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Original Article

Kaempferia galanga L. Extract Administration Attenuate Aquaporin-4 Expression in Traumatic Brain Injury: An Experimental Study in Rats

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ABSTRACT

Introduction: Traumatic brain injury (TBI) is still a major health problem in the world. It might cause long-term disability that affect socio-economic life and become nation health burden. Post-traumatic cerebral edema might develop and commit to an unfavorable prognosis. Aquaporin 4 (AQP4) is water channel protein and a key regulator of water metabolism in the brain. Although the mechanism of AQP4 in the regulation of post-traumatic brain edema remains controversial, AQP4-lacking mice show better survival and decreased brain edema. Thus, novel strategies that suppress AQP4 become a potential field. We hypothesized that *Kaempferia galanga L.* may suppress brain expression of AQP4 following TBI and possibly limit the development of cerebral edema due to its neuroinflammation properties. **Method:** We conducted TBI to experimental rats, then given *Kaempferia galanga L.* extract at a dose of 600 mg/kg BW and 1200 mg/kg BW. Evaluation intensity of AQP4 expression by immunohistochemistry was performed 24 and 48 hours later to see its therapeutic effect. **Results:** Administration of *Kaempferia galanga L.* extract at a dose of 1200 mg/kg BW showed weak expression of AQP4 in all samples, both 24 and 48 hours following traumatic brain injury treatment. **Conclusions:** Intensity of AQP4 expression in rats' brain was lower at 24 and 48 hours after TBI in rats receiving *Kaempferia galanga L.* extract with dose 1200 mg/kg BW compared to the other groups. Our result indicates that *Kaempferia galanga L.* might affect the expression of brain AQP4 in a dose-dependent manner.

Key words: Post-traumatic cerebral edema, Neuroinflammation, Neurotrauma, Ayuverdic medicine.

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INTRODUCTION

Rapid surges in urbanization and development construction industries lead to an increase in TBI-related motor vehicle accidents and falls.[1] It is the most common cause of neurological deterioration and mortality worldwide.[2] Post-traumatic cerebral edema might develop and commit to an unfavorable prognosis.[3] To date no medical therapies effectively attenuate cerebral edema and the efficacy of invasive neurosurgical procedures to ease brain swelling remains limited. The need for novel approaches to limit post-traumatic edema is crucial.

Aquaporin 4 (AQP4) is a key regulator of water metabolism categorizes as a family of water-channel proteins which permit selective, bidirectional water movement in response to the osmotic gradient.[4,5] It is widely distributed in glial cells, ependymocytes and capillary endothelial cells in the brain. Although the mechanism of AQP4 in the regulation of post-traumatic brain edema remains controversial, AQP4-lacking mice show better survival and decreased brain edema.[6] Thus, novel strategies that suppress AQP4 become a potential field.

Neurological deterioration after traumatic brain injury might occur as a result of inflammation.[7,8] Pro-inflammatory cytokine and interleukin-1β (IL-1β) increase following brain injury which correlates with elevated intracranial pressure and neurological outcome.[9] Il-1β is found to have a role in the development of cerebral edema. In IL-1 type 1 receptor (IL-1R)-deficient mice show a reduction in both vasogenic and cellular edema after mild hypoxia-ischemia.[10] IL-1β is also found increased AQP4 expression via activation of pro-inflammatory transcription factor, nuclear factor κB (NFκB).[11] Based on those results, IL-1β possibly promotes cerebral edema by way of the regulation of AQP4 after TBI.

Kaempferia galanga L., is an aromatic ginger that has been consumed by humans for centuries in Asia population as an anti-inflammatory and antioxidant in Ayurvedic medicine.[12] Indonesian, without exception, have been taking it for generation because Indonesia is a tropical country rich in various traditional plants. A study showed extract of Kaempferia galanga L. might hamper pro-inflammatory cytokines such as IL-1β.[13] Its lipophilic properties help to penetrate the brain blood barrier.[14] Administration of Kaempferia galanga L. shows the change in expression of brain-derived neurotrophic factor (BDNF) and malondialdehyde (MDA) following TBI which indicates its neuroprotective behavior.[15,16] We hypothesized that *Kaempferia galanga L.* may suppress brain expression of AQP4 following TBI and possibly limit the development of cerebral edema.

