NEURO-PROTECTION AND NEURO-THERAPY EFFECTS OF *Acalypha indica* Linn. WATER EXTRACT *EX VIVO* ON *Musculus gastrocnemius* Frog

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Abstract

The studies of neuro-protection and neuro-therapy effects of *Acalypha indica* Linn. water extract *ex vivo* on *Musculus gastrocnemius* frog have already done at three Departments in Faculty of Medicine, University of Indonesia. The experimental studies were done on 2 groups of frog for neuro-protection and neuro-therapy effects. Each group of frog was divided into 7 subgroups of application, 4 samples each. There were 5 subgroups of doses: 5; 10; 15; 20; 25 mg and 2 subgroups as control. Pancuronium bromide 0.2%, 4 mg, was used for a positive control as muscle relaxant. Neuro-protection study was done as follow: ringer – extract – pancuronium bromide, and neuro-therapy study was ringer – pancuronium bromide – extract, respectively. The parameters measured in these studies were the electrical activities such as amount and duration (second) of re-polarization; depolarization, resting potential, and the height of spike after electrical stimulation at 5 mV. Neuro-protection effect of extract was determined by the ability of muscle to show the electrical response after incubating with pancuronium bromide for 10 minutes, and after incubating with extract for 10 minutes for neuro-therapy effect. In the dose of 15 mg and 20 mg/mL of *A. indica* Linn. extract showed better activities than the dose of 25 mg of extract, both as neuro-protection and neuro-therapy effects, but statistically its have not a significant difference. This study should be followed by an *in vivo* experiment on frog and it would be done in pharmacokinetic and pharmacodynamic studies on other animal models.

Keywords: Acalypha indica Linn*. ex vivo, neuro-protection, neuro-therapy*

1. Introduction

At the last decade, the incidence of the central nervous system's disorder that usually known as stroke tend to be increased which is associated with hypertension. Misbach and Kalim have reported that the disease [1], now, was occur on younger patient and it's to be the third killer disease after coroner disease and cancer.

In the year of 2006, the morbidity of stroke caused by thrombus occlusion on the brain was 83%, but 70% from hypertension patient usually could suffer stroke after bleeding in their brain.

Out cases of stroke patient could be treated without any sequelae, but the rest patient have had suffered paralyze, hemi or paraplegia which it could not be treated by a conventional drug, such as piracetam.

That convention drug is more expensive and more side effects rather than herbal medicine, such as anxiety, insomnia, and depression [2].

To solve that problem, more patient using the alternative drugs, not only acupuncture treatment but also using the traditional herbs, such as sambiloto (*Andrographis panniculata* Burms.f), mahkota dewa (*Phaleria macrocarpa* Scheff.), and mengkudu (*Morinda citrifolia* L.) [3-5]. All treatment using herbs generally used to reduce hypertension, not for hemi or paraplegia as neurological disorders.

The traditional herbs that empirically reducing the hypertension complication is *A. indica* Linn. which is known in Indonesia as akar kucing. It has already been used by whom with suffer hemi or paraplegia, but it lacks reporting data.

Although several data of their efficacy have proven as anti-urecemic [6], and anti diabetic agent [7], their effects on neuromuscular junction as neuro-protection and neuro-therapy, *ex vivo* and *in vivo*, have not been proven yet.

On the preliminary study, this extract at the dose of 25 mg showed neuro-protection and neuro-therapy effects

on neuromuscular junction of frog. It was defined as neuro-protection because the extract was given before incubating with d-tubocurare 2% as a muscle-relaxant, and vice versa for neuro-therapy effect [8].

Based on the preliminary study, this study were proposed to prove the neuro-protection and neuro-therapy effects of the extract in the dose of 5 mg to 25 mg, *ex vivo* on *M. gastrocnemius* frog.

