The nerve protection and in vivo therapeutic effect of Acalypha indica extract in frogs

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Abstract

**Aim** To demonstrate nerve protection and/or treatment effect of Acalypha indica Linn. extract on nerve paralysis induced by subcutaneous injection of pancuronium bromide on frog’s back.

**Methods** The study was performed on sixty frogs (Bufo melanostictus Schneider) that divided into two groups, i.e. the neuro-protection and neuro-therapy group. Each group was divided further into 6 sub-treatment groups: negative control group treated by water and positive control group treated by piracetam, treatment groups received the extracts 200, 300, 400, 500 mg/Kg BW. Pancuronium bromide 0.2% was injected subcutaneously as muscle relaxant. The protective effect was studied by giving the extract orally, 1 hour prior to injection; while the therapeutic effect of the extract was studied by 10 minute treatment after injecting pancuronium bromide solution. The parameters measured were the onset and duration of paralysis (in minutes) and the recovery time (time needed to recover into normal condition).

**Results** The study showed significantly different protective effect of Acalypha indica Linn. root water extract at 400 and 500 mg/Kg BW compared to negative control group and positive control group (piracetam); while the therapeutic effect was obvious at the dose 200-500 mg/Kg BW compared to negative control group (p<0.05). There was no significant difference compared to positive control group (piracetam), except at 300 mg/Kg BW (p=0.012).

**Conclusion** These results have proven that the water extract of Acalypha indica Linn. root has comparable protective and treatment effect on nerves system, as piracetam, but further studies should be performed to provide more evidences particularly pharmacokinetic and pharmacodynamic studies on two animal models that commonly used. (Med J Indones 2010; 19:96-102)

**Key words:** Acalypha indica Linn, Bufo melanostictus Schneider, nerve-protection

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In 2006, the morbidity rate of stroke incidence in patients with hypertension due to cerebral thrombosis was about 83%, while 70% of these cases were caused by cerebral hemorrhage. One third of these patients recovered successfully from remaining symptoms, but two thirds of them had still persistent neurological disorders characterized by musculoskeletal paralysis such as hemiplegia or hemiparese.1

To treat such persistent symptoms, various conventional chemical drugs have been used despite their high cost, uncertain or still questionable efficacy between their benefit and risk, and/or aggravating side effects such as sleeping disorder, anxiety and depression, e.g., piracetam.2

Hypertensive patients often turned their choice to alternative or complementary traditional herbal medicine, such as celery (Apium graveolens Linn.),3 garlic (Allium sativum Linn.),4 and mahkota dewa (Phaleria macrocarpa Scheff.).5 These herbs are consumed separately or in combination to treat hypertension but not as remedies for paralysis or hemi-/paraplegia that may have occurred as a result of the hypertension-caused stroke and its remaining post-stroke symptoms.

In some Indonesian communities, root extract with boiling water of akar kucing (Acalypha indica Linn.) has been used traditionally to reduce or even heal such remaining symptoms. However, no data has been established as a reference on the beneficial magnitude of Acalypha indica in the treatment of neurological disorders in vivo.

Previously, it was demonstrated that the aqueous root extract from Acalypha indica Linn. has neuro-protective and neuro-therapeutic effects on gastrocnemius muscles of frogs in ex vivo studies6. Therefore, a similar experimental in vivo study in frogs was performed. The frogs were considered as an experimental animal model based on the data of these previous studies which used isolated frog nerves and muscles ex vivo in order to examine membrane transport and activity of neurotransmitters.3,8

In a preliminary study4, pancuronium bromide was used as a model substance to induce neural paralysis and its dosage was adjusted to 2 mg/ml (0.2%) at 1:20 dilution for subcutaneous (s.c.) administration, equivalent to 0.1 mg per frog.

The dosage of root extract was determined based on the ex vivo studies to 6, 9, 12, 15 mg or 200, 300, 400, 500 mg/kgBW, respectively, at an average body weight per frog of 30 grams.

From the ex vivo studies, it was expected that the results of the present study would also demonstrate protective and/or therapeutic effects of the root extract from Acalypha indica L. on the frogs’ nerve system in vivo. Therefore, this animal model was chosen as a novel access in this preliminary study.