MATERIALS AND METHODS

Animals

Ethical clearance for animal studies was approved by the Animal Care and Use Committee (ACUC)

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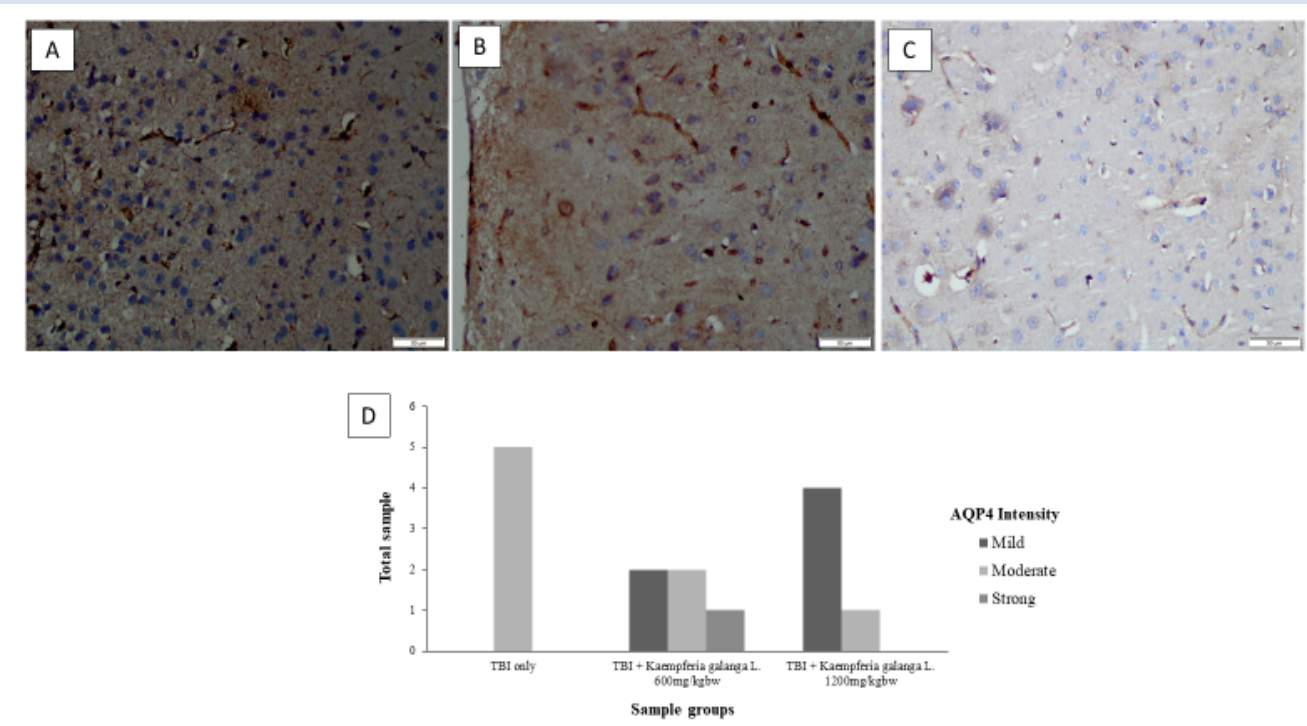

Figure 1: AQP4 expression 24 hours following TBI in TBI group only showing moderate intensity (++) (A), group with TBI-administration *Kaempferia galanga L*. extract 600 mg/kg BW show moderate intensity (++) and group with TBI-administration *Kaempferia galanga L*. extract 1200 mg/kg BW show mild intensity (+), mild and moderate intensity were seen in both groups which given *Kaempferia galanga L*. extract 80% samples in TBI-administration *Kaempferia galanga L*. extract 1200 mg/kg /BW exhibit mild intensity of AQP4 expression.