Because of the side effects of d-tubo-curare, this drug was replaced by pancuronium bromide 0.2% as a muscle relaxant. The first drug, now, is unavailable at the drug dispensary. The other reasons are that pancuronium bromide 0.2% is more potent and safer than d-tubo-curare [9].

Neuro-protection and neuro-therapy effects of *A. indica* water extract in this study were a novel study. Hopefully, their results would be used to solve the neurological disorder from hypertension complication such as hemi or paraplegia. This study should be developed to declare the mechanism of action of this extract do to pharmacokinetic and pharmaco-dynamic effects and that its would be followed by a clinical study.

If this extract have been proven as neuro-protection and neuro-therapy effects, this study would be continued to prove whether it's also has the same effect *in vivo* on frog. The last result hopefully to be used in patient with neurological disorders to increase their quality of life. Meanwhile, this herb could be cultured in and promoted to their status from wild herbs to standardized herbs or may be come phyto-pharmaca. Their effects could be used in patients who have any neurological disorders.

The Aims of this Study. General: To prove that *A. indica* water extract have neuro-protection and neuro-therapy effects *ex vivo*. **Specific**: To prove that *A. indica* water extract have neuro-protection and neuro-therapy effects *ex vivo* on *M. gastrocnemius* frog which have paralyzed with pancuronium bromide 0.2%.

2. Methods

This study has been done at the Department of Medical Pharmacy, Physiology, and Department of Medical Physics Faculty of Medicine, Indonesia University (FKUI), for 10 (ten) months, since June 2007.

Material. Fifty six frogs were purchased from Dep. of Physiology FKUI; Pancuronium bromide 0.2%; Sterile Ringer sol. and aquabidest, reagents; Herbs *A. indica* were obtained from Depok, West Java and it's already determined by Bogoriensis Laboratory LIPI, Bogor.

Equipment. Rotavapor Bűchi, disposable syringe 3 mL, electrical muscle activity recorder (ECG/Oscilogram modification), minor surgery, spectrophotometer, interface 750 data studio program.

Research Steps

Step I. **Extract Preparation:** Simplisia (dry root of *A. indica*) 10% in aquabidest, were decocted twice for 30 minutes; Extract was dried using rotary evaporator, and weighted to obtain its rendement; The dried extract was diluted with aquabidest, to make a colloidal solution, for obtaining a final concentration of 5 – 25 mg/Ml; Part of the dried extract was studied for standard phyto-chemistry

Step II. *Ex Vivo* Study: Fifty six frogs were divided into 2 groups for neuro-protection and neuro-therapy studies. Each group were divided into 7 subgroups as follows: 2 subgroups of negative control, consist of a group without extract and a group without pancuronium bromide; 5 subgroups of doses: 5 mg; 10 mg; 15 mg; 20 mg; and 25 mg. Based on Federer formula, every subgroup consisting of 4 frogs; The dose of pancuronium bromide was 4 mg/sample; *M. gastrocnemius* and *Nervus ischiadicus* were excised and immersed immediately in Ringer solution before studying. The point area of studying is a neuromuscular junction.

Group I. Neuro-protection study: 1) *M. gastrocnemius* and *N. ischiadicus* left and right were immersed in Ringer solution before studying; 2) Throw the solution out and then stimulate the nerve with electrical sources of 5 mV (See Fig.1). The electrical muscle activities were recorded computerized in Interface 750 Data Studio Program; 3) Throw the extract into special a equipment and left in for 10 minutes. Stimulate the nerve with electrical sources of 5 mV. The electrical muscle activities were recorded and repeated 4 times every minute. Throw the extract out and washed it with Ringer solution; 4) Four milligram (2 mL) of pancuronium bromide were throw in to sample and left in for 10 minutes. Stimulate the nerve with electrical sources of 5 mV. The electrical muscle activities were recorded and

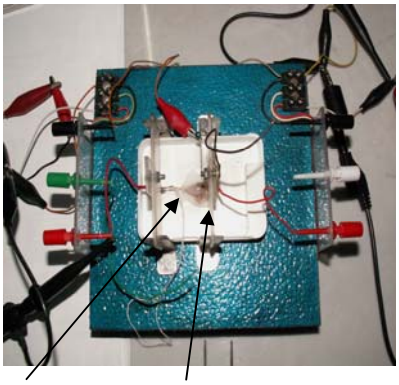


Figure 1. The Equipment of the Electrical Muscle Stimulation that Has Connected to Interface 750 Data Studio Program

repeated 4 times every minute; 5) The lower dose of extract which has an electrical muscle activity will be used next to *in vivo* study.

