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DOSAGE AND ADMINISTRATION LENGTH OF *CENTELLA ASIATICA* (L.) URBAN DECREASE THE LEVEL OF SOD AND MDA AND IMPROVE BRAIN HISTOLOGICAL CONDITION OF RATS

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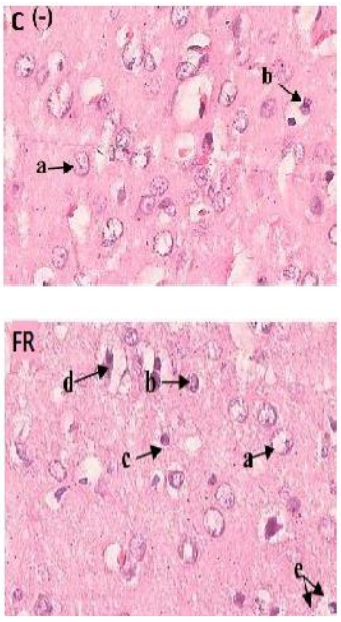
Graphical abstract



Abstract

Medicinal plants have been used for treating many kinds of illness due to their safety, cheap and easy to be accessed by all level of society. This study was aimed to determine the effect of the dosage forms (fresh, boiled and ethanol extracted) and the duration (24 and 42 days) of medicinal plant *Centella asiatica* to the levels of superoxide dismutase (SOD), malondialdehyde (MDA) and the histopathology of diabetic rat brain. The results showed that administration of various dosage forms of *C. asiatica* were able to increase the number of pyramid cells and neuroglia in the cerebrum significantly (*P <0.01*) but had no significant effect (*P>0.01*) at the administration length and the interaction between both treatments. This finding resulted in the new traditional herbal medicine that can be used for treating degenerative disorders on nervous system.

Keywords: Daylight factor %DF, window to wall ratio (WWR), window to floor ratio (WFR)

1.0 INTRODUCTION

Centella asiatica (L.) Urban is one of the natural ingredients which is commonly used as a traditional medicine. It has been widely recognized and easily obtained in the world such as India, Sri Lanka, China, Malaysia, South Africa and Madagascar. *C. asiatica* was reported to having complete and potential properties such as antioxidant, wound healing, anticancer, and anti leprotic [1].

Some of reasons in using *C. asiatica* as object of study in neurology are about its versatile ability to stimulate the growth of dendrites of nerve cells degeneration and neuronal cells due to impaired memory. It is also proofed as therapy for insomnia sufferers and people or animal with mental disorders, increasing Intelligence Quality (IQ), mental abilities, as well as tackling mental weakness and improving memory and overcome fatigue [6].

The ability of *C. asiatica* in repairing cell damage and degeneration in brain was successfully identified. The anti-oxidative property of *C. asiatica* may play an important role in reducing the activity of reactive oxygen species (ROS) in causing neuron cell damage in the brain [1]. The chemical properties such as Betulinic acid, thankunic acid, and isothankunic acid, triterpenoids asiatic acid and asiaticoside and medassic acid were the important compounds to protect neurons from damage.

Some of study reported that there is correlation between diabetic condition and formation of ROS and then causing cell necrosis. High blood sugar levels in rats are also affecting the neurons necrosis, and the condition of hyperglycemia can experience auto oxidation of glucose by producing a number of Reactive Oxygen Species (ROS). This excessive amount of ROS causes lipid peroxidation which produces malondialdehyde (MDA) and can reduce the capacity of the intracellular antioxidant enzymes, such as superoxide dismutase (SOD) [3, 4].

The use of *C. asiatica* as neuro-protective agent has been widely performed, but the effect of traditional extraction method or dosage forms (fresh, boiled or ethanol extracted) and the length of administration did not found. Therefore, our study was focused on the effect of dosage forms and the length of administration of *C. asiatica* on the levels of superoxide dismutase (SOD), Malondialdehyde (MDA), and histopathology condition in diabetic rat brain respectively.

2.0 LITERATURE REVIEW

This research base on Medicinal plants have been used for treating many kinds of illness due to their safety, cheap and easy to be accessed by all level of society. Focus this research was aimed to determine the effect of the dosage forms (fresh, boiled and ethanol extracted) and the administration length (24 and 42 days) of medicinal plant Centella asiatica to the levels of superoxide dismutase (SOD), malondialdehyde (MDA) and the histopathology of diabetic rat brain.

