Optimization of *Centella asiatica* (L.) Urban Dosage in Improving Memory of Mice with Brain Necrosis Model

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Abstract. Brain necrosis in mice can be triggered by injection of streptozotocin (STZ), causing nerve cell injury that can trigger neurodegenerative diseases. *Centella asiatica* (L.) Urban is one of the herbs whose triterpenoid compounds have neuroregenerative activity, so it is widely used as *Centella asiatica* (L.) Urban extract (EkCa). The aim of this study was to determine the optimization of EkCa dose in improving memory of mice in brain necrosis model. The design of this study was completely randomized design with 36 male Balb/C strain mice weighing 25–30 g with 6 treatments (control, EkCa 20, 30, 40 mg/kgBW, and metformin 25 mg) with each treatment repeated 6 times. Brain necrosis mice were made by injecting multiple doses of STZ intraperitoneally at a dose of 40 mg/kgBW for 3 days and 60 mg/kgBW for 2 consecutive days. Memory was obtained from the calculation of retention time (RT-LT $>$ 0). Data analysis used One Way Anova test followed with Duncan test. The results showed that the optimum dose was 30 mg/kgBW ($P < 0.05$). From the results of the study, it can be concluded that EkCa has neuroregenerative activity so that it can improve the memory of mice with brain necrosis model.

Keywords: Brain necrosis · *Centella asiatica* (L.) Urban extract (EkCa) · Memory · Streptozotocin

1 Introduction

Brain necrosis is cell death due to acute cell degradation or trauma [1]. Brain necrosis can be triggered with Streptozotocin (STZ). Streptozotocin (STZ) is a diabetogenic agent that has toxic ability and can destroy pancreatic cells. STZ induction triggers oxidative stress in the hippocampus which causes an imbalance in the levels of free radicals and antioxidants. Conditions of hyperglycemia cause neurocognitive decline which is

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associated with a reduction in the volume of white matter and grey matter in the brain STZ is a glucose analog that selectively accumulates in pancreatic beta cells via the glucose transporter Glut 2 in the plasma membrane [2, 3].

STZ injection in experimental animals can cause the accumulation of free radicals in the brain that trigger oxidative stress and the release of inflammatory mediators [4]. The release of inflammatory mediators causes inflammation in nerve cells which can trigger nerve cell injury. Nerve cell injury results in memory impairment, progressive cholinergic deficits, neurodegenerative, and oxidative stress resulting in cognitive decline and an increase in aggregated cerebral Aβ fragments. If this happens within a certain time interval, it can have implications for the pathology of Alzheimer's disease [5–7].

Centella asiacatica (L.) Urban (CA) is one of the herbs that spread in Southeast Asia, including Indonesia [8]. According to previous research, this shows that aquadest CA extract has biological effects that are improve memory, learning, and anti-aging [9–11]. This is because CA extract contains secondary metabolites such as tritepenoids, flavonoids, tannins, alkaloids, saponins, cardiac glycoside, and etc [12, 13]. It can be seen in several studies that these compounds can be useful as antioxidants [14, 15], anti-aging agents [16, 17], and neuroprotectives [18, 19]. Muchtaromah et al (2016) reported that CA was able to regenerate brain cells in brain necrosis model mice so that it could improve memory. Various kinds of CA preparations in the form of extracts of 300 mg/kgBW, fresh and boiled are both able to improve the memory of rats with brain necrosis to approach the ability of normal rats [20]. Matthew et al (2019) stated that the CA treatment for memory enhancement with the use of a CA extract at a dose of 200 mg/kg could improve cognitive function in Alzheimer's model mice [21].

Another study also reported that administration of crude methanol extract of CA at a dose of 50 mg/kg/day for 14 days could increase antioxidant enzymes such as catalase, SOD, and glutathione peroxide [22]. CA made casoside compounds at doses of 10, 20, and 40 mg/kg can increase the weight of rats, but it can also reduce infiltration of inflammatory cells and provide protection against joint damage [23]. Therefore, in the study using CA extract at doses of 20, 30, 40 mg/kgBW to improve memory of brain necrosis model mice. Based on the above background, the purpose of this study was to determine the optimal dose of EkCa in improving memory in mice model of brain necrosis.

2 Methods

2.1 *Centella asiatica* (L.) Urban Extract (EkCa)

100 g of CA simplicia was put into a glass beaker, then 500 mL of 70% ethanol was added and macerated for 24 h (ratio 1:5). After that the sample was homogenized using a shaker (speed 130 rpm). The sample was then filtered with filter paper and re-macerated 3 times. The filtrate was then concentrated using a rotary evaporator (temperature 50 °C with a speed of 65 rpm) [24].

2.2 Animals

This study used experimental male mice Balb/C strain 2–3 months old and weighing 25–30 g. Thirty six mice used were placed in 6 plastic cages with each cage containing 6 mice

and had been acclimatized for 2 weeks. This research has received another certificate of research ethics number: 021/EC/KEP-FST/2020.

2.3 Sample Group Distribution

This study was divided into 6 treatment groups, each treatments repeated 6 times. The distribution of treatment groups as follows:

a. Positive control group (K+): using STZ-induced mice without CA extract;
b. Negative control group (K−): using healthy mice (not induced by STZ);
c. Treatment group M: using STZ-induced mice and given 25 mg of metformin;
d. Treatment group P1: using STZ-induced mice and given CA extract 20 mg/kgBW;
e. Treatment group P2: using STZ-induced mice and given the CA extract 30 mg/kgBW;
f. Treatment group P3: using STZ-induced mice and given CA 40 mg/kgBW extract.

