

The Neuroprotective Effect of *Bacopa monnieri* against Pilocarpine Induced Status Epilepticus in Rats

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ABSTRACT

Objective: To examine the therapeutic potential of Brahmi extract as a neuroprotective treatment for status epilepticus (SE).

Methods: After the oral administration of 160 mg/kg BM extract for 2 weeks, SE was induced by an i.p. injection of pilocarpine, and spatial learning and memory were tested on the next day. After the behavioral testing, all animals were sacrificed, and the brains were removed for histological examination to determine the amount of surviving neurons in the hippocampal subregions.

Results: SE caused spatial learning and memory impairment, and significant reduction of surviving neurons in all subregions of the hippocampus. Pre-treatment with BM extract could attenuate the spatial learning and memory deficit and reduced neuronal death in all subregions of the hippocampus.

Conclusion: BM extract demonstrated the neuroprotective effect against SE induced spatial learning and memory deficit and neuronal death in the hippocampus.

Keywords: Status epilepticus (SE); seizure; pilocarpine; *Bacopa monnieri* (Brahmi); learning and memory; hippocampus (Siriraj Med J 2017;69: 345-350)

INTRODUCTION

Status epilepticus (SE) – one of the neurological emergencies – is defined by prolonged and self-sustaining seizure related with high mortality rate.¹ It is a severe clinical manifestation of epilepsy consequences resulting in brain damage and chronic epilepsy.² The pilocarpine induced SE is a well-established animal model for SE and shares many characteristics of human epilepsy. For example, interictal activity spontaneously generated in the hippocampus of epileptic patients was found in rats with pilocarpine induced seizure.³ Upregulation of neurotrophins in the hippocampus was evident in both epileptic patients, and pilocarpine-injected rats.⁴ Cognitive and memory impairments found in the patients were also present in the pilocarpine-injected rats.⁵ Previous studies of SE in rats demonstrated the impairment of

spatial learning and memory in Morris Water Maze (WMW) test.⁶ The impairment caused by SE was in agreement with neuronal loss in many brain areas such as amygdala, thalamus, and hippocampus which is critical for learning and memory.^{7,8} The neuronal loss was the results of many pathological processes such as glutamate toxicity, inflammation, and oxidative stress.⁹ Although anticonvulsants can control the seizures, they cannot prevent the neurodegeneration, and might even cause cognitive impairments.¹⁰ The potential for antiepileptic drugs to adversely affect cognitive capacities is significant.¹⁰ Currently, the herbal medicines have been recognized for their benefits, and are being investigated for their application in many illnesses. *Bacopa monnieri* (Brahmi) is a small, creeping herb found in many Asian countries, where it is used in Ayurvedic medicine for cognitive

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Received 5 July 2017 Revised 3 August 2017 Accepted 4 August 2017

doi:10.14456/smj.2017.65

enhancement¹⁰ and anti-convulsion.¹¹ In Thailand, Brahmi has also been commercialized as a food supplement for learning and memory deficit by the Government Pharmaceutical Organization (GPO). Its scientific name is *Bacopa monnieri* Wettst. The active constituents in Brahmi are saponins which can be classified into two groups: jujubogenin glycoside and pseudojujubogenin glycoside.¹² The administration of Brahmi extract in adult male rats with the doses of 160 mg/kg for at least 2 weeks enhanced learning and memory abilities.¹³ Additionally, the extract could prevent neuronal damage after the exposure to many neurological injuries.^{14,15} For example, the extract demonstrated the protective effect in chronic cerebral hypoperfused rats through its anti-oxidative, anti-apoptosis and cholinergic modulation effects.¹⁶⁻¹⁸ While Brahmi extract is widely used for several conditions related to the central nervous system, there are very few studies reporting the effect of Brahmi extract on SE. The present study aimed to investigate the possibility of using Brahmi extract to prevent brain damage, and learning and memory deficit caused by SE.

MATERIALS AND METHODS

Animals

Eighteen adult male rats, weighing 250 to 300 g, were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom. Rats were maintained in the 12-hour light/dark cycle and were housed individually with free access to food and water. All experiments were approved by the Animal Ethics Committee, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand (SI-ACUP 020/2559).