Group II. Neuro-therapy study: 1) The sequent of 1 – 2 on group I was followed similarly; 2) Four milligram (2 mL) of pancuronium bromide were throw in to sample and left in for 10 minutes. Stimulate the nerve with electrical sources of 5 mV. The electrical muscle activities were recorded and repeated 4 times every minute. Throw pancuronium bromide out and washed it with Ringer solution; 3) Throw the extract into a special equipment and left in for 10 minutes. Stimulate the nerve with electrical sources of 5 mV. The electrical muscle activities were recorded and repeated 4 times every minute.

The measuring variable are: the amount and duration (second) of depolarization, re-polarization, and resting potential, and the height of spike after stimulation.

Statistical Analysis. The electrical muscle activities were analyzed using one way Analysis of Varian with the probability values (p) of 0.05 [10].

3. Results and Discussion

The water extract of *A. indica* from three times preparation to give rendement as 1.85%; 2.4%, and 1.9% respectively. These results have different in other study from FMIPA UI that has achieved 12.3% [6]. The differences may be caused by several reasons, i.e. the age, the location, and the dried processing of herbs.

From the third samples of *A. indica*, all samples have shown Phyto-chemistry results as same as other results that contains [6,11]: alkaloid, saponin, and tannin, although its not prepared at the same day and not obtained from the same location (Table 1).

The roots of *A. indica* were used in this study because its proven empirically for curing by whom with neurological disorder. Unfortunately, all results have not recorded yet, so that, we can not determine first, the dose and second, the duration of their application to the research models. Other researcher has succeded to isolate the active component of *A. indica* which is called acalyphin (Figure 2) and the active component from their root is stigmasterol [11].

Table 1. Phyto-Chemistry Results from Three Kinds of Water Extract of *A. indica*

Extr.	Alkaloid	Terpenoid/ steroid	Flavonoid	Saponin	Tannin
A	+ + +	- / -	-	+ +	+ + +
B	+ + +	- / -	-	+ +	+ + +
C	+ + +	- / -	-	+ +	+ + +

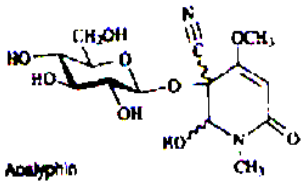


Figure 2. The Chemistry form of Acalyphin [11]

