Pharmacology and Clinical Pharmacy Research **ISSN:2527-7322 | e-ISSN: 2614-0020**
Volume 8 No. 2 August 2023 **doi: 10.15416/pcpr.v8i2.48162**

Effects of *Centella Asiatica* (L) Urb. on Cognitive Function in Hypothyroid Mice Offspring

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Abstract

Centella asiatica (*C. asiatica*) is widely used in traditional medicine due to its numerous health benefits. Among its reputed advantages are improved memory, intelligence, and neural protection. Impairment of cognitive function as a center for memory processing occurs due to perinatal hypothyroidism. Although several studies have shown that *C. asiatica* extract may improve memory function, the effectiveness of its extract as a memory enhancer for patients with perinatal hypothyroid is less unknown. Therefore, this study aimed to determine the effects of ethanol extract of *C. asiatica* (EEC) leaf as a memory enhancer in perinatal hypothyroid mice model. *C. asiatica* leaves were extracted by the decoction method, and the ethanol extract was administered to mice. The hypothyroid mouse model was developed by administering antithyroid agents to pregnant mice from gestational day (GD) 18 to postnatal day (PND) 21. The hypothyroid mice were administered either donepezil 5 mg/kg BW/mL (positive control) or treatment group (EEC 2 mg/kg BW/mL) from PND 21 to PND 35 (14 days). The light-dark test (LDT) and memory tests of offspring were conducted on PND 36. We found that EEC improved the cognition and memory of perinatal hypothyroidism mice. This study contributes to the foundational research for developing memory-enhancing supplement preparations, mainly targeting children with perinatal hypothyroidism

Keywords: *Centella asiatica*, ethanol extract, memory enhancer, perinatal hypothyroid

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Received: 05 February 2023 **Revised**: 1 July 2023 **Published:** 7 July 2023

Introduction

Centella asiatica (*C. asiatica*) is an herbal plant that thrives in tropical climates and is renowned for its medicinal properties. It has been traditionally used to treat various ailments affecting the brain, endocrine system, skin, respiratory system, and gynecological issues.[1,2] *C. asiatica* has also been to improve memory which is attributed to the neuroprotective and neurotrophic factors found in it. The phytochemical analysis of *C. asiatica* has identified several bioactive compounds, including isoprenoids (sesquiterpenes, sterols, pentacyclic triterpenoids, and saponins) and phenylpropanoid derivatives (eugenol derivatives, caffeoylquinic acid, and flavonoids).[3]

Thyroid hormones (TH) play an important role in the development and functional maintenance of the central nervous system. During the development stage, these hormones regulate the growth and morphogenesis of brain and nerve cells by influencing the dendritic growth of cerebellar Purkinje cells, proliferation, and migration of granules. Consequently, thyroid hormone deficiency during development leads to impaired motor coordination in adulthood.[4,5] Moreover, hypothyroidism negatively affects the hippocampus, leading to impaired granule cell migration and dendritic growth of pyramidal cells. This disruption in synaptic function contributes to decreased memory and cognitive processes.[6,7]

Therefore, this study aimed to assess the efficacy of *C. asiatica* leaf extract as a memory enhancer in perinatal hypothyroid mice. Through careful observation of behavioral changes in mice with perinatal hypothyroidism following the administration of EEC, we aimed to evaluate the impact of the extract on memory function. The results of this study would be the primary basis for further research to develop the preparation of EEC as a dietary supplement to improve memory in children, especially in children with a history of perinatal hypothyroidism.

Methods

Extraction and Phytochemicals Screening

C. asiatica leaves were macerated using 70% ethanol. A total of 200 grams of C. asiatica leaves were dissolved in 2L of solvent and soaked for 24 hours, followed by filtration. The resulting filtrate was immersed three times, with each cycle lasting 24 hours and involving the replacement of the solvent. The collected filtrate was combined and concentrated with a rotary evaporator at a temperature of 45°C. The evaporation time was ± 3 hours. After evaporation, the extract was weighed to determine the yield of the extract, then stored in a refrigerator (± 4°C) in a light-tight bottle until it was used. After obtaining the C. asiatica extract, a phytochemical screening process was carried out to determine the content of secondary metabolite compounds such as alkaloids, flavonoids, saponins, steroidal tannins, and terpenoids in the extract.[8,9]

In vivo Analysis

The experimental animal protocol in this study followed directions from the Program Study of Pharmacy, Faculty of Mathematics and Natural Sciences, Bandung Islamic University, Bandung-Indonesia and Departement of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Bandung-Indonesia.