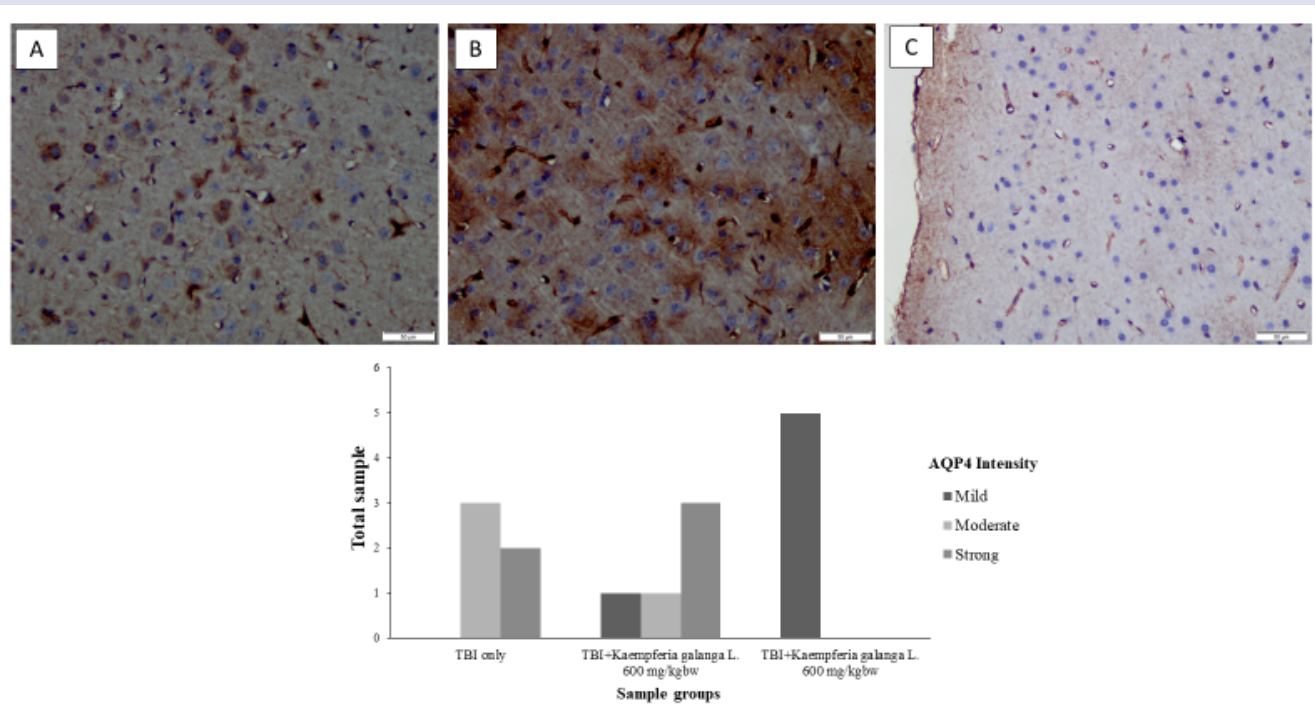

Figure 2: AQP4 expression 48 hours following TBI. (A) TBI group only showing moderate intensity (++), (B) group with TBI-*Kaempferia galanga L*. extract 600 mg/kg BW show strong intensity (+++) and (C) group with TBI-*Kaempferia galanga L*. extract 1200 mg/kg BW show mild intensity (+), (D) All samples (100%) in TBI-*Kaempferia galanga L*. extract 1200 mg/kg BW exhibit mild intensity of AQP4 expression.

