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NEUROPROTECTIVE EFFECTS OF PHYLLANTUS DEBILIS ON RAT'S HIPPOCAMPUS

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Phyllanthus debilis has the potential as an antioxidant and anti-inflammatory, suitable for application in neurodegenerative disorders such as cognitive impairment. This study aimed to examine the effect of P. debilis dichloromethane extract as a neuroprotector in rats treated with electroconvulsive shock (ECS). The rats were given the test substances for 20 days p.o, and ECS induction was held from day 15 to day 19. The Morris water maze (MWM) test determined the cognitive function, while hippocampus homogenates were used for biochemical studies. P. debilis showed the ability to reduce cognitive impairment in ECS-induced rats. The biochemical tests on the rat hippocampus showed a significant increase in catalase (CAT) activity and lipid peroxidase inhibition. The results aligned with anti-inflammatory activity, where TNF-α concentration showed significantly lower results than the ECS control. Overall results demonstrated the ability of P. debilis as a neuroprotective agent through the reduction of oxidative stress and inflammation.

Keywords: *Electroconvulsive shock, cognitive impairment, oxidative stress, inflammation.*

INTRODUCTION

Cognitive impairment is characterized by memory loss, inability to concentrate, difficulty learning, and difficulty processing thoughts[1]. Cognitive impairment can occur due to trauma to the brain, ischemic stroke, and other degenerative diseases that impair hippocampus function[2]. Naturally, aging also can cause cognitive decline, interfering with the quality of life of the elderly[3]. The primary mechanisms causing cognitive impairment include increased oxidative stress and inflammation in the brain, especially the hippocampus [4]. As is well known, the hippocampus is part of the limbic system that plays a role in encoding (making new memories)[3]. Treatment of cognitive impairment currently leads to natural ingredients, which could prevent or slow down patient memory decline. Many neuroprotectors have been developed with antioxidant and anti-inflammatory properties[5–7].

P. debilis has traditionally been used as an antidiabetic and antidiarrheal, especially in Sri Lanka. It was also used to treat wounds, scabies, and sores[8]. The ethanol extract of *P. debilis* contain steroids, saponins, alkaloids, tannins, phenols, flavonoid, and terpenoid. Moreover, the gas chromatography-mass spectrometry (GC-MS) analysis of *P. debilis* extract showed the presence of phytol and flavone, which were known to have antioxidant and anti-inflammatory activities[9]. Petroleum ether extract of *P. debilis* also showed potent analgesic and anti-inflammatory activities in albino mice at 50 mg/kg[10]. Previous research also showed that this plant had better antioxidant potential than other Phyllanthus species, such as *P. urinaria, P. virgatus, P. madespatensis,* and *P. amarus*[11]. Moreover, one of the compounds of *P. debilis*, debelalactone, showed antioxidant properties that could attenuate anti-hepatotoxicity in rats[12].

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This study aimed to discover *P.debilis* activity in animals with ECS-induced cognitive impairment. This method activates the microglia-neuroinflammation-oxidative stress response, leading to neuroapoptosis and cognitive decline[13]. In many studies, ECS has been used to produce cognitive impairment animal models, resulting in oxidation stress and elevation of Interleukin (IL)-1β and Tumour Necrosis Factor (TNF)-α in the hippocampus[14–17]. An increase in pro-inflammatory cytokines could activate microglial cells and cause neuroinflammation[18], similarly to oxidative stress, which causes neurodegeneration and cell damage and play a significant role in neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's diseases[19].

MATERIAL AND METHODS

Extract preparation

The fresh plant was obtained from Balai Penelitian Tanaman Rempah dan Obat (BALITRO) (Bogor, Indonesia). The aerial part of *P. debilis* was washed and put in the oven at 50°C for three days. Dried *P. debilis*, then powdered and sifted with mesh no. 20. A total of 500 grams of powder was soaked in 30% potassium hydroxide (KOH) (Merck, Germany) and dried up for up to 24 hours. The powder was then sonicated with dichloromethane (1:10) for 5 minutes, then filtered [20]. The extract was dried with a rotary evaporator at 50°C, followed by water bath drying. The total extract was 13.42 grams, with a yield of 2.68%.