Preparation of Brahmi extract

Brahmi extract is commercially available at any well-known drug stores. It is extracted, prepared, and commercialized by the Government Pharmaceutical Organization (GPO). Briefly, the aerial parts of Brahmi plant are cut into small pieces and dried at 50°C for 12 hr. The dried plant material is milled to obtain crude powder. The crude powder is then extracted by percolation method with 95% ethanol with the ratio of 1 g of Brahmi: 7 ml of ethanol. The crude powder is dried again, and then repeatedly extracted with 95% ethanol. The obtained extract is dried with a rotary evaporator under reduced pressure. The extract contains approximately 5% of saponins (w/w), the active constituent of the extract.¹⁹

Experimental design

All rats were randomly divided into 3 groups. The first group (sham group), rats were fed with 1 ml of 0.9%

normal saline without SE induction. The second group (SE group), rats were fed with 1 ml of 0.9% normal saline as a negative control before SE induction. The third group (SE+BM group), rats were fed with Brahmi extract 160 mg/kg of body weight before SE induction. This Brahmi extract dose was selected according to its maximal neuroprotective effect against 2VO induced cerebral ischemia.¹⁶ Each group daily received 1 ml of either 0.9% normal saline or Brahmi extract by intragastric gavage for 14 days before the induction of SE. After the animals recovered from the SE, the assessment of spatial learning and memory was performed for 7 days followed by histological examination at the end.

Pilocarpine-induced seizure

Initially, scopolamine methylnitrate (1 mg/kg) was subcutaneously injected in order to minimize the peripheral cholinergic effects, so it would reduce the severity of symptoms that was not associated to seizures such as respiratory distress or fine involuntary movement of muscles. Thirty minutes later, pilocarpine hydrochloride (380 mg/kg) which was dissolved in 0.9% saline solution, was intraperitoneally (i.p.) injected.²⁰ After the animals had shown seizure activities such as mouth and facial movements, head nodding, forelimb clonus, rearing, and falling for 30 min, the seizure event was terminated with diazepam (4 mg/kg i.p.). The behavioral manifestation of SE was monitored by VDO recording for 5 min before the pilocarpine administration as a baseline, and for 2 hours after the administration. In the case of wild running and jumping of the animals, the SE was interrupted with diazepam (4 mg/kg i.p.). Animal behaviors were monitored until they completely returned to normal. The severity of seizures was graded using Racine scale stage 1-5 which were stage-1: facial automatism, stage-2: head nodding, stage-3: unilateral forelimb clonus, stage-4: bilateral forelimb clonus, stage-5: rearing, falling and generalized convulsions.

Morris water maze test

MWM is a spatial learning and memory test, consisting of 3 training trials: visible platform, acquisition, and probe trials. Rats received the same training trials 4 times per day. In the visible platform trial, the platform location was clearly identified by a marker rising above the water, and the trial was performed on the first day of behavioral training to determine the sensorimotor performance and motivation to escape from the water. Time spent to find the platform (escape latency) was recorded and analyzed. The acquisition trial was performed consecutively for 5 days after the visible platform trial. Animals had to learn

to use visual cues to find the platform hidden under the water. The escape latency was recorded and analyzed to determine the spatial learning and memory performance. In the probe trial performed on the last day of training, the platform was removed, and time spent in the target quadrant (retention time) was analyzed to determine the amount of memory retained in the animals.

Histological examination for neuronal loss in the hippocampal subregions

After the behavioral training, all rats were sacrificed and the brains were removed. The paraffin sections of the brain were processed and cut at 7 μm thickness. In each brain, a series of 3 sections spaced at an interval of 200 μm was stained with 0.1% cresyl violet to examine neuronal damage. The hippocampus was viewed under a light microscope (Carl Zeiss Axio Imager M2, Germany), and images of CA1, CA3, and DG subregions were taken. Then, three representative images of each subregion were cropped into a 0.1 mm^2 square (345 x 300 μm^2). The numbers of hippocampal neurons (surviving and dead) in all subregions of the representative images were manually counted using the free UTHSCSA Image Tool 3.0 program (University of Texas Health Science Center at San Antonio).

Statistical analysis

The data were analyzed using SPSS 16.0. All results were expressed as mean \pm SEM. The mean escape latency was analyzed using the repeated-measure ANOVA and followed by Fisher's least significant difference post hoc test. The other data were analyzed using one-way ANOVA and followed by Fisher's least significant difference post hoc test. P values of 0.05 or less were considered significant.

