ANTI CONVULSANT EFFECT OF *Centella asiatica* FRACTIONS AND HISTOPATOLOGY STUDY OF LIVER AND KIDNEY

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Abstract

Background. *Seizure is an neurologik issue that relatively much be found. Treatment with conventional chemical remedies are more expensive beside their limited distribution. conventional remedies have a lot of side effects. Therefore, needed to find a new alternative treatment that more safe, effective and selective to suppress seizure as anti convulsant. Pennywort (Centella asiatica (b) urb) has effect as anti convulsant. Ethanol extract of Pennywort herb also known could have sedative effects on mice because it contains with brahminoshide and brahmoshide glycosides using their cholinergic mechanism.*

Objective. *The aim of this research is to know whether ethyl acetat fraction and unsoluble ethyl acetate fraction of pennywort herb can be used as anti convulsant.*

Methods. *This research conducted using mice which divided in eight groups which consisted of 7 mices per group. The classification of the group consists of negative control (suspension of CMC 5 %), ethyl acetate fraction group with dose 100mg/KgBW, 200mg/KgBW, 400mg/KgBW, unsoluble ethyl acetate fraction group with dose 100mg/KgBW, 200mg/KgBW and 400mg/KgBW, and positive control group (fenobarbital 100mg/KgBW). To make convulsion condition, mice induced using PTZ dose 80Kg/BW. This test lasted for 7 days. The result was analyzed by using post hoc test and Mann Whitney method were also used to compare each sample. The significant value was accepted if $P<0.05$.*

Outcome measured. *The parameters of anti convulsant including time of duration. The histopatology tested on liver and kidney.*

Results. *Unsoluble ethyl acetate fraction dose 400 mg/KgBB has the ability to reduce duration time. Histopathology test showed that ethyl acetat fraction dose 100mg/KgBW and unsoluble ethyl acetat fraction dose 400mg/KgBW significantly increase repairment of kidney damage induced by PTZ.*

Conclusion. *Conclusion that not all fractions have the ability as anti convulsant.*

Keywords : *Anti convulsant, Centella asiatica, ethyl acetate fraction, unsoluble ethyl acetate fraction, Phentylenetetrazole (PTZ).*

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INTRODUCTION

A seizure is a neurologic issue relatively much be found. Almost 5 % child under 16 years old at least experienced once seizure during his life (Schweich and Zempsky, 1999). Seizure treatment with anticonvulsant drug sometimes affect on cognitive (Aldenkamp *et al.,* 1993). Although it is known that the result of seizures conventional treatment is so beneficial clinically but there has cognitive side effect of the drug at dose of therapy. Because their side effects and the treatment cost, the new treatment options that are safe, effective and selective to suppress seizures is crucial to trying to accomplish.

One of medicine plant which alleged has an effect as anticonvulsant is pennywort (centella asiatica (b.) Urb.). Some studies on the pharmacology effects of pennywort, it is known that those plant indicates the activity as anticonvulsant with the additional benefit of preventing cognitive decline (Gupta *et al.,* 2003).

The ethyl acetat and unsolube ethyl acetat fractions of pennywort against onset time, duration time and the number of seizures, mortality, and also liver and kidney histophatologi on male mice induced PTZ was studied in this reserach. So it can be used as medicine alternative which effective as anticonvulsant drug and can be useful in the development of traditional medicine in Indonesia.

METHODS

Materials and Instruments

Materials: Pennywort herb (*Centella asiatica* (L) Urb), male *Swiss* mice (5-6 weeks) with body weights approximately 25-35 g, ethanol 70%, ethyl acetat, penthilenetetrazole (PTZ) (Sigma Co), phenobarbital (Bratachem) dan CMC Na (Bratachem), NaCl 0,9%, formaldehyde 10%, hematoxilin-eosin stain.

Instruments: milled machine, sieve which mesh number 40, oral injection, vacum rotary evaporator, analytical scale, spectrophotometer UV-1800 Shimadzu.

