

# ANTICONVULSANT ACTIVITY OF ETHANOL AND CHLOROFORM EXTRACT OF *Centella asiatica* (L) URB ON PENTYLENETETRAZOLE INDUCED SEIZURE IN MICE

Moch.Saiful Bachri, Fitri Setyaningsih, Nita Dwi kurniawati

Faculty of Pharmacy Ahmad Dahlan University Yogyakarta Indonesia

## Abstract

**Background.** *Centella asiatica* (L.) Urb. (CA) is a traditional herb has many properties such as antianxiety, antistress, sedative and anticonvulsant. The aim of this study is to prove the ethanol extract and chloroform extract of CA have the anticonvulsant activity.

**Method.** This study used 35 Swiss strain male mice aged 6-8 weeks, weight 25-35 g were divided into 7 experimental groups at random. Group I (control) were given 0.5% Na CMC, group II, III, IV were given doses of ethanol extract of the herb CA 100 ; 200 and 400 mg/kg BW, respectively. Group V, VI, VII were given doses of chloroform 100; 200; and 400 mg/kg BW respectively, and group VIII was given phenobarbital doses of 100 mg/kg BW orally. Samples were administered for 1 week. At the 7th day, 1 hour after sample administration, Pentylenetetrazole was induced by intraperitoneally. Generalized tonic clonic was observed. Anticonvulsant effect parameter was collected by observation of onset, duration, frequency of seizures, and the number of death of each group. The data were analyzed with Kruskal-Wallis test, continued by Mann Whitney test.

**Result.** The Data of onset and duration seizures showed result significant difference between groups ethanol extract of CA doses of 200 and 400 mg/kgBW compare to control. The chloroform extract at doses of 200 and 400 mg/kgBW showed significant difference compare to control. Observation duration of seizures showed result that significant difference between groups of chloroform extract of CA herb 100 mg/kg BW, 200 mg/kgBW, and 400 mg/kg BW compare to the control. The frequency of seizure between ethanol and chloroform extract groups compare to control showed no significant difference. While the number of deaths showed result significant difference between groups of ethanol extract of CA doses of 400 mg/kg BW and chloroform extract doses of 400 mg/kgBW compare to the control.

**Conclusion.** The results showed that extract ethanol and chloroform extract of *Centella asiatica* (L.) Urb. Doses of 400 mg/kgBW has anticonvulsant activity.

**Key word :** *Centella asiatica* (L.) Urb., Anticonvulsant, Seizure, Pentylenetetrazole

## INTRODUCTION

Seizures are a neurological problem encountered relatively large. Nearly 5% of children aged less than 16 years had experienced at least one seizure during their lives. Seizures can be caused by many factors, such as illness, fever, electroshock stimulation or influence of chemicals (Ardian, 2008). One of the chemicals used to stimulate the occurrence of seizures is Pentylentetrazole (PTZ) (De Lima and Rae, 1991). PTZ is often used in the research model for the induction of seizures and provides a system for the study of the incidence of epilepsy (Becker et al., 1992).

Seizures therapy with anticonvulsant medication sometimes affects cognition (Tonny et al., 1993). Because of side effects and therapy costs are relatively expensive, so a new treatment option that is safe, effective and selective to suppress seizures is critical to the effort. Treatment using plants to treat seizures became more popular (May et al., 1992).

*Centella asiatica* (Umbelliferae, CA) has been used in various parts of India for different ailments like headache, body aches, insanity, asthma, leprosy, ulcers, eczemas and wound healing (Chatterjee et al., 1992; Chopra et al., 1956; Shukla et al., 1999; Suguna et al., 1996). In the studies of pharmacological effects, CA showed anticonvulsant activity caused to prevent cognitive decline (Gupta et al., 2003). Ethanol extract of CA have sedative effects (Amalia, 2009).

Katere and Ganachari (2001) have reported that the CA has an anticonvulsant activity with an associated decrease in the oxidative stress in the lithium pilocarpine model of status epilepticus. The administration of CA (300 mg/kg orally) decreased the PTZ-kindled seizures and showed improvement in the learning deficit induced by PTZ kindling as evidenced by decreased seizure score and increased latencies in passive avoidance behavior (Gupta et al., 2003).

Therefore, the present study was aimed to evaluate the effect of ethanol and chloroform extract of CA on male mice induced PTZ.

## METHOD

### Animal

Study was carried out using male swiss mice weighing 25-35 g. The mice were group housed in cages and were adapted for 1 week. They were allowed free access to get fed, tap water ad libitum. The testing and observations of anticonvulsant effects were done at Pharmacology Laboratory of Pharmacy Faculty of Ahmad Dahlan University Yogyakarta.

### Maceration

CA was obtained from Ambarawa Central Java, were sorting, washing, drying, and making powder. The powder then macerated with 70% ethanol, and chloroform 3 times for 3 days, then filtered and evaporated to form a thick extract.