RESULTS

1) Effects of pilocarpine induced status epilepticus on mortality rate

In this study, pilocarpine induced status epilepticus in all animals. Five SE+BM rats reached stage 4, and one SE+BM rat reached stage 5, whereas all SE rats achieved stage 5. Pilocarpine induced SE caused death in 40% of SE animals, but only 25% of SE+BM animals. The results of both groups were quite similar to the mortality rates reported in the previous studies.²¹⁻²⁴ The results suggest that BM extract has a protective effect against SE by reducing the mortality rate in the SE+BM animals (Fig 1).

2) Effects of Brahmi extract on sensorimotor performance and motivation in rats after status epilepticus

Sensorimotor performance and motivation to escape

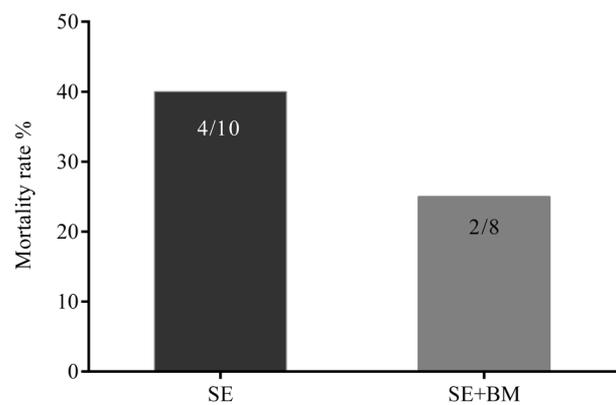


Fig 1. The mortality rate of SE rats (4 deaths out of 10 animals) and SE+BM rats (2 deaths out of 8 animals)

from the water of the rats were determined in the visible platform trial in which the platform was made observable. The results showed the mean escape latencies were not significantly different among all groups ($F(2, 15) = 1.795$, $p = 0.200$) (Fig 2), suggesting that the sensorimotor performance and motivation were not affected by either the Brahmi extract treatment or pilocarpine induced status epilepticus.

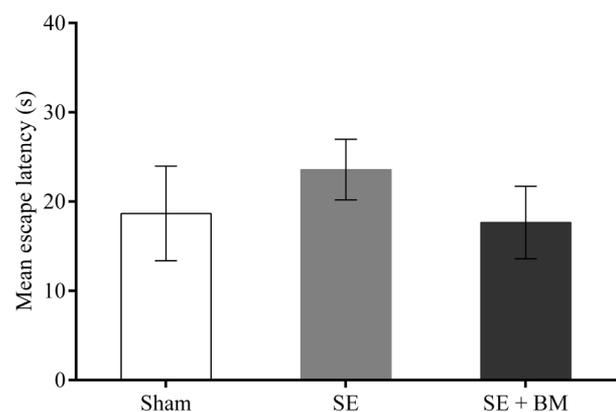


Fig 2. Effects of status epilepticus on sensorimotor performance and motivation to escape in the visible platform trial. Data are shown as mean \pm SEM (n = 6 per group).

3) Effects of Brahmi extract on spatial learning and memory in rats after status epilepticus

Rats were tested for their spatial learning and memory performances in the acquisition trial. The data illustrated that the mean escape latencies were significantly different between groups ($F(2,15) = 41.024$, $p < 0.001$). SE rats had the mean escape latency (30.80 ± 3.15 sec) significantly higher than the sham group ($p < 0.001$), indicating the impairment of spatial learning and memory caused by the pilocarpine induced SE. The SE rats treated with Brahmi extract (SE+BM group) had the mean escape latency (12.53 ± 1.67 sec) significantly lower than the SE group

($p < 0.001$) (Fig 3). Brahmi extract could significantly decrease the mean escape latencies when compared to the SE group. The results suggest that Brahmi extract could attenuate the spatial learning and memory deficit induced by status epilepticus.