Animal and experimental design

The experiment was approved by the ethics committee of Universitas Muhammadiyah Prof. DR HAMKA with number: 03/21.08/02172. The rats were given *P. debilis* extract (PD) 200 mg/kg, citicoline (C) 200 mg/kg as a standard drug, carrier for ECS, and normal control, for 20 days. From day 15 to day 19, the rats were treated with ECS. Induction was held with the intensity of 200 mA for 0.5 seconds using digital electroconvulsiometer EC-02 (Orchid scientific). In the following days, the rats were sacrificed. The brains were removed, and the whole hippocampus was isolated. The hippocampus was then homogenized with cold phosphate buffer saline (PBS) (pH 7.4) to make 10 % homogenate.

Morris Water Maze (MWM) test

A 150 cm diameter and 100 cm high pool was used for the study. With an imaginary line, the pool was divided into four quadrants. The platform with a 15 cm diameter was placed in one of the quadrats. The water was filled up to 2 cm below the platform in the training phase. The rats were allowed to swim from 4 insert points from each quadrant. The rats were guided to the platform if they failed to find it within 60 seconds. While in the test phase, the water was colored with white non-toxic paint, and the platform was submerged 2 cm below the water. The water temperature was maintained at 25 ± 1°C, and the experimental room's light and temperature remained consistent throughout the study. The data was presented as escape time, the average time for rats to find the platform from 4 insert points.

Lipid Peroxidation Test

Tetraethoxypropane (TEP) (Merck, Germany) was used as the standard and made serial concentrations in PBS. 2.5 mL of 20% Trichloroacetic acid (TCA) (Merck, Germany) was mixed with 2.5 mL homogenate or TEP to precipitate the protein. For the blank, PBS was added instead of homogenate. Then 5 mL of 0.67% Thiobarbituric Acid (TBA) (Merck, Germany) was added. Finally, the mixture was heated for 15 minutes at 95-100 °C, then centrifuged. The supernatant was measured at 532 nm. Malondialdehyde (MDA) levels are expressed in nm/gram brain tissue.

Catalase (CAT) Activity Test

First, 100 µL of the sample was added with 200 µL of H_2O_2, homogenized, and incubated for 2 minutes at 37°C. Next, 200 L of H_2O_2 was vortexed and incubated for 2 minutes at 37°C. The mixture was then added to the working solution, consisting of 100 mL cobalt (II) solution (Pudak scientific, Indonesia), 100 mL sodium hexametaphosphate solution (Loba Chemie PVT.LTD, India), and 1800 mL sodium bicarbonate solution (Pudak scientific, Indonesia). The mixture was then homogenized and left in the dark at room temperature for 10 minutes. The standard was given the same treatment, replacing the sample with

aquabidest. The absorbance was measured at 440 nm[21].

Determination of TNF-α Levels

TNF-α levels were determined using an ELISA kit (BT Lab, China). The procedure was carried out according to the manufacturer's manual.

Statistic analysis

The data were tested for homogeneity and normality, then continued with the ANOVA test.

RESULTS AND DISCUSSION

Results

The ECS induction could result in paralyzed animals, indicated by tonic-clonic seizures, and cause spinal fractures [22]. In this study, the intensity of 200 mA for 0.5 seconds caused tonic seizures for approximately one minute without paralysis. ECS treatment for five consecutive days resulted in significant cognitive impairment on the second day of induction, which was indicated by the significantly higher escape time of the ECS control group compared to other groups (Figure 1). In contrast, the group that received PD showed improved cognitive function during the experiment. However, PD does not show better results than the standard drug (C).

Biochemical tests of hippocampal homogenates showed that PD could significantly increase CAT activity compared to control ECS (Figure 2.A). The antioxidant ability of PD was also seen in the results of the lipid peroxidase test, where PD could inhibit lipid peroxidase as indicated by a significantly lower MDA level than the ECS control (Figure 2.B). Furthermore, PD could significantly reduce the TNF-a level in the hippocampus (Figure 2.C).