3.0 METHODOLOGY

Female wistar rats (*Rattus norvegicus*), 4 months of age with body weight (BW) of 200-300 g, were used in this study. The animals were acclimated in the laboratory for 2 weeks. To make the rat brain neurons undergo necrosis, the rats were induced by alloxan at a dose of 65 mg/BW as single dose for 2 times. Before injection, the rats were fasted for 24 hr. At day of 8 after the first injection, again the rats were induced by alloxan monohydrate with a dose of 65 mg/kg BW. After 5 days, the rats that had suffered necrosis were divided into treatment groups.

To determine the period of neuronal damage from rat brain to the human, the age of human was converted to the rat, where 10-year period in humans is equal to 1 month (4 weeks) period of rats. It is estimated that over a period of 4 weeks, microvascular damage occurred in diabetic rat brain neurons.

Thirty female rats were divided into 10 treatment groups; each treatment group consisted of 3 rats as replicates. The groups were divided as follows:

a. Group I (C-1): Non diabetic rats and then dissected on day 29.
b. Group II (C-2): Non diabetic rats and then dissected on day 43.
c. Group III (C+1): Diabetic rats without giving of *C. asiatica* dissected on day 29.
d. Group IV (C+2): Diabetic rats without giving of *C. asiatica* dissected on day 43.
e. Group V (EX1): Diabetic rats with administration of *C. asiatica* extract at a dose of 300 mg/kg bw per day for 28 days and then dissected on day 29.
f. Group VI (EX2): Diabetic rats with administration of *C. asiatica* extract at a dose of 300 mg/kg bw per day for 42 days and then dissected on day 43.
g. Group VII (FR1): Diabetic rats with administration of fresh *C. asiatica* leaves as much as 0.2 g/kg bw per day for 28 days and then dissected on day 29.
h. Group VIII (FR2): Diabetic rats with administration of fresh *C. asiatica* leaves as much as 0.2 g/kg bw per day for 42 days and then dissected on day 43.
i. Group IX (BO1): Diabetic rats with administration of boiled *C. asiatica* leaf as much as 0.64 ml/kg bw per day for 28 days and then dissected on day 29.
j. Group X (BO2): Diabetic rats with administration of boiled *C. asiatica* leaf as much as 0.64 ml/kg bw per day for 42 days and then dissected on day 43.

C. asiatica dosage forms administered orally to female rats at 6 weeks after injection of alloxan monohydrate. Giving of some *C. asiatica* dosage forms were made during 28 days and 42 days as prescribed and predetermined volume so as not to exceed the capacity of the rat gastric, and then on days 29 and 43 to the organ removal surgery brain to analyze the levels of antioxidant superoxide dismutase (SOD) and the levels of Malondialdehida (MDA).

4.0 RESULTS AND DISCUSSION

The ANOVA results revealed that the induction of alloxan significantly ($P<0.01$) reduced the concentration of SOD as showed at Figure 1. A, in which the treatment C+ possessed lowest level of SOD at the duration of 28 and 42 days, respectively. The administration of *C. asiatica* also significantly increased the level of SOD ($P<0.01$) at the

administration length of 28 days but insignificant effect for 42 days. The dosage forms of *C. asiatica* have significant effect (*P<0.01*) to the level of SOD in comparison with normal rat (C-), but have no significant among pretreatments (extract, fresh or boiled preparation) in case of 28 as well as 42 days treatment. The dosage form of boiled (BO) provides the highest levels of SOD (47.61 ± 1.27) on the provision of 28 days and about 34 units lower (13.3 ±2.08) on giving 48 days. This fact showed that the administration length affecting significantly the work of antioxidant in diabetic rat. The same pattern also could be seen at the treatment of extract (EX) and fresh form (FR) whereas the level of SOD were 45.86 ±1.28; 14.85 ±0.30; 43.52 ±1.91 and 14.03 ±0.61 units for 28 and 42 days treatments respectively. This suggested that the traditional use of *C. asiatica* by consuming freshly as "lalapan" and boiled did not reduce the usefulness of antioxidant properties in comparison with the extracted dosage form.

The previous study reported that the reaction of alloxan produced toxic ions in vivo and resulted decreasing of antioxidant system accompanied by decreasing of SOD [3]. However the administration of *C. asiatica* reported in this study could increase SOD level significantly as reported by Hussin *et al.* [3]. These effects may be attributed to the antioxidant components and polyphenol substances present in *C. asiatica*. The phenolic compounds (quercetin and catechins) present in the *C. asiatica* may have functional property as scavenging of ROS and inhibition of the production of free radicals and chain-breaking activity [1, 5]. These findings suggest that the *C. asiatica* has a good effect and increase antioxidant mechanism involved inside the body [4]. Number of Reactive Oxygen Species (ROS) as results of aloxan induction will stimulate synthesis of small proteins, later is well known as an endogenous antioxidant, that play a role in cell defense as a result of the body's mechanism which is in homeostasis condition. Production of endogenous antioxidants cause reduced though the use of exogenous antioxidants from outside of the body [1].