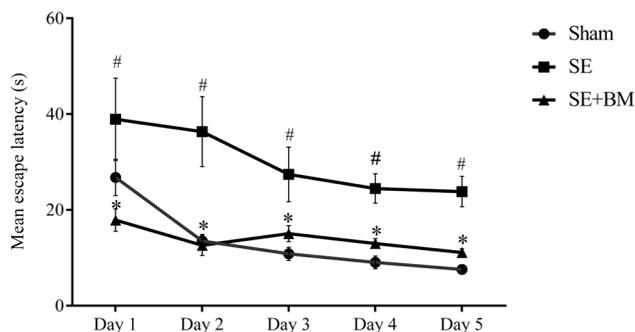


Fig 3. Changes in the mean escape latency in the acquisition trial. Data are shown as mean \pm SEM ($n = 6$ per group). “*” indicates the significance at $p < 0.05$ between SE+BM group versus SE group on each training day. “#” indicates the significance at $p < 0.05$ between SE group versus sham group on each training day.

Furthermore, memory retention was assessed by measuring the time spent in the target quadrant during the probe trial (Fig 4). The SE group had significantly lower mean retention time (34.42 ± 3.30 sec) than the sham group (51.67 ± 5.01 sec) ($F(2, 15) = 10.133$, $p = 0.001$), indicating the deficit in the memory retention caused by SE, and the deficit was prevented in the SE+BM group since there was no significant difference between the sham and SE+BM groups. The data further support the neuroprotective effect of Brahmi extract against SE induced learning and memory impairment.

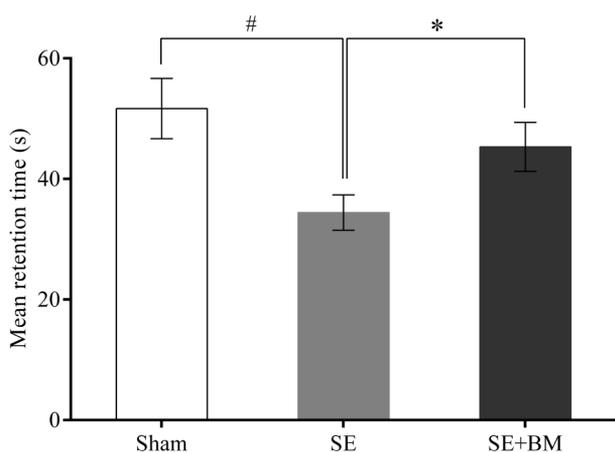


Fig 4. Mean retention time in probe trial. Data are shown as mean \pm SEM ($n = 6$ per group). “*” indicates the significance at $p < 0.05$ between SE+BM group versus SE group. “#” indicates the significance at $p < 0.05$ between sham group versus SE group.

4) Effects of Brahmi extract on the number of surviving neurons in hippocampal subregions

The number of surviving neurons in CA1, CA3

and DG subregions of the hippocampus was assessed in order to examine the effect of SE and Brahmi extract on the neuronal survival. The surviving neurons were characterized by large nuclei and prominent nucleoli (Fig 6). The numbers of all neurons (surviving and dead) per total area (0.3 mm^2) were counted in each area, and the percentages of surviving neurons to all neurons were calculated. In SE group, the percentages of surviving neurons in CA1, CA3 and DG significantly decreased when compared to the sham group (CA1: $F(2, 6) = 35.338$, $p < 0.001$; CA3: $F(2, 6) = 37.611$, $p < 0.001$; DG: $F(2, 6) = 27.354$, $p < 0.001$). After the administration of Brahmi extract at the dose of 160 mg/kg of BW, the percentages of surviving neurons in the CA1, CA3 and DG significantly increased when compared to the SE group (CA1: $F(2, 6) = 35.338$, $p = 0.002$; CA3: $F(2, 6) = 37.611$, $p < 0.001$; DG: $F(2, 6) = 27.354$, $p = 0.001$) (Fig 5).

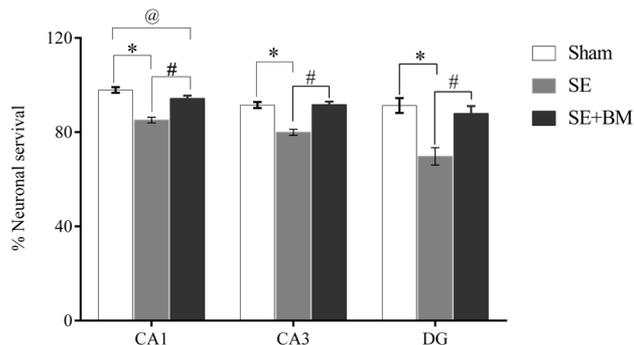


Fig 5. The percentages of surviving neurons of the hippocampal CA1, CA3 and DG subregions. The results are shown as mean \pm SEM ($n = 3$ per group). “*” indicates the significance at $p < 0.05$ between sham group versus SE group. “#” indicates the significance at $p < 0.05$ between SE+BM group versus SE group. “@” indicates the significance at $p < 0.05$ between sham group versus SE+BM group.