at the faculty of veterinary medicine of Universitas Airlangga. Male Wistar rats (*Rattus novergicus*), aged 2,5-3 months old, weighing 200-250 grams were utilized in the experiment. They were housed in a controlled temperature environment (22±2°C), 12 hours' light-dark cycle and with free access to food and water. A total of 33 rats were used and sustained intervention TBI protocol. We separate the study subject into four groups. The first group the animal obtained no TBI intervention and therapy. The second group received intervention of TBI protocol without administration of extract Kaempferia galanga L. The third and fourth groups had intervention of TBI protocol and administration of a single dose of *Kaempferia galanga L.* extract *via* orogastric tube 30 minutes following intervention (600 mg/kg BW and 1200 mg/kg BW, respectively).

Preparation of extracts of *Kaempferia galanga* L.

Extract of *Kaempferia galanga* L. was made at the Faculty of Pharmacy Universitas Airlangga. First, it was sliced and dried at 70°C before being mashed into a simplicia. The simplicia was macerated and rotatory-evaporated before being mixed with ethanol 70% in a 100 mL solution. Before usage, the *Kaempferia galanga* L. extract in ethanol was evaporated. The next procedure is to make a 0.5% suspension by dissolving the extract *Kaempferia galanga* L. in 100 cc of water.[17] It is known that the major chemical constituents of the volatile oil from dried *Kaempferia galanga* L. were ethyl-p-methoxycinnamate (31.77%), methylcinnamate (23.23%), carvone (11.13%), eucalyptol (9.59%) and pentadecane (6.41%), respectively.[12] We used two different dosages, 600 mg/kg and 1200 mg/kg of plant extract, which proven showed significantly (P<0.001) as anti-inflammatory in carrageenan model and cotton pellet granuloma model in comparison to control.[14]

Traumatic brain injury animal model

TBI animal model in rat was performed as previously described by Barzo and colleges.[18] We used this model to derive conditions that mimic closed head injury (CHI) accompanied by cerebral edema. After induction of anesthesia, the mouse was placed onto the platform directly under the weight drop device. A scalp incision was made in the midline longitudinally until the skull was exposed. Identification of corona suture to determine impact area which is 2 mm behind the suture. A 10 mm in diameter and 3 mm in thickness of stainless-steel helmet was mount into the skull using bone glue. The falling height of the free-falling weight with 450-g brass was set at 100 cm. The impact was hit directly the helmet. This experimental conditions result in no mortality with low incidence of skull fracture.[19]

Aquaporin 4 expression evaluation using immunohisto-chemistry

Immunohistochemical evaluation were performed on paraffin sections. Tissue was stained with monoclonal rabbit anti-mouse AQP4 diluted 1:200 (cat. no. 16473-AP; Wuhan Sanying Biological Technology Co., Ltd., Wuhan, China). The section was sealed between a slide and cover slip, and observed under microscope with a microfire digital camera. Microscopic description and semiquantitative evaluation of the expression and the intensity of AQP4 immunoreaction was performed blinded by a neuropathologist. Normal choroid plexus, subcortical cerebellum and perivascular area show strong expression of AQP4 that used for positive control for AQP4, labeled as three positive marks (+++). The AQP4 expression was classified according to the positive control: (+) mild staining, (++) moderate staining and strong staining (+++). The analysis was conducted within the same day and was repeated up three time to ensure consistency.

RESULTS

In this study we analyze administration of single dose of *Kaempferia galanga L.* extract with expression of AQP4 in brain following TBI. Results for AQP4 expression were evaluated in 24- and 48-hour following intervention (Fig. 1 and 2, respectively). IHC expression of AQP4 at 24 hours in TBI only group showed homogenous result, all sample (100%) exhibit moderate intensity (++) while TBI-*Kaempferia galanga L.* group with dose 600 mg/kg BW showed varying result with only one (20%) sample exhibited strong intensity. In group TBI-*Kaempferia galanga L.* group with dose 1200 mg/kg BW, 80% of sample showed mild intensity (+).