Sample Preparation

The pennywort powder sifted by using mesh 40 then will be extracted. The process of maceration is carried out by using ethanol 70% for 24 hours until the solvent is clear sight. Fractination of ethanol extract using ethyl acetate solvent, where there will be two parts those are the soluble part of ethyl acetate fraction and insoluble ethyl acetate fraction.

The sample of ethyl acetat and insoluble ethyl acetat fractions tested for flavonoid total using spectrophotometer UV-1800 Shimadzu, where determination of the flavonoid total according to the Chang *et al* method's (2002).

Adaptation of the Mice

Adult male *Swiss* mice with body weights approximately 25-35g were kept in room temperature and standard (natural) photoperiod of approximately 12h of light alterating with approximately 12h of darkness. The mice were maintained on standard mice feed and potable water which were made available *ad libitum*.

The test of this research was conducted to 8 groups of mices which consisted of 7 mices per group. Each group consisted of positive control (phenobarbital 100mg/KgWB), negative control (CMC 0,5%), ethyl acetat fraction group with doses 100mg/KgBW; 200mg/KgBW; 400mg/KgBW, insoluble ethyl acetat fraction group with doses 100mg/KgBW; 200mg/KgBW; 400mg/KgBW. Factions was given for seven days while positive control group phenobarbital given at the 7th. On the 7th day one hour after PTZ induction, observation was done on onset, duration, number of seizures, mortality and histopathology observation of liver and kidney.

Statistical analysis

Statistical analysis was done by using Mann-Whitney Results are expressed as the

mean ± SD. Statistical significance was defined as $P < 0.05$.

RESULTS AND DISCUSSIONS

From the maceration process, rendemen of extract was obtained 18,54%. Fractination was done on pennywort extract using ethyl acetat which having rendemen value about 4,12%. Insoluble ethyl acetat fraction has rendemen 13,4%. Rendemen value of insoluble ethyl acetate more higher than rendemen of ethyl acetate fraction. This is because ethanol 70% extract more polar than ethyl acetate fractions. Was known that the largest component in ethanol extract compound is triterpen compounds that are polar.

Total Flavonoid

The levels of total flavonoids was measured on ethyl acetat and insoluble ethyl acetat fractions using UV-Vis spectrophotometer. The purpose of determination of flavonoid total levels is to fullfil standardization extract or faction. Differences of growing places and environmental conditions affect the levels of active substances from plants even on the same plant species. The largest of total flavonoid level contained in ethanol extracts there is 3,419%. Total flavonoids of insoluble ethyl acetate fraction (1,465%) have higher levels than ethyl acetate fraction (0,323%). The polarity of ethyl acetate is lower than ethanol 70%, so not all of the extract could dissolved in ethyl acetate solvent which called insoluble ethyl acetat fraction.

Have been researched previously by zainol, et al (2009) that some compounds of flavonoids contained in pennywort are naringin, routine, quercetin, catechin, luteolin, and apigenin, kaemferol. This flavonoid compounds have the effect as an antioxidant. Flora and gupta's (2007) research concluded that flavonoid of a fraction pennywort to give the effect protection against toxicity cells of the neurons in mice by antioxidant mechanism.

So can be concluded that high flavonoid can increase the activity of the neuron cell protection, in the end can serve as agents of an anti seizure.

Ethyl Acetat and Insoluble Ethyl Acetat Fractions of Pennywort Herb As Anticonvulsant

Has been researched before by Ganachari *et al* (2004) that ethanol extract 100mg/KgBW showed potential to extend sleeping time on experiment animals were given sodium pentobarbiton and has the activity as anticonvulsant. Hopefully when ethanol extract was fractionated with ethyl acetat, it will get specific compound that will enhance the activity of pennywort herb as an anticonvulsant.

None of the groups that have different values significantly to negative control, this indicates that all the factions have not been able to reduce onset time of seizure activity. Insoluble ethyl acetate fraction group dose 400mg/KgBB showed decreasing the time of duration better than the other groups. It characterized by significant differences of duration time compared to a negative group.