Treatment

A schematic of the animal study schedule is shown in Figure 1. The hypothyroid mouse model (Dam, n= 8) was conducted by administering 100 ppm propylthiouracil (PTU) in drinking water.[6] The hypothyroid mice then were administered either donepezil 5 mg/kg BW/mL (positive control) or treatment group (EEC 2 mg/kg BW/mL) from PND 21 to PND

ISSN:2527-7322 | e-ISSN: 2614-0020

35 (14 days). The light-dark test (LDT) and memory tests of offspring were conducted on PND 36.

Behavior Test

The light/dark test

This test was carried out to see the anxiety-like behavior in mice. All sessions were recorded on video, and exploration time was measured manually with a stopwatch. The parameters calculated are the time of animal exploration in the light compartment and the number of transitions between each compartment.[10] (Figure 2)

Rotarod Test

The accelerating rotarod test assessed motor coordination and motor learning.[11] Mice were placed on a cylinder drum of a rotarod apparatus. The surface of the drum was covered with hard chloroethylene, which does not permit gripping on the surface. Prior to the test, the mice were habituated to remaining on the stationary drum for 1 min. The apparatus was started at an initial speed of 4 to 40 rpm over 2 mins. Mice performed five trials per day, and the test was repeated for three consecutive days to assess motor learning.[12] (Figure 3)

Object Recognition Test (ORT and Object-in-Location Recognition Test (OLT)

This test was carried out to see the memory function in mice. ORT/OLT was done by storing two identical objects. All sessions were recorded on video and exploration time was measured manually with a stopwatch. (Fig. 4) The discrimination ratio is determined by dividing the time spent exploring new objects by the total time exploring both objects during the test session.[6,7,13]

Statistical Analysis

Statistical comparisons were performed by one- or two-way ANOVA followed by the Bonferroni post hoc test using SPSS Software version 22.0 (IBM SPSS, Armonk, New York). Differences were considered significant at $p < 0.05$. All values are presented as the mean ± SEM.

Results and Discussion

Extraction and Phytochemical Screening

The simplicia extraction process was carried out by the maceration method. The maceration method was chosen because it is simple and widely used. Maceration is also the most suitable method to avoid the destruction of thermolabile compounds. The solvent used is

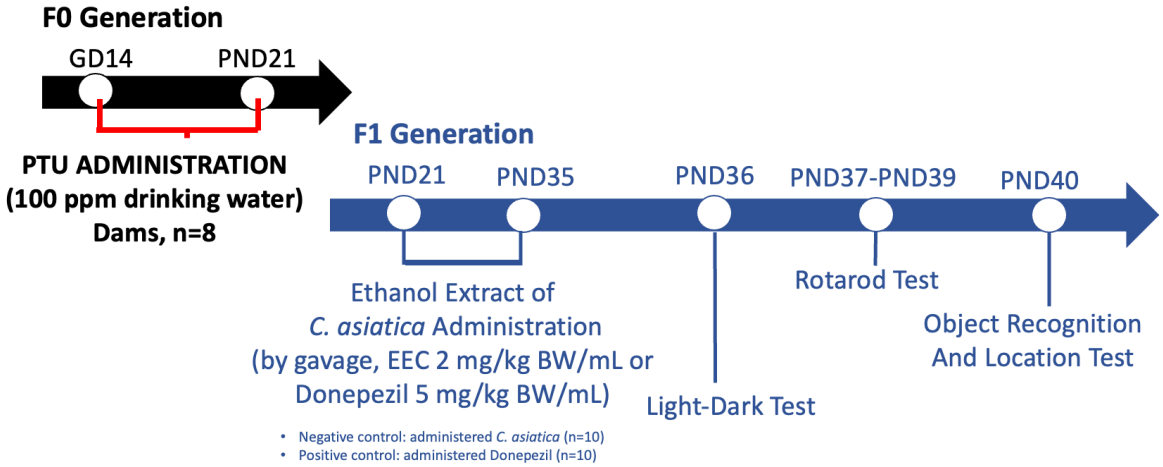

Figure 1. Schematic Drawing of Experimental Procedure

70% ethanol. The extraction process yielded a total of 33.36 grams of thick extract, indicating that approximately 16.68% of the weight of the sample/simplicia was successfully dissolved or obtained during the extraction process. Following the extraction, a phytochemical screening test was conducted to qualitatively determine the chemical classes in the sample/plant material. This screening method predominantly relied on color test reactions with specific color reagents. Detailed results of the phytochemical screening for both the simplicia and the 70% ethanol extract of *C. asiatica* can be found in Table 1.