The general objective of this study was to prove that the root extract of Acalypha indica Linn. (akar kucing) has neural protective and therapeutic effects in vivo.

The specific objective was to prove that the root extract of Acalypha indica Linn. in a dose range of 6, 9, 12 and 15 mg or 200, 300, 400, 500 mg/kgBW has protecting and therapeutic effects on the nerves innervating the frog musculoskeletal system in vivo paralyzed by subcutaneous injection of pancuronium bromide.

METHODS

The animal model: This in vivo study was performed with 60 frogs in a weight range of 30±3.0 grams at the Department of Medical Pharmacy and Department of Physiology, Faculty of Medicine, University of Indonesia, from December 2007 to April 2008. The frogs identified as Bufo melanostictus Schneider by Bogoriensis Laboratory of LIPI, Bogor, were provided by the Department of Physiology, Faculty of Medicine, University of Indonesia.

The extract dosage was adjusted to 200, 300, 400, 500 mg/kgBW or 6, 9, 12, 15 mg for each frog, respectively, and piracetam as the positive control at 30 mg/frog or 1,000 mg/kgBW. Pancuronium bromide 0.1 mg per frog was administered by subcutaneous (s.c.) injection. This dosage had been optimised experimentally in a preliminary study6.

Group I: Neuroprotective effect

This part of the study was conducted in six subgroups: negative control (water); positive control (piracetam); the aqueous root extract of Acalypha indica Linn. with doses of 6 mg; 9 mg; 12 mg; 15 mg per frog, respectively, and piracetam as the positive control at 30 mg/ frog or 1,000 mg/kgBW. Pancuronium bromide 0.1 mg per frog was administered by subcutaneous (s.c.) injection. This dosage had been optimised experimentally in a preliminary study6.
Group II: Neurotherapeutic effect

This part of the study was also conducted in six subgroups: negative control (water); positive control (piracetam); the aqueous root extract of *Acalypha indica* Linn. with doses of 6 mg; 9 mg; 12 mg; 15 mg per frog, were given orally, 10 minutes after subcutaneous injection of pancuronium bromide. The duration of paralysis (in minutes) and scored recovery time to normal condition were the parameters measured.

Statistics

Each group in this study consisted of 5 frogs, thus SD was evaluated from n=5. The onset of action (only for protection), duration of paralysis and recovery time (in minutes), both for protective or therapeutic effects were analyzed statistically by using one-way Anova with limit of significance at p=0.05.

RESULTS

Group I: Neuroprotective effects (Figs.1-3).

Neuroprotective effects in frogs paralyzed with 0.1mg pancuronium bromide *in vivo* showed a maximum at dose 12mg or 400mg/kgBW, but the entire dose range from 6mg to 15mg (or 200-500mg/kgBW) had equivalent or even stronger effects than the positive control group with piracetam 30mg.

Onset of paralysis

Figure 1 shows that frogs which obtained the extract one hour before injection of pancuronium bromide, experienced paralysis within 14-25 minutes, whereas in the negative control group onset of paralysis was after 9±1.4 minutes and in the positive control group of piracetam after 11±1.6 minutes. In other words, onset of paralysis was slower over the whole range dose from 6mg to 15mg of extract as compared to both, negative and positive controls. The peak of efficacy in this experiment is at 25±2.2 minutes with 12mg of extract (Fig.1). The protective effect of 12mg and 15mg (22±2.0 min.) of extract is significantly different from both negative and positive controls (p<0.05).

Duration of paralysis

During an observation time of 2 hours, 12mg of extract showed a maximum protective effect on the duration of paralysis (59±2.0 min.) induced by pancuronium bromide followed by 9mg with a duration time of 77±2.5 minutes. This is significantly (p<0.05) shorter than negative controls (112±5.0 min) but only 12mg of extract shortens duration of paralysis also significantly towards positive control of piracetam (81±2.8 min, p<0.05). The other treatment groups with 6mg and 15mg of the extract showed 83±3.4 and 83±3.2 min. duration time of paralysis comparable to piracetam. In other words, duration of paralysis was shorter over the whole range of dosage from 6mg to 15mg of extract as compared to negative control, with 12mg being also significantly more effective than piracetam 30mg (Fig.2).
Complete recovery

Complete recovery took about 2 hours in the control group which had only been given water through the nasogastric tube, i.e., these frogs did not wake up before 2 hours of observation. Based on this data, the groups were categorized as follows: unawake after 2 hours (>120 minutes) = score 5; awake between 90-120 min = score 4; 60-90 min = score 3; 30-60 min = score 2; and less than 30 minutes = score 1.