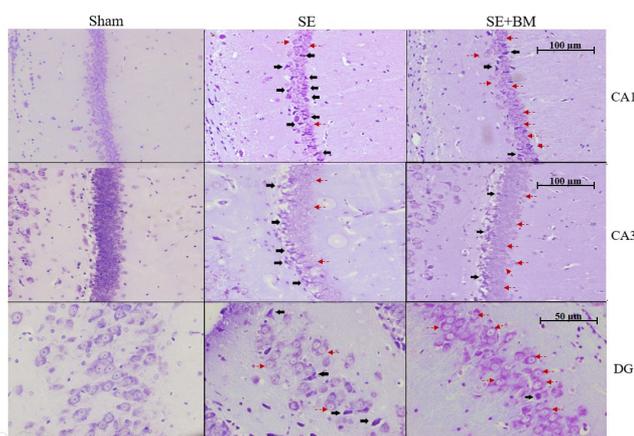


Fig 6. The 7 μm thick slides of each representative section in various subregions of the hippocampus. The pictures from the left to the right panels represent the different animal groups (sham, SE, and SE+BM, respectively). The pictures from the top to the bottom represent the hippocampal subregions. The black arrows indicate dead neurons and the red arrows indicate living neurons.

DISCUSSION

In this experiment, status epilepticus was induced by systemic pilocarpine injection and the potential for neuroprotective effect of *Bacopa monnieri* (Brahmi) extract was investigated. Status epilepticus can cause neuronal damage in hippocampal areas.⁷ The hippocampus, a brain area critical for spatial learning and memory, is one of the highest susceptible brain areas during seizures-related brain injury.²⁵ Neuronal injury during SE could result from various mechanisms such as glutamate-mediated excitotoxicity, oxidative stress and inflammation, leading to neuronal damage.²⁶ In this experiment, besides having the high mortality rate, SE rats had significantly higher mean escape latency, and lower mean retention time than the sham animals in the acquisition and probe trials, respectively. This spatial learning and memory deficit along with the significant reduction of surviving hippocampal neurons in all subregions supported the deleterious consequence of SE. Daily administration of Brahmi extract for 2 weeks before the SE event could prevent the deleterious effect of SE in SE+BM animals by showing significantly lower mortality rate, lower mean escape latency, and higher mean retention time than the SE animals. The results suggest the neuroprotective effects of Brahmi extract against status epilepticus. The mechanisms underlying the neuroprotective effect of Brahmi extract on spatial learning and memory were examined by assessing the number of surviving hippocampal neurons which remained after the status epilepticus. It is well known that the successful spatial learning of the MWM task highly depends on the function of hippocampal neurons.²⁷ The results indicated that Brahmi extract prevented the reduction of surviving neurons in CA1, CA3 and DG subregions after status epilepticus. Brahmi extract may exert its neuroprotective action via anti-oxidant, anti-oxidative stress, anti-inflammation, and glutamate modulator mechanisms. Studies demonstrated that inflammatory processes within the brain might be a common and key mechanism in the seizure and epilepsy pathophysiology.²⁸ The administration of Brahmi extract could decrease pro-inflammatory cytokine levels such as interleukin-1 β and tumor necrosis factor- α (TNF- α)²⁹, supporting anti-inflammatory activity of the extract which might have led to the neuroprotective effect in the present study.

CONCLUSION

Brahmi extract showed neuroprotective effects against status epilepticus induced neuronal damage and death in the SE rat model. *Bacopa monnieri* treatment could attenuate the spatial learning and memory impairment,

and neuronal death, suggesting the potential therapeutic value of *Bacopa monnieri* in status epilepticus treatment. However, it would be necessary to explore the specific functions of its active compounds, and their detailed underlying mechanisms in the future.

ACKNOWLEDGMENTS

The present study was supported by Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand, Grant Number (IO) R016031034. We would like to thank Ms. Namphung Thongta, Ms. Unchalee Vattarakorn, and our research assistants, for their help in many aspects during the experimental period. This study would not have been achieved without their help.

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