2.4 Brain Necrosis Model Mice

Mice were made with chronic diabetes by intraperitoneal injection of STZ at a dose of 40 mg/kgBW for 3 days and 60 mg/kgBW for 2 consecutive days. Then the mice were left for 9 days without any treatment to get the mice in a state of chronic diabetes. Day 14 the blood sugar levels was checked [25].

2.5 Administration of EkCa

The EkCa that had been dissolved in citrate buffer was then given to mice orally at a dose of 20 mg/kgBW, 30 mg/kgBW, and 40 mg/kgBW every day for 28 days.

2.6 Observation of Cognitive Ability

Observation of learning and remembering behavior of mice was measured using a modified passive avoidance test from the Jarvic method. The test equipment consists of 2 chambers, small and large. Washroom 25 × 15 × 50 cm, transparent and bright (25 W) with parallel wire floor. A large dark room measuring 50 × 50 × 50 cm with a floor of 1 cm wire woven wire which is supplied with 5 mA of electric current. The two rooms are connected by a small door measuring 10 × 7.5 cm. Mice were put in the restroom which were expected to passively enter the large room through the connecting door. When mice enter the large room, the animal is shocked by the electric current that flows through the floor of the room [20].

The test consists of learning test and retention test. The time gap between the two tests was 24 h, indicating the mice's short-term memory ability. The time it took the mice from the restroom to enter the large room was recorded, and then they were shocked by electric shocks to their legs once for 10 min. Then the mice were injected intraperitoneally with scopolamine. A day later the retention test was carried out. The retention time indicates the difference between the learning test time and the retention time so that it can be assumed as the ability to learn and remember. Measurement time for 600 s. The ability to remember is categorized as good if the subject's retention test has not entered the dark room for up to 600 s, and if the RT-LT > 0 [20].

Table 1. Retention time

Retention time of Mice Brain Necrosis Model	
K+	144 ± 5^{a}
K−	310 ± 4^{d}
M	323 ± 3^{b}
P1	474 ± 6^{c}
P2	492 ± 3^{d}
P3	489 ± 5^{d}

Descriptions: K+ = Sick Mice; K− = Normal Mice; M = Metformin; P1 = EkCa 20 mg/kgBW; P2 = EkCa 30 mg/kgBWt; and P3 = EkCa 40 mg/kgBW. Different notations show significant differences.

2.7 Data Analysis

Data analysis in this study used the One Way Anova parametric test followed with Duncan Multiple Range Test using IBM SPSS software.

3 Results

The results of the memory abilities study were obtained from the retention time shown in Table 1.

Based on Table 1, K+ treatment had the lowest retention time compared to other treatments (144 ± 5). Treatments P1, P2, and P3 have an average that is not far away. K+ has a low mean value because this treatment group was only injected with STZ. This shows that the effect of STZ has an effect on the retention time of mice.

4 Discussion

STZ is a diabetogenic agent that can trigger brain necrosis. Due to its toxicity, STZ can damage pancreatic beta cells by generating free radicals. STZ induction can increase oxidative stress, ROS production and mitochondrial dysfunction. ROS are mediators that can increase the production of free radicals [26, 27]. Increased production of free radicals triggers cell and tissue damage. The effect on the brain will be nerve injury that causes degeneration of nerve cells that affect brain function or cognitive dysfunction [5]. Based on the above exposure, the results of the study showed that STZ-induced mice without ca extract had the lowest retention time compared to other treatment groups.

The high retention time in the treatment group that was given EkCa in various doses showed that EkCa in this study could improve memory in the model mice. Table 1 shows that the results of giving EkCa at a dose of 20 mg/kgBW were significantly different from EkCa at a dose of 30 and 40 mg/kgBW. Meanwhile, EkCa doses of 30 and 40 mg/kgBW were not significantly different from the K-treatment group.

The high retention time in the treatment given EkCa indicates that EkCa has neuroregenerative activity. This is because CA contains triterpenoids which have neuroprotective properties [21, 22, 28]. These compounds maintain normal neuronal function and are able to limit age-related degeneration [31, 32]. Flavonoids have potential as Alzheimer's herbal medicine [33]. CA also contains asiatic acid from the triterpenoid group as a neuroprotective agent [34–36]. CA functions as an antioxidant agent and free radical scavenger that can prevent lipid peroxidation and DNA damage [37, 38].

Oxidative brain damage, memory dysfunction and neuronal cell death due to STZ injection are responsible for disrupting cognitive memory. CA can increase mitochondrial enzyme activity and reduce caspase-3 and Bax/bcl-2 (anti-apoptotic factor) levels in the hippocampus. *Centellaasiatica* (L.) Urban was able to inhibit apoptotic neuronal death in the hippocampus by influencing the AKT/GSK3β sinyal signaling pathway [39].

Nerve growth factor is important for hippocampal synaptic plasticity and nerve growth and memory formation. CA was able to increase BDNF levels in the rat hippocampus to reduce Alzheimer's symptoms [40–42]. Choligernichypofunction is significantly implicated in memory impairment due to STZ injection. Krebs activity in CA was able to increase the transcription of cholinergic markers, choline acetyltransferase and vesicular acetylcholine transporter. CA inhibits nitric oxide synthase activity thereby increasing cholinergic neurotransmission, which is important in memory formation [32, 43].

5 Conclusion

Based on the results of this study, it can be concluded that the variations in the dose of CA extract could affect the memory of the brain necrosis model mice. The optimal dose in this study was a dose of 30 mg/kgBW, while the retention time of brain necrosis model mice was almost the same as the retention time of healthy mice.

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