The negative control group thus scored 4.25 and the piracetam group as positive control scored 3.8. Administration of 12 and 15 mg of extract resulted in recovery scores of 2.4 and 2.5, respectively (Fig. 3), which values are significantly different from both negative control and piracetam groups (p < 0.05). The other two treatment groups with 6 mg and 9 mg of extract and a score of 3.0 were also more effective than piracetam, although the difference is not significant. However, all treatment groups differ significantly from the negative control group. Also in this experiment, the administration of 12 mg of extract shows a maximum effect (Fig. 3). Statistical evaluation was performed with the original values in minutes before scoring.

The therapeutic effect of Acalypha indica Linn. extract is characterized by a maximum at the group which obtained 9 mg per frog. The difference in duration time of paralysis (20±1.2 min vs 115±5.4 min in negative controls) is highly significant (p=0.000) and also vs the positive control group which had received 30 mg of piracetam (40±2.0 min; p=0.012).

All other values in the dose range of 6–15 mg of extract per frog also demonstrated significantly shorter duration times of paralysis between 44±2.2 and 65±3.0 minutes (p=0.000-0.003) vs negative controls (115±5.4 min), comparable to the positive control group which had obtained piracetam (40±2.2 min; p=0.145-0.455). Hence, except for the group with 9 mg of extract, no significant difference to the piracetam group was observed (Fig. 4).

Group II: Neurotherapeutic effect (Figs. 4 and 5).

Duration of paralysis

This experiment resembles that one shown in Fig. 2, the difference is that the extract was now administered 10 min after the injection of pancuronium bromide. The results are depicted in Figure 4.

The therapeutic effect of Acalypha indica extract in frogs

![Figure 3. Recovery score within 2 hours of observation (protective effect)](image)

* p<0.05 vs (negative) control; $ p<0.05 vs piracetam

![Figure 4. Therapeutic effect on duration of paralysis during 2 hours of observation. (115±5.4; 40±2.0; 57±2.6; 20±1.2; 44±2.2; 65±3.0 min.)](image)

* p<0.000 vs (negative) control; $ p<0.012 vs piracetam

Complete recovery

After 2 hours of observation, all values in the dose range of 6–15 mg of extract per frog demonstrated significantly shorter complete recovery times from paralysis between 27±1.4 min for the group with 12 mg and 44±1.6 min for the doses of 9 mg and 15 mg (p=0.000-0.003) vs negative controls (115±5.6 min) and positive controls piracetam (58±2.0 min; p=0.000-0.316). These results were scored in the same way as explained in the context of Fig.3 and are depicted in Fig.5: negative control=score 4.25; piracetam=3.1; extract 6 mg=2.3; extracts 9 mg and 15 mg=1.7; extract 12 mg=1.4.
Summary

A significant neuroprotective effect of *Acalypha indica* Linn. extract on frogs paralyzed with pancuronium bromide *in vivo* has been shown at a dosage of 12mg per frog or 400 mg/kgBW, while the whole dose range of extract (6-15mg or 200-500 mg/kgBW) exerted a significant therapeutic effect compared to the negative control group with a maximum effect at 9-12mg or 300-400 mg/kgBW.

**DISCUSSION**

*Acalypha indica* Linn. (*akar kucing*)

Nahrstedt et al. isolated acalyphin from *Acalypha indica* Linn. and published its structure in 1982 10 and it was considered the active compound 11 although a variety of other compounds have been isolated from *Acalypha indica* 12,13. Various extracts from this plant had been shown to have diuretic 14, antimicrobial 15, scavenging activity 16, and antifertility 17 effects, however not all extracts were decocts from the plant’s roots as proven for the antiurisemic activity in rats18. In our study, a standardised aqueous extract from the roots was applied.