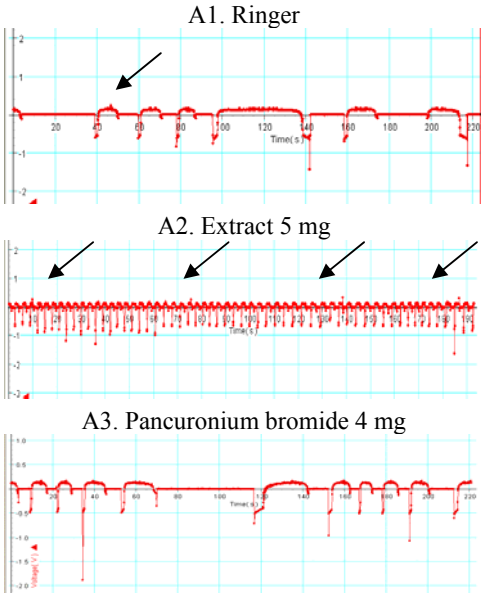


Figure 3A. A1-A3 = in the Dose of 5 mg

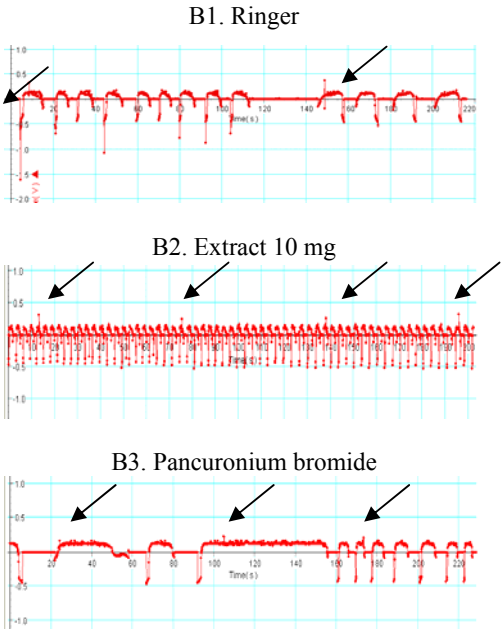
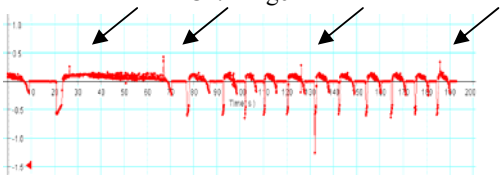
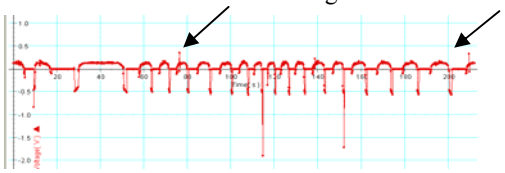


Figure 3B. B1-B3 =in the Dose of 10 mg

C1. Ringer



C2. Ekstrak 15 mg



C3. Pancuronium bromide

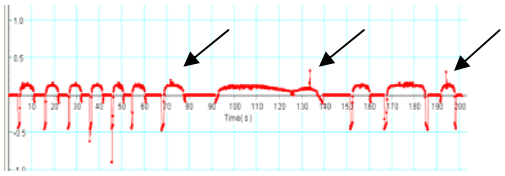
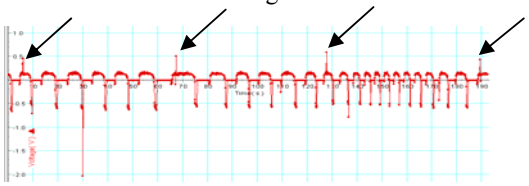


Figure 3C. C1-C3 = in the Dose of 15 mg

D1. Ringer



D2. Extract 20 mg

D3. Pancuronium bromide

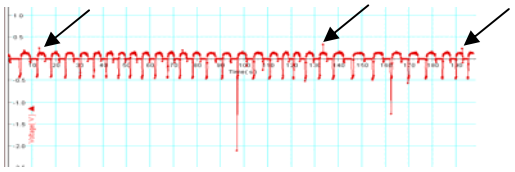


Figure 3D. D1-D3 = in the Dose of 20 mg

Neuro-therapy effects have recorded as follows: ringer – pancuronium bromide – extract. These results were shown at Figure 4.

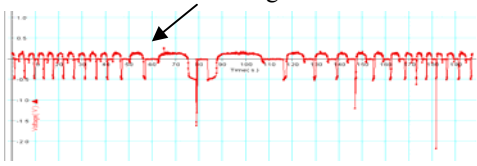
Neuro-protection effects were recorded from samples as follows: ringer – extract – pankuronium bromide. The results were shown at Figure 3A-3E. The arrow sign was the spike that has obtained from muscle after stimulation to there nerve.

Neuro-protection and neuro-therapy studies which have done on *m. gastrocnemius* frog were based on several researchers who already used this model to prove the several kinds of study, such as the electrolyte effect or the drug effect on muscle, nerve, or on neuromuscular junction of frog [12,13].

