analysis

Data were processed using GraphPad Prism 7 software and the results were provided as a mean ± standard deviation (SD). After conducted normality test by Shapiro-Wilk test, the statistical significance of the difference between the BDNF level seen in those four groups was tested by one-way analysis of variance (ANOVA). The significance of a difference was considered in p-value < 0.05.

3 RESULTS AND DISCUSSION

3.1 Results

The level of BDNF and protein content was measured in each subject separately. BDNF level was then divided by the concentration of protein to get the representative BDNF level for further identification.

As seen in Figure 1, there were differences in the numbers of BDNF levels in each group. By calculating the mean of BDNF levels in each group, it has been found that treatment group *Acalypha*

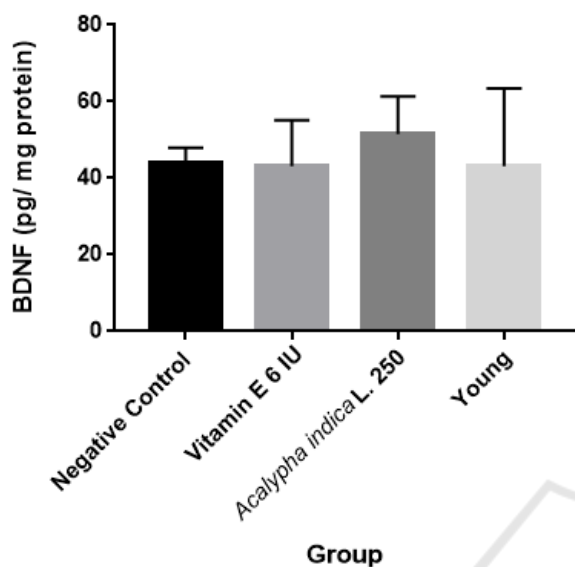


Figure 1: BDNF Level Expression in Negative Control, Vitamin E (Positive Control), *Acalypha indica* L., and Young Group.

indica L. root extract 250 mg/kg BW had the highest BDNF content which reached 51.57 ± 9.713 pg/mg protein, while the lowest was shown by positive control group vitamin E 6 IU which was 43.09 ± 11.99 pg/mg protein. BDNF contained in the negative control group was 44.09 ± 3.854 pg/mg protein. This value was slightly higher than the value of BDNF level in the young group, which was 43.18 ± 20.28 pg/mg protein. Despite the differences, these data were statistically insignificant with p-value 0.6545.

3.2 Discussion

This study showed that the root extract of *Acalypha indica* L. could provide anti-oxidative potential when given to aged subjects. This role was facilitated through the increment of BDNF level of brain tissues.

The high concentration of BDNF in the group receiving *Acalypha indica* L. is synergic with many preceding studies that have proven its positive correlation. For example, when compared to standard antioxidant L-ascorbic acid, *Acalypha indica* L. provided moderate antioxidant activity with different expressions depending on the material

used: root 53.27% and leaves 31.14%. This study clearly indicated that the antioxidant expression of *Acalypha indica* L. was higher in root extract (Shanmugapriya, Ramanathan et al., 2011). Bioactive constituents contained in the extracts, such as phenolic and flavonoid components were known to give radical scavenging activities by neutralizing reactive oxygen species (ROS) which later prevent neuronal damage and improve viability and proliferation of neuronal cells (Vauzour, Vafeiadou et al., 2008).

BDNF concentration was observed as the lowest in the group with vitamin E supplementation, which corresponded with a previous study on rats done by Sakr, Abbas et al. (2015). BDNF gene expression was known to be decreased in rats with sustained hypoxia and chronic exercise when exposed to vitamin E supplementation. Vitamin E given in this research is 100 mg/kg BW of the doses and injected intraperitoneally. This suggested that BDNF expression of the cortical neuron was related to oxidative stress induced by hypoxia and exercise. However, this research was limited in young rats aged 4 weeks.

According to one research which studied the correlation between age and plasma BDNF level, there were significant differences in BDNF levels between younger and older subjects after undergo horizontal bed rest for 14 days. In younger subjects, BDNF levels were smaller (34.36 ± 15.24 pg/mL) than older subjects (62.02 ± 18.31 pg/mL). These results were affected by the resistance of the brain to counteract acute stressors, which was declined as the age increased. It contributed to a bigger increment in BDNF level as a compensatory mechanism (Soavi, Marušić et al., 2016). Therefore, this strongly correlates with such differences of BDNF level shown in both old and young groups observed in this study.

As stated in a study on sixty frogs (*Bufo melanostictus Schneider*) aimed to identify the therapeutic effect provided by *Acalypha indica* L., there was a significant difference ($p < 0.05$) of neuroprotective effect among treated frogs when compared to control group. In the experiment, the extracts were made into four groups with various amount of doses. It concluded that the neuroprotective effect was observed highest at 200-500 mg/kg BW of dose (Purwaningsih et al., 2010). This range of dose was also synergic with a study detecting another pharmacological activity of *Acalypha indica* L. This study demonstrated that its anti-inflammatory effect was observed best at dose 250 mg/kg BW which seen by maximum inhibition

that was comparable to phenylbutazone 100 mg/kg BW as a standard drug (Saha, Ahmed et al., 2017).

Moreover, one *in vitro* study using hypoxic primary cell culture of hippocampus obtained from 9-10 weeks of age rats stated the possible mechanism underlying *Acalypha indica* L. role in BDNF increment. It directly impedes protein damage which contributes to the production of new endogenous BDNF. Through experiment in which the root extract of *Acalypha indica* L. with different exposure of doses used, it was known that proliferation of the neuronal cells was linear with increasing doses applied (Ibrahim, Rahadian et al., 2012).

Therefore, it might be a limitation of this research that made the results insignificant. It could be occurred due to relatively small doses of *Acalypha indica* L. used or the course of the experiment that was too short. In addition, there is a possibility that the molecular mechanism of increased BDNF level in both young and aged subjects are not the same. Hence continued research to gain more level of confidence in research results is suggested.

We can conclude that *Acalypha indica* L. root extract with the optimum doses can improve neuronal cell survival by increasing BDNF levels in aged subjects.

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