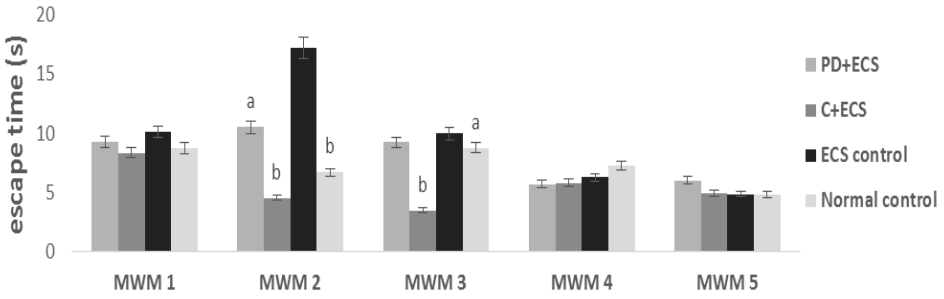

Fig. 1: Cognitive function test results of *P. debilis* (PD), citicoline (C) on MWM test. The data showed as the mean ± SEM (n = 7, each group). [a]significantly different from ECS control (p<0.01), [b]significantly different from ECS control (p< 0.001).

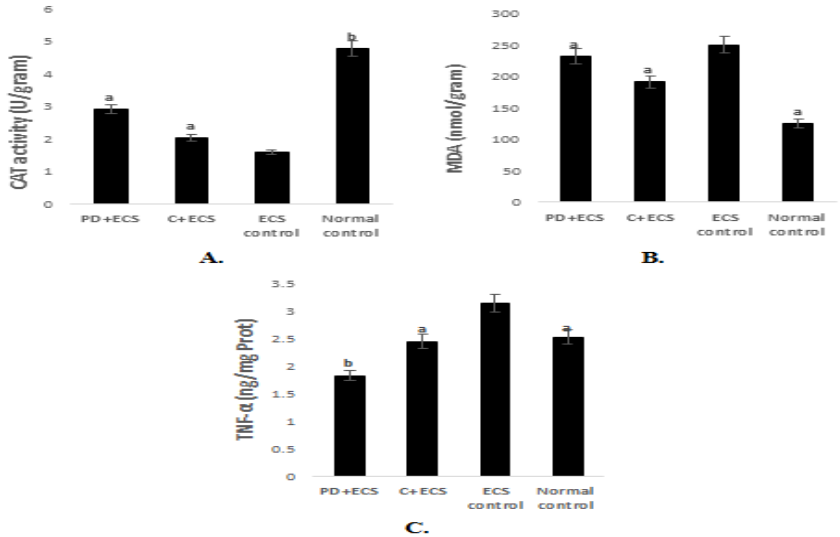

Fig. 2: *P. debilis* (PD) effect on hippocampal CAT (A), lipid peroxidation (B), and TNF-α levels (C) compared to citicoline (C), ECS control, and normal control. The data showed as the mean ± SEM (n = 7, each group). [a]significantly different from ECS control (p<0.05), [b]significantly different from ECS control (p<0.001).

Discussion

The results showed the ability of *P. debilis* as a neuroprotector, indicated by the ability to prevent cognitive impairment in animals induced by ECS through antioxidant and anti-inflammatory mechanisms. The previous study demonstrates that ECS treatment could cause learning and memory impairment[14&15&23]. In the present study, ECS treatment was shown to impair cognitive function in the MWM test, despite the reversible effect. Studies showed that repeated ECS treatment could result in inflammation and oxidative stress in the brain's hippocampal region, leading to memory deterioration[24].