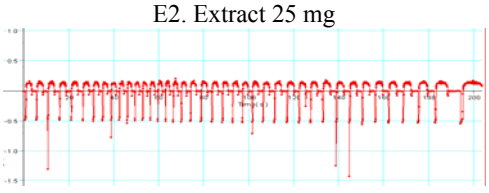
The arrow sign in Figure 3 and 4 is the sign of muscle contraction after electrical stimulating of 5 mV, but the height of spike has not shown optimally because of the problem of the equipment. The electrical stimulation was installed manually, that's why the spike, time by time, has not achieved and recorded accurately. There fore, statistically, these results have not given a significant different, although in the dose of 15 – 20 mg, the electrical muscle activities showed better than other dose of 5 mg and 25 mg. The possibility reason that's why at the dose of 25 mg has not shown optimally activity is that their receptor of the neurotransmitter, especially in neuromuscular junction, could not able to uptake the large dose of the extract.

For that reason, this research should be continued to prove it and other research would be proposed for achieving the best result. So, we have to improve the standardized of the herbs, the equipment, and the animal models.

E1. Ringer



E2. Extract 25 mg



E3. Pancuronium bromide

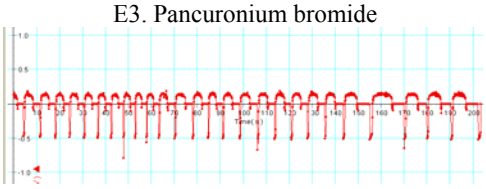


Figure 3E. E1-E3 = in the Dose of 25 mg

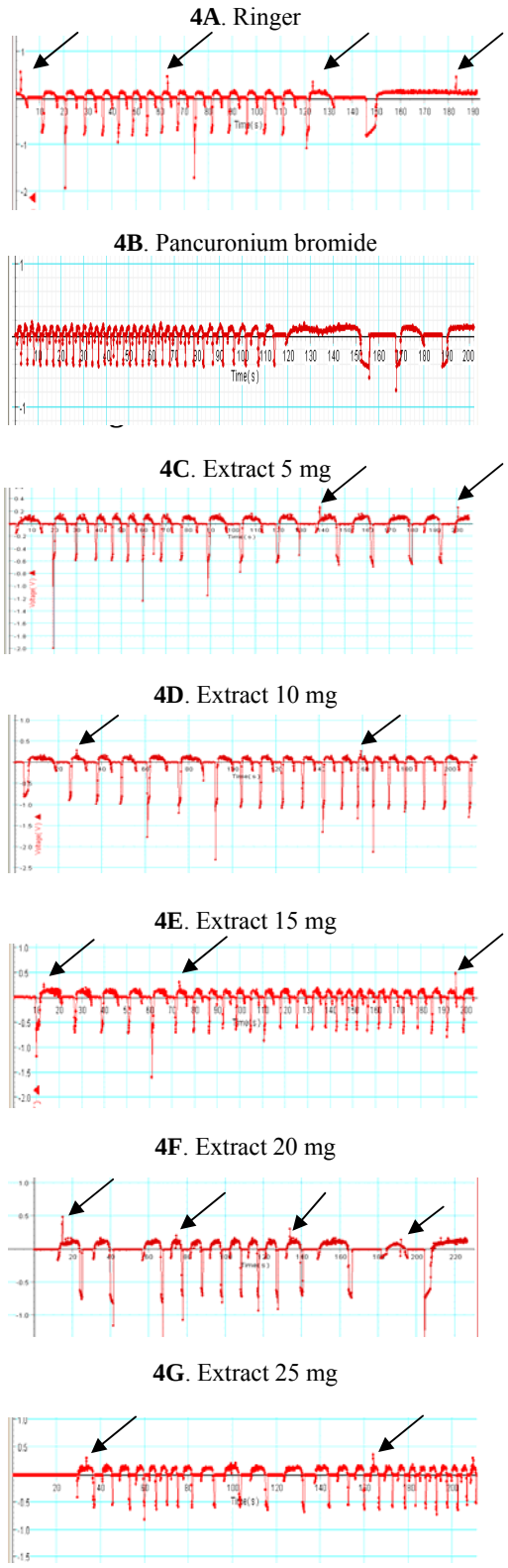


Figure 4. **4A-4G** = Neuro-Therapy Effects Have Recorded as Follows: Ringer – Pancuronium Bromide – Extract