Animal Model

The hypothyroid mouse model was successfully carried out by giving 100 ppm PTU to the mother mice. The hypothyroid status of the mice was seen from the body weight of hypothyroid mice, which tended to be lower than the control, and the behavioral test results were consistent with the previous study.6 A recent study increased the dosage of PTU to 100 ppm to mimic severe hypothyroidism in clinical cases. PTU was administered to pregnant dams through water bottles from GD 14 to PND 21. On PND 21, dams and female offspring were sacrificed, while the male offspring were continuously administered EEC for 14 days at a dose of 2 mg/kg BW/mL. Gray et al showed that water extract of *C. asiatica*, administrated at the same dose, improved performance in all behavioral tests of aged mice. These findings suggested the potential effects of *C. asiatica* on memory related to the hippocampus and cortex and executive function mediated by the prefrontal cortex.[3]

Behavioral Test Results

We first performed the light-dark offspring. The light/dark test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodents in response to mild stressors, that is, novel environments and light.[14] Activity in the open field was monitored for 10 min. Figure 2a shows that the EEC-treated group stayed longer in the light compartment than the control (control negative= 47.44 ± 12.12 seconds; control positive= 112.17 ± 14.92; EEC= 130.23 ± 8.87. by Bonferroni test, $p<0.001$). Moreover, the EEC-treated group showed less transition between light and dark compartments. (Fig.2b)

Table 1. Results of Simplicia Phytochemical Screening and Pegagan Extract

No.	Compounds	Stain Viewer	Simplisia	Extract
1.	Poliphenolat	FeCl3	+	+
2.	Antraquinone	NaOH	+	+
3.	Flavonoid	Mg Powder and HCl	+	+
4.	Tanin Catechist	Steasny Reagent (formaldehyde, 30% : HCl 2:1	+	+
5.	Tanin Galat	Sodium Acetate + FeCl3	-	-
6.	Triterpenoid and steroid	Liebermann - Burchard	+	+
7.	Alkaloid	Dragendorf	-	-
		Mayer	-	-

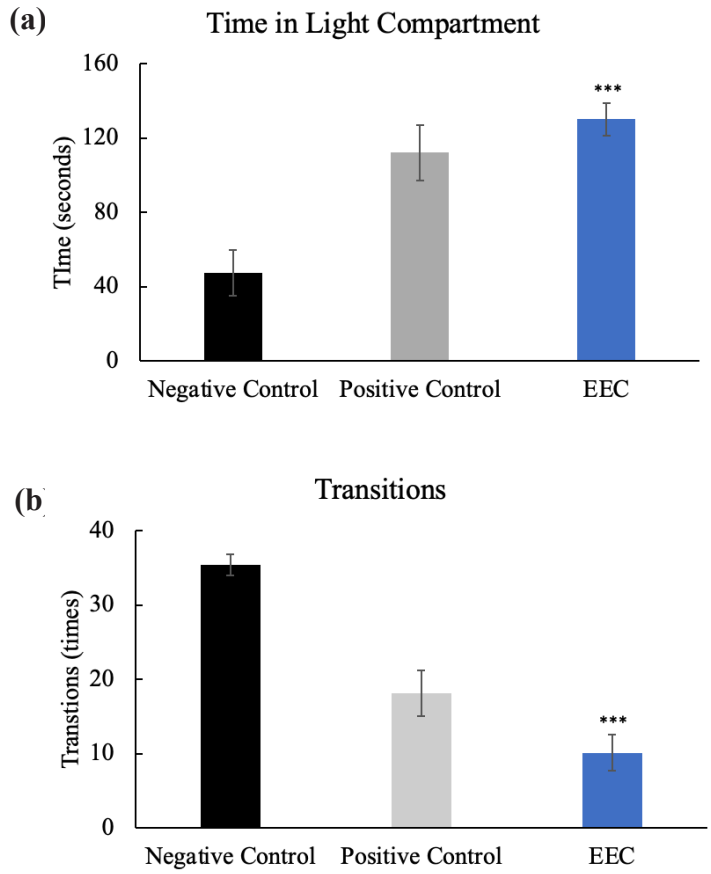

Figure 2. The Effect of EEC Administration in Light-Dark Test.