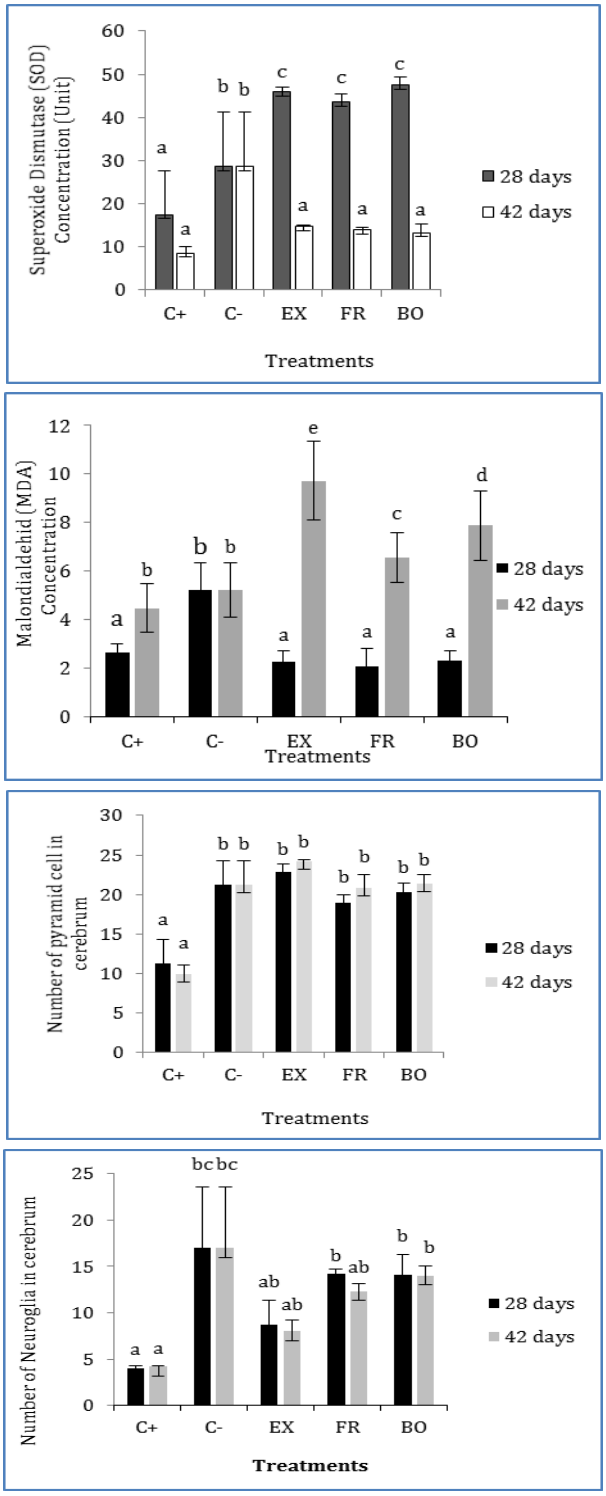


Figure 1 The Effect of dosage forms and administration length of *C. asiatica* to the levels of Superoxide dismutase (SOD) (A), malondialdehid (MDA) (B), pyramid cells (C) and neugrelia in female rat brain induced with aloxan. (C+: positive control, diabetic rat without *C. asiatica*, C-: negative control, normal rat without administration of C. asiatica, the notation EX: extract preparation, FR: Fresh and BO: boiled, different notation above the bars indicated significantly different based Duncan Mean Range Test)

MDA as indicator of ROS formation as a result of lipid peroxidation was also determined in this study. The results showed that the dosage of *C. asiatica* have insignificant effect at the 28 days administration, but significantly (*P<0.01*) at the 42 days administration, in which the fresh form showed the lowest level of MDA in comparison with Extract (EX) and Boiled (BO) dosage. This suggested that the longer *C. asiatica* is consumed, the higher of MDA level is obtained. These results, again, indicated that the administration of *C. asiatica* should be at the length of 28 days continuously not 42 days, to acquire the best effect of *C. asiatica* in reducing Reactive Oxygen Species formed inside the diabetic rat brain.