4. Conclusion

This study has proved that the water extract of *A. indica* (akar kucing) have neuro-protection and neuro-therapy effects *ex vivo* on *M. gastrocnemius* frog at the dose of 15-20 mg, but statistically its have not shown significantly different.

Suggestion. Although the extract of *A. indica* (akar kucing) have proven as neuro-protection and neuro-therapy, this study should be continued to prove their effects *in vivo* on frog or other animal models. The study in pharmacokinetics and pharmaco-dynamic on two kinds of animal model are also needed. All results are needed to increase the status of akar kucing from the wild herbs to be come the standardized herbs or phytopharmaca. For all above, we have to improve the standardized of the herbs, the equipment, and the animal models.

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Reference

1. Misbach Y, Kalim H. *Stroke Mengancam Usia Produktif*. (online). *http://www.medicastore.com/stroke/≠atas*. 2007.
2. Reynolds JEF (ed.). *Piracetam* In: Martindale's The Extra Pharmacopeia. 31st ed. London: 1995: 1742.
3. Dalimarta S. *Atlas Tumbuhan Obat Indonesia.* Jilid 1. Jakarta: Trubus Agriwidya, 2006: 120.
4. Dalimarta S. *Atlas Tumbuhan Obat Indonesia.* Jilid 3. Jakarta: Puspa Swara, 2005: 62.
5. Dalimarta S. *Atlas Tumbuhan Obat Indonesia*. Jilid 4. Jakarta: Puspa Swara 2006: 56.
6. Azizahwati, Wiryowidagdo S, Prihandini E. Efek penurunan kadar asam urat darah pada tikus putih jantan dari rebusan akar tanaman akar kucing (*Acalypha indica* Linn.). Jurnal Bahan Alam Indonesia ISSN 1412.2855. 2005; 4(1): 213-18.
7. Das AK, Ahmed F, Biswas NN, Dev S, Masud MM. Diuretic activity of *Acalypha indica.* Dhaka Univ *J. Pharmaceutical Sciences* 2005; 4(1): 1-3.
8. Purwaningsih EH, Ibrahim N. Uji pendahuluan ekstrak rebusan akar kucing pada *M. gastroknemius* katak. *Tidak dipublikasikan.*

9. Taylor P. Agents acting at the neuromuscular junction and autonomic ganglia. In: Gilman AG. The Pharmacological Basis of Therapeutics. 11th ed. New York: McGraw-Hill, 2006: 217-236.
10. Norman GR, Streiner DL, editor, *Biostatistics: the Bare Essentials*. London: Mosby-Year Book Inc., 1994: 56-58, 182-201.
11. Windra IGNA. Klasifikasi *Acalypha indica* Linn. (online). *http://image.toiusd.multiply.com/attachment/O/RjtU4QoKCp8AAAif7ao1/acalypha%20indica.doc?nmid 19377666*. 2008.
12. Mȇme W, Léoty C. Cyclopiazonic acid and thapsigargin reduce Ca^{2+} influx in frog skeletal muscle fibres as a result of Ca^{2+} store depletion. *Act Physiol Scand* 2001; 173(4): 391-399.
13. Shirokova N, Rios E. Caffeine enhances intramembranous charge movement in frog skeletal by increasing cytoplasmic Ca^{2+} concentration. *J. Physiol* (lond) 1996; 493: 341-356.