(a) EEC-treated group stayed longer in the light compartment than the control (by Bonferroni test, $p<0.001$).

(b) The EEC-treated group showed less transition between light and dark compartments. (by Bonferroni test, $p<0.001$).

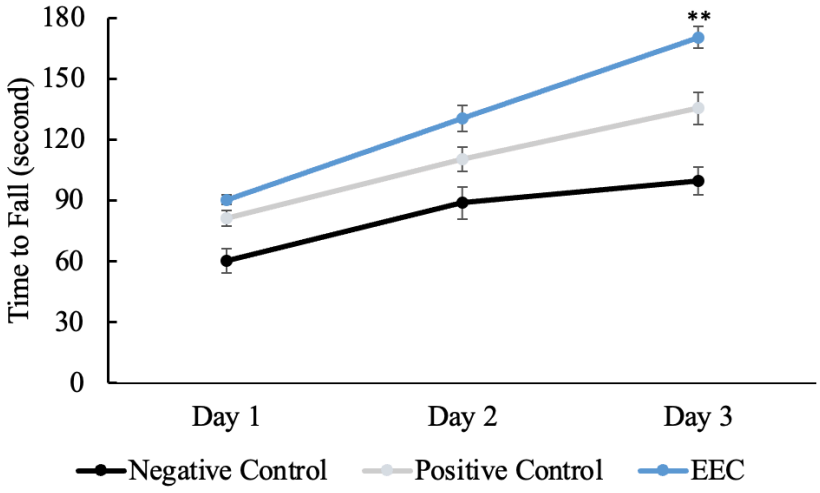

Figure 3. The Effect of EEC Administration in Rotarod Test.

The EEC-treated group showed higher time spent on the rotarod until three consecutive days than the control group (by Bonferroni tes, $p<0.01$)

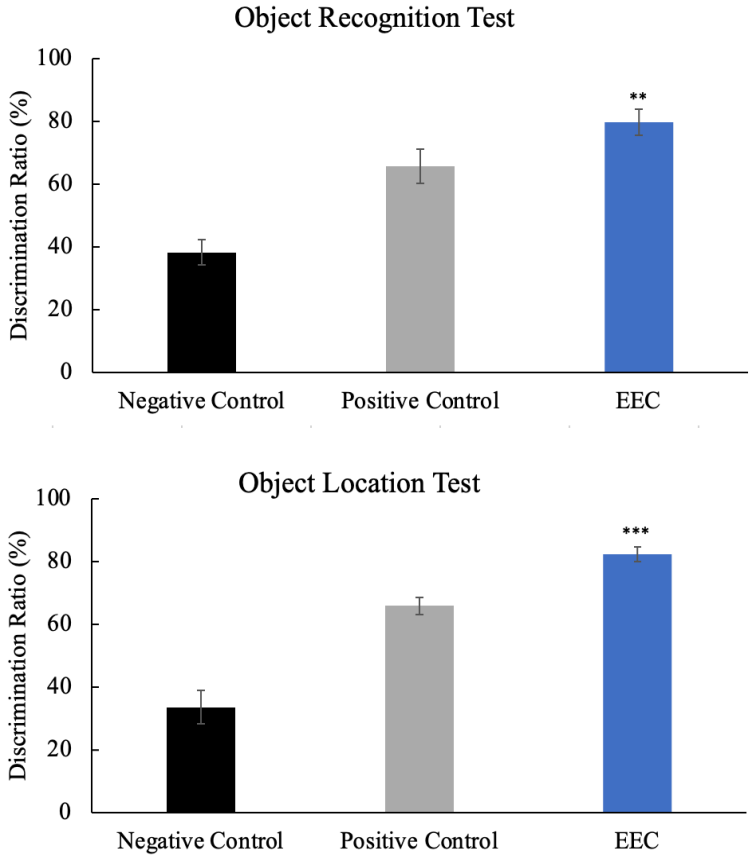

Figure 4. The Effect of EEC Administration in Obejct Recognition Test and Object-in-Location Test.