Table I .Onset and Duration Average Time

Group	Onset (second)	Duration(second)
NEGATIVE CONTROL	172,14±133,74*	333,33±86,22*
POSITIVE CONTROL	0,00±0,00#	0,00±0,00#
EAF 100mg/KgBB	120,83±48,21*	530,00±29,44*#
EAF 200mg/KgBB	130,00±20,98*	507,50±137,75*
EAF 400mg/KgBB	155,00±28,87*	460,75±120,98*
IEAF 100mg/KgBB	220,00±98,99*	569,00±209,36*
IEAF 200mg/KgBB	166,00±108,77*	700,00±124,90*#
IEAF 400mg/KgBB	139,17±80,40*	138,00±131,42*#

*p<0,05 significantly different to positive control (phenobarbital), # p<0,05 significantly different to negative control.

The content of compounds such as brahmoside, brahminoside and other triterpen compounds in insoluble ethyl acetate fraction have possibility role to decline duration time on seizure mice

(Amalia, 2009). In addition, a polar compounds such as flavonoids also have anticonvulsant effect and lowering anxiolytic (Almeida et al., 2008).

Number of Seizures and Mortality

Table II. Number of Seizures and Mortality percetae in Each Group

Group	Number of Seizures	Mortality (%)
NEGATIVE CONTROL	1,71±0,95*	100*
POSITIVE CONTROL	0,00±0,00#	0#
EAF 100mg/KgBB	2,29±0,76*	71*
EAF 200mg/KgBB	2,00±0,58*	57*
EAF 400mg/KgBB	2,00±1,00*	43#
IEAF 100mg/KgBB	2,43±0,79*	100*
IEAF 200mg/KgBB	1,50±0,55*	57*
IEAF 400mg/KgBB	1,57±0,98*	71*

*p<0,05 significantly different to positive control (phenobarbital), # p<0,05 significantly different to negative control.

From the data showed that none of the giving fraction group can decrease the frequency of seizures. Meanwhile, the group that showed an improvement in the percentage of mortality is ethyl acetate fraction group dose 400mg/KgBW. The certain mechanism of levels of mortality on the fractions giving are different wasn't found. The limited number of mice using in research allows one of many determinant steadiness factor of data.

Liver and Kidney HistopatologyTest

Examination using Hematoxylin-Eosin staining (HE) and the organs are carried out in the laboratory of Pathology Anatomy Faculty of Medicine Gadjah Mada University to made histology preparations of liver and kidney. Liver histopathology test in all groups there were no significant differences at both the negative and positive control.

Table III. Rapairment Percentage of Liver and Kidney

Group	Hepar Histo(%)	Kidney Histo (%)
NEGATIVE CONTROL	0	0*
POSITIVE CONTROL	20	80#
EAF 100mg/KgBB	20	100#
EAF 200mg/KgBB	20	20
EAF 400mg/KgBB	40	40
IEAF 100mg/KgBB	20	40
IEAF 200mg/KgBB	60	80#
IEAF 400mg/KgBB	40	100#

*p<0,05 significantly different to positive control (phenobarbital), # p<0,05 significantly different to negative control.

Whereas in kidney histopathology test, ethyl acetate fraction group dose 100mg/KgBW and insoluble ethyl acetate fraction dose 400mg/KgBW given a very significant improvement over the negative control group.

Low percentage of liver repairing happent in entire group include the positive control group, it indicate that the induction of PTZ has damage to liver or leaning to hepatotoxic. Several groups of fractions also shows the percentage of bad improvement on the kidney. Besides it hepatotoxic characteristic, PTZ also allows to nefrotoxik on mice kidney.

CONCLUSION

Insoluble ethyl acetate fraction dose 400 mg/KgBW has the ability to reduce duration time however not be able to extend onset time, minimizing frequency of seizures, and decrease the number of mortality. Mortality significantly decreased at dose 400mg/KgBW of ethyl acetate fraction. Ethyl acetate fraction dose 100mg/KgBW and insoluble ethyl acetate faction dose 400mg/KgBW showed improvements against kidney damage induced by PTZ. However, do not indicate a significant improvement of liver damage

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