Malondialdehyde or MDA is a marker of free radicals in the body. Free radicals are a substance that will trigger if the amount is excessive or exacerbate disease that exists in the body. Levels of free radicals in the body can be inhibited by eating foods that contain antioxidants. Several studies have also mentioned that the water decoction of *C. asiatica* contain a number of flavonoids [2]. The level of lipid peroxidation can be suppressed by the presence of antioxidants. Thus, decreased levels of MDA indicate the inhibition of lipid peroxidation. However, the provision of 42 days has increased MDA allegedly caused by too excessive antioxidant activity in the cell so that it becomes a new problem with high levels of MDA. So based on the parameters of the high and low levels of MDA, administration of *C. asiatica* in a variety of dosage forms quite effectively lower the MDA with provision for 28 days instead of 42 days. This result is also consistent with previously reported by Dhanasekaran *et al*. [2] that the administration of antioxidants (inside *C. asiatica*) will decrease MDA levels in diabetic mice.

Histological brain due to the administration of *C. asiatica* was assessed by evaluating the number of pyramid cells and neuroglia found in the cerebrum of the brain of white rats. The result showed that positive control (C+) gave the lowest number of pyramid cells, which is about 10 cells. This means that induction of aloxan damaged the pyramid cells in the cerebrum. *C. asiatica* extract dosage forms (EX) provided the highest pyramid cell count for about 22.83 cells compared with C+, the dosage form of fresh (18.92) and boiled (20.33) in the duration of 28 days and that is not much different from the amount obtained in the administration for 42 days (Figure 1C). The treatment *C. asiatica* also significantly affected the total neuroglia cerebrum of diabetic rat. The results showed that the dosage form of *C. asiatica* affecting the number of neuroglia cerebrum significantly (P<0.01) but the administration length has insignificant effect (P>0.01) as well as the interaction between each treatment (Figure 1D).

The histological observation of pyramid cells and neuroglia in treated rat's cerebrum was provided in Figure 2. The Figure shows the cross-sectional histological structure of the cerebrum with a magnification of 400×. It could be seen that in negative control (K-) group were found to have a pyramid cell dendrites ramify through both poles of the normal cell and neuroglia. However on the positive control pyramid cells and neuroglia looks experiencing piknosis (K+). It is characterized by a core smeared darker. In the treatment group, EX, FR, and BO are looked the improvement of histological profile characterized by increasing of cell amount and the presence of cells undergoing mitosis. This is caused cells regeneration as administration of Centella asiatica treatment. This study showed that *C. asiatica* extract are able to prevent and repair the damage of neuronal cells, and this result supported the previous research which proved that the antioxidant compounds play a role in the regeneration of neurons and neuroglia in diabetic rats [4].

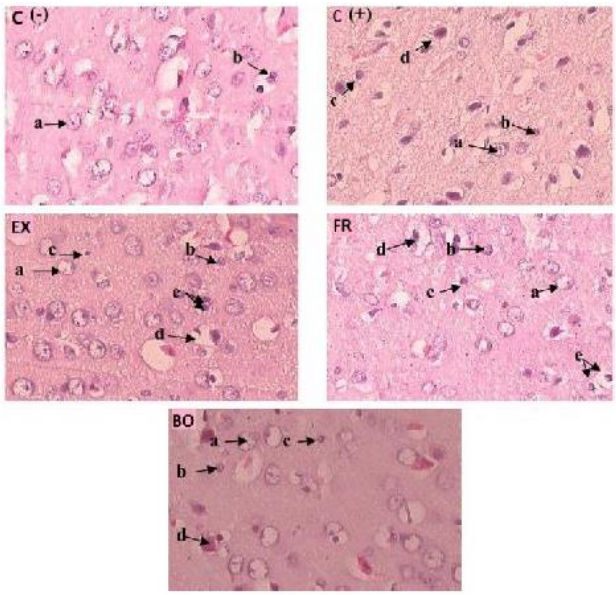


Figure 2 Observation Results of Histological preparation of cerebrum rat (magnification of 400×). Note: (a) Normal pyramid cell, (b) normal neuroglia, (c) necrotic neuroglia cell in picnosis, (d) necrotic pyramid cell in picnosis phase, (e) mitotic phase

5.0 CONCLUSION

The t administration of various dosage forms of *C. asiatica* have significantly increased the levels of superoxide dismutase (SOD) and decrease the levels of malondialdehyde (MDA) of diabetic rat brain as well as the duration of administration (28 days and 42 days). Interaction between *C. asiatica* dosage forms and duration of administration also have high significant effect. The administration of various dosage forms of *C. asiatica* was also able to increase the number of pyramid cells and neuroglia in the cerebrum significantly (*P <0.01*) but had no significant effect (*P>0.01*) at the administration length and the interaction between both treatments. This suggested that the traditional use of *C. asiatica* by consuming freshly as "lalapan" and boiled did not reduce the

usefulness of antioxidant properties in comparison with the extracted dosage form. This study is promising a new medicinal plant that is useful for curing nervous system disorders

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