### Anticonvulsant Test

This study used 35 Swiss strain male mice aged 6-8 weeks, weight 25-35 g were divided into 7 experimental groups at random. Group I (control) were given 0.5% Na CMC, group II, III, IV were given doses of ethanol extract of the herb CA 100 ; 200 and 400 mg/kg BW, respectively. Group V, VI, VII were given doses of chloroform 100; 200; and 400 mg/kg BW respectively, and group VIII was given phenobarbital doses of 100 mg/kg BW orally. Samples were administered for 1 week. At the 7th day, 1 hour after sample administration, Pentylentetrazole was induced by intraperitoneally. Generalized tonic clonic was observed for thirty minute. Anticonvulsant effect parameter was collected by observation of onset, duration, frequency of seizures, and the number of death of each group (Visweswari, et al, 2010) and modified with research Amabeoku (1998).

**Statistical analysis**

The data are represented as mean±SD. The data was analyzed by Kruskal wallis test followed by Man Whitney U test.

**RESULT AND DISCUSSION**

Anticonvulsant effects of CA can be seen from the time of onset, duration, number of seizures and the number of deaths in test animals each group. The average onset, duration, number of seizures and the number of deaths (mortality) in the treated group compared with controls. The mean ±SD of onset, duration, frequency of seizures and the percentage of the number of deaths (mortality) in each group are shown in Table I.

smaller duration than the control group and significantly different.

On the frequency of seizures, all doses of EtOH extract of CA; CHCl<sub>3</sub> extract (200 and 400 mg/kgBW) and phenobarbital group had a smaller frequency than the control group. But statistically not significantly different

The administration of EtOH and CHCl<sub>3</sub> extract of CA decreased the number of deaths compared with control. At doses of 400 mg / kgBW of EtOH extract and CHCl<sub>3</sub> extractsof CA reduced the mortality by 80% and very significant reduction in mortality when compared with the control group that all animal tests was death. It can be demonstrated that administration of EtOH extract and CHCl<sub>3</sub> extract of CA delayed onset, reduced the

**Tabel I. Mean ± SD onset, duration, frequency seizure and percent of mortality on male mice induced PTZ**

Group	Doses	General tonic clonic seizure			Frequency seizure
		Onset (second)	Duration (second)	Frequency seizure	
Control	-	85,00±2,65	887,00±233,50	2,40±0,89	100
EtOH extr	100	84,00±6,08	632,67±128,75	2,20±0,84	60
	200	110,33±1,16*	487,67±143,97	2,00±0,71	40
	400	116,00±3,46*	25,00±6,22*	1,40±1,14	20*
CHCl <sub>3</sub>	100	75,20±8.82	357,00±139.10	2,60±0,55	60
	200	99,30±6,02*	282,00±17.38*	1,80±0,84	60
	400	112,80±5.32*	190,30±32.73*	1,50±1,00	20*
Phenobarbital	100	1800,00±0,00*	0,00±0,00*	0,00±0,00*	0*

\* = P <0.05 significantly different compared with control group.

Table I showed the administration of EtOH extract of CA (200; 400 mg / kgBW), CHCl<sub>3</sub> extract of CA (200; 400mg/kgBW) and phenobarbital groups on onset significantly different compare to control group. The treatment of ethanol and chloroform extract indicated can delay the onset of seizures.

The EtOH extract of CA (400 mg / kgBW); CHCl<sub>3</sub> extract of CA (200; 400 mg/kgBW), and phenobarbital group had a

duration and frequency of seizures and decreased the mortality caused by PTZ treatment. The effect showed dependent doses.

The control group was treated with 0.5% CMC-Na has no effect as an anticonvulsant. Balamurugan, et al (2008) reported 0.5% of CMC-Na did not affect seizures. The control group cannot delay the onset, decreased duration, reduced frequency of seizures and mortality in PTZ-induced male mice.

Phenobarbital showed significant differences with other groups. Phenobarbital has the greatest anticonvulsant effect compared with other groups characterized by the occurrence of seizures in this group of mice and was able to delay the onset, shorter duration, and reduce the frequency of seizures, and decreased the mortality in PTZ-induced mice. Phenobarbital has been clinically proven to have anticonvulsant activity by supporting the inhibitory effect of GABA (Kondziella et al., 2002).

Pentylentetrazole (PTZ) was a chemical convulsant frequently used in the study of seizures was known the seizure mechanism by decreasing the inhibitory activity of GABA (Bradford, 1989). The reduced inhibition of the GABA neurotransmitter caused a seizure (Manno, 2003). PTZ injection intraperitoneally in mice lead to general tonic-clonic seizures (Brito, et al., 2006). In PTZ-induced seizure, the EtOH extract and CHCl<sub>3</sub> extract of CA can protect mice against seizures.

The results of the study have demonstrated that EtOH extract and CHCl<sub>3</sub> possessed anticonvulsant activity on the animal models investigated and this provides a rationale for its use in traditional medicine for the management of epilepsy.

## CONCLUSION

The results showed that extract ethanol and chloroform extract of *Centella asiatica* (L.) Urb. Doses of 400 mg/kgBW has anticonvulsant activity.

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