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**Frogs as animal models**

Frogs have been used classically for demonstration of neuro-musculoskeletal effects in the laboratories of physiology and pharmacology and numerous studies generally using this *ex vivo* neuro-musculoskeletal model support its validity 3,4. Therefore, also our working group used this *ex vivo* model in a preliminary study.6 The present study demonstrating protective and therapeutic effects of *Acalypha indica* Linn. (*akar kucing*) extract in living frogs provides evidence that frogs can be used as an *in vivo* animal model for innervation, both in peripheral and central nervous systems.

**Piracetam**

From innumerous publications on the effects of piracetam, only few which may be considered in the context of “positive control” in the present study are selected. Clinically doses of piracetam up to grams are applied in therapy and the official safety data sheet denotes LD50(*mouse*) of 2,000g/kgBW. There for, in our study the dosage of 1,000mg/kgBW was chosen, equivalent to 30mg per frog. Piracetam is thought have impact on acetylcholine via muscarinic cholinergic receptors and on NMDA glutamate receptors and may modulate ion channels and thus membrane permeability 19-22. Furthermore, in animal models there appears to be an effect on cerebral/neural mitochondrial energy metabolism, electron transport and ATP synthesis 23-24.

**Dosage finding of the extract**

The effective dose in frogs *in vivo*, 20 times the dose of *ex vivo* study (200-500 mg/kg BW or 6-15 mg/frog, at an average body weight of 30 g), was lower than *ex vivo* study using neuromuscular junction’s model i.e. 10; 15; 20; 25 mg/sample, because of their body weight, both the protective and therapeutic effect 6.

**Effects**

In the neuroprotective set of experiments, the extract is superior over piracetam 30mg which is even statistically significant at higher extract dosages of 12-15mg. From these experiments different from the plant extract, piracetam does not exert protective efficacy.

In the therapeutic set of experiments, piracetam exerts a significant effect vs controls, as does the plant extract at all dosages from 6-15mg. However, at a dose of 9mg, the extract is significantly more effective than 30mg of piracetam.

In both sets of experiments, the plant extract exerts a bell-shaped pharmacodynamic action with peaks at 12mg for the protective effect and at 9mg for therapy. These dosages are also significantly superior over our reference compound piracetam at the applied dose of 30mg.
These results are interpreted that the extract acts as a competitive inhibitor to pancuronium bromide in neuromuscular junction both in neuroprotective and neurotherapeutic sets of experiments, while piracetam did not show comparable neuroprotective effect. Hence, in our model, piracetam can be used as neurotherapeutic compound at neuromuscular junction, but its mechanism of action appears to be different from the Acalypha indica extract. It is not clear whether one of the “active” compounds of Acalypha indica like acalyphin, is responsible for the effects seen in our sets of experiments or rather their metabolites. Furthermore, as often in herbal medicines, it is the specific composition of extracts that causes pharmacological effects.

The effective dose of this extract as neurotherapeutic agent was lower (300 mg/kgBW) than its neuroprotective dosage (400-500 mg/kgBW). Since the effect was better than that of piracetam the extract of Acalypha indica Linn. will be promising to be chosen as complementary or alternative herbal medicine to treat neurologic disorders, especially in central nervous system although the mechanism of action cannot yet fully be explained.

Although frogs can be used at least as a first or preliminary animal model as demonstrated in this study, further studies have to be conducted with another animal model using mice or rats. Furthermore, the mechanism of action of the extract as a neuroprotecting and neurotherapeutic agent has to be demonstrated yet in central and peripheral nervous systems. To this end a model not using pancuronium bromide should be used in order to exclude that the extract might just counteract this compound in terms of a competitive antagonist. The aim of future studies should also be done to improve the extract as a standardized herbal medicine for the treatment of central or peripheral neural paralysis, either in the central nervous system or in neuromuscular junction.

In conclusion, the results of this study indicate that the extract of Acalypha indica Linn. has neuro-protective and neurotherapeutic effects in frogs in vivo. From our interpretation it is suggested that the extract acts as a competitive inhibitor to pancuronium bromide in neuromuscular junction, both in neuroprotective and neurotherapeutic sets of experiments. However, further studies should be performed in other animal models to prove its mechanism of action in preclinical pharmacokinetic and pharmacodynamic studies.

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