P. debilis has been studied to have antioxidant and anti-inflammatory activities [10,25]. This study further tested the effects of *P. debilis* as an antioxidant and anti-inflammatory in the brain, especially the hippocampus of the cognitive impaired rats. The result shows the ability of PD to increase CAT activity, the enzymatic antioxidants that break down hydrogen peroxide into water. This enzyme works synergistically with superoxide dismutase (SOD), which converts reactive oxygen species (ROS) into hydrogen peroxide[26]. The result was linear with the lipid peroxidation test, as indicated by lower MDA levels in the PD group. MDA is the final product of polyunsaturated fatty acid peroxidation[27]. Increases in ROS production result in the overproduction of MDA, as seen in the ECS control group. In addition to oxidative stress, inflammation in the brain could activate microglia and astrocytes, causing CNS cell demyelination and neurodegeneration, triggering further ROS secretion and pro-inflammatory cytokines and chemokines[1&28]. TNF-α is a marker of cognitive decline and physical frailty in the older population[29]. This cytokine is known to cause reduced hippocampus volume through TNF receptor superfamily member 1A (TNFRSF1A) pathway [29]. TNF-α and other pro-inflammatory cytokines, IL-1β and IL-6, could modify synaptic plasticity and neurogenesis, leading to neuropsychiatric illnesses like dementia and major depression[30].

Interestingly, in terms of anti-inflammatory activity, PD showed better results than citicoline, with lower TNF-α levels. Citicoline or cytidine-5'-diphosphocoline (CDP-choline) was used as the standard drug in this study. This drug affects the cholinergic system and acts as a choline donor for acetylcholine synthesis[31]. Citicoline has been studied to reduce cognitive impairment in various animal models such as brain ischemic, scopolamine-induced rats, and ECS-induced rats[32–34]. The drug was also used as a cell membrane stabilizer and reduced free radicals in traumatic brain injury, stroke, Parkinson's disease, and vascular dementia[31].

The activity of natural ingredients has been widely studied related to neurological diseases. The primary mechanism related to antioxidants and anti-inflammatory is often related to the content of phenols and flavonoids[35]. The lignan content, such as phyllantin and hipophyllantin, and other phenolic compounds like gallic acid, rutin, corilagin, furosin, and geraniin have been studied to the major compounds of *P. debilis*[9], which might be responsible for the neuroprotective effect in this study. This research was limited to antioxidants and anti-inflammatory mechanisms, while cognitive disorders can also be influenced by other activities such as antiapoptotic or effects on the cholinergic system, so further research is needed.

Conclusions

P. debilis demonstrated neuroprotective ability through increased antioxidant and decreased inflammation in ECS-induced cognitive impairment in rats.

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التأثيرات الوقائية العصبية ل فيلانتم ديبيليس على الحصين فى الجرذان

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فيلانتم ديبيليس لديه القدرة على أن يكون مضادا للأكسدة ومضادا للالتهابات ، ومناسب للتطبيق في الاضطرابات التنكسية العصبية مثل الضعف الإدراكي. هدفت هذه الدراسة إلى فحص تأثير مستخلص دايكلوروميثان فيلانتم ديبيليس كحامي عصبي في الجرذان المعالجة بالصدمة الكهربائية (ECS). أعطيت الجرذان مواد الاختبار فمويا لمدة ٢٠ ، وتم إجراء المعالجة بالصدمة الكهربائية (ECS) من اليوم ١٥ إلى اليوم ١٩. حدد اختبار متاهة موريس المائية (MWM) الوظيفة الإدراكية ، بينما تم استخدام تجانس الحصين للدراسات الكيميائية الحيوية. أظهر فيلانتم ديبيليس القدرة على تقليل الضعف الإدراكي التي تسببها المعالجة بالصدمة الكهربائية (ECS) في الجرذان. أظهرت الاختبارات الكيميائية الحيوية على حصين الجرذان زيادة كبيرة في نشاط الكاتلاز (CAT) وتثبيط بيروكسيديز الدهون. تتماشى النتائج مع النشاط المضاد للالتهابات ، حيث أظهر تركيز TNF-α نتائج أقل بكثير من مجموعة التحكم ECS. أظهرت النتائج الإجمالية قدرة فيلانتم ديبيليس كعامل وقائي للأعصاب من خلال الحد من الإجهاد التأكسدي والالتهابات.