The EEC-treated group showed a higher discrimination ratio than the control than the control group (ORT, by Bonferroni test p<0.01; OLT, by Bonferroni test p<0.001)

TH plays a critical role in regulating important neurotransmitters, including GABA, serotonin, and norepinephrine. These neurotransmitters are essential for various brain functions and are influenced by TH levels. In hypothyroidism, there is a disruption in the balance of neurotransmitters, which can lead to feelings of depression and anxiety.[15]

Increased anxiety due to hypothyroidism in utero and postnatal and in a thyroid receptor knockout mouse model has been previously reported. A study in mouse adult-onset hypothyroidism in male mice produces a mild anxiogenic effect, possibly due to unliganded receptor actions.[16] The present study confirmed that EEC minimizes the risk of anxiety-like behavior in hypothyroid mice. This result is in line with a study conducted by Wanasuntronwong et al, which showed that the administration of C. asiatica developed an anxiolytic effect in acute and chronically stressed mice.[17] Moreover, human studies have shown that *C. asiatica* supplements can improve mood and arousal in healthy individuals,[18] reduce anxiety-related disorders and stress and depression phenomena significantly.[19] Thus, it can be concluded that *C. asiatica* has good potential in reducing anxiety disorders in hypothyroid mice.

We conducted the rotarod test to examine motor coordination and motor learning. The EEC-treated group showed higher time spent

on the rotarod until three consecutive days than the control group. (Day 1 to Day 3. Negative control: 60.3 ± 6.06 seconds, 88.95 ± 7.95 seconds, 99.55 ± 6.96 seconds; Positive control: 81.2 ± 4.04 seconds, 110. 37 ± 6.00 seconds, 135.31 ± 7.91 seconds; EEC: 90.32 ± 2.45 seconds, 130.21 ± 6.44 seconds, 170.3 ± 5.32 seconds. By Bonferroni test $p<0.01$).

Several brain regions are involved in motor coordination, but the cerebellum plays a major role. TH regulates cerebellum function. Perinatal hypothyroidism reduced the growth and branching of Purkinje cell dendrites and the number of synapses between Purkinje cell dendrites and granule cell axons.[13] The recent study found that the EEC-treated group showed higher time spent on rotarod and developed better motor learning in three consecutive days. The specific mechanisms by which *C. asiatica* enhances motor coordination and learning require further investigation. However, a study conducted by Lee et al.[20] using multiple stroke models in rats suggested that asiatic acid caused a significant reduction in infarct volume and improved neurological outcomes. Further studies are necessary to examine the effect of *C. asiatica* on motor coordination and learning.

We evaluated memory function in mice by performing ORT and OLT. The EEC-treated group showed a higher discrimination ratio than the control (ORT: Negative control= 38.30 ± 4.07%; Positive control= 65.77 ± 5.44%; EEC= 79.75 ± 4.12%. OLT: Negative control= 33.53 ± 5.25%; Positive control= 65.91 ± 2.75%; EEC= 82.30 ± 2.30%).

Previous studies have reported that developmental thyroid hormone insufficiency impairs spatial learning memory in rodent models[7,21,22] This study showed that the administration of EEC improved memory function. The beneficial effects of *C. asiatica* on neuronal health and cognitive function have been well-known both in vitro and in vitro.[2,3,23,24] The previous study declared that the improvement in the object-in-location test might cause by asiatic acid, a major triterpene component of *C. asiatica*,[25] which improves performance in the same task in healthy and impaired rodents. Moreover, improved memory can be associated with increased ARE gene expression, particularly in the hippocampus, suggesting a possible reduction in oxidative stress in addition to the natural cellular response to pathology.[26] This study clearly described that *C. asiatica* improves cognitive function in hypothyroid mouse models.

Conclusion

In conclusion, the findings of this study provide evidence for the effectiveness of *C. asiatica* in reducing anxiety levels and improving cognitive function in a hypothyroid mouse model. In contrast, the donepezil-treated group exhibited slightly better outcomes than the EEC-treated group. Furthermore, our study suggests that *C. asiatica* has the potential for further development as a memory enhancement supplement for individuals with hypothyroidism. Further research is warranted to explore the underlying mechanisms and optimize its formulation for therapeutic use in hypothyroid cases.

Funding

Nil

Conflict of Interest

None declared

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