In 48 hours setting, expression of AQP4 in TBI only group showed moderate intensity in 60% of sample. In TBI-*Kaempferia galanga L.* group with dose 600 mg/kg BW showed varying results, 60% with strong intensity and one sample each showing mild and moderate intensity. Homogenous result was seen in TBI-*Kaempferia galanga L.* group with dose 1200 mg/kg BW, all sample (100%) showed mild intensity. Other evaluation according dose-based classification of *Kaempferia galanga L.* extract showed nine out of ten samples that received 1200 mg/kg BW dose expressed mild intensity (+) of AQP4. That finding was quite different compare with TBI group that received 600 mg/kg BW of *Kaempferia galanga L.* extract.

DISCUSSION

This study showed intensity of AQP4 expression in brain was lower at 24 and 48 hours after TBI in rats receiving *Kaempferia galanga L.* extract with dose 1200 mg/kg BW compared to the other groups. Our findings indicate that *Kaempferia galanga L.* might affect the expression of brain AQP4 in a dose-dependent manner. To the best of our knowledge, our report illuminates the possibility of a novel therapeutic for anti-edema drugs following TBI, since administration of *Kaempferia galanga L.* extract decreased AQP4 brain expression.

TBI is still a major health problem in the world. It might cause long-term disability that affect socio-economic life and become nation health burden.[20] New therapeutic options are continuously being explored with the aim of improving the outcomes of patients with traumatic brain injury. Many preclinical studies have been carried out by trying to use experimental animal models that are as similar as possible to the pathological conditions in humans. The weight-drop brain injury model described by Borza and colleges is a method that can be used to obtain diffuse closed brain injury in experimental animals.[19] This model is widely used compared to other brain injury models because closed head injury accounts for the highest incidence of brain injury cases.[21] The drop-out rate of death due to this method was also found to be lower compared to other models of brain injury.[22]

Brain edema is a devastating complication that can occur following traumatic brain injury. It will rise intracranial pressure leading to the high mortality and disability in traumatic brain injury patients.[23,24] Brain edema can be broadly divided into two types: cytotoxic edema (intracellular edema) and vasogenic edema (extracellular edema).[25] Cytotoxic edema occurs within hours of injury and dominates the initial phase of traumatic brain injury (2-24 hours).[26] Damage to the blood-brain barrier results in vasogenic edema that increases 24 to 72 hours after injury and approximately resolves two weeks later.[27]

AQP4 is a water channel of membrane protein that widely distributed in the brain and play as key regulator of water metabolism that changes in its expression following brain injury. It was influence by type of brain edema that occurs.[6] Traumatic brain will express AQP4 that increase up to 72 hours post-injury. It associated with high rates of neurological deficits and positively correlated with brain edema.[28] That theory is in line with our results, the increase in AQP4 expression in the study group tends to increase linearly with time. Our study evaluated the expression of AQP4 at 24 and 48 hours after brain injury. This time point is reported to be the ideal time to assess brain edema and post-injury AQP4 expression in experimental animals.[29] The group that was

decapitated at 48 hours after injury tend to show higher intensity AQP4 expression when compared to the other groups that were decapitated at 24 hours. We also found variability of AQP4 expression in each research group in dose-dependent manner. The rat group that received *Kaempferia galanga L.* extract at a dose of 1200 mg/kg BW showed weaker AQP4 expression compared to either the group that did not receive *Kaempferia galanga L.* extract or the group that received a dose of 600 mg/kg BW.

Kaempferia galanga L., is an aromatic ginger that has been consumed by humans for centuries in Asia population. It is believed has strong anti-inflammatory and antioxidant benefits in Ayurvedic medicine.[12] It is known that the major chemical constituents of the volatile oil from dried *Kaempferia galanga L.* were ethyl-p-methoxycinnamate (31.77%), methylcinnamate (23.23%), carvone (11.13%), eucalyptol (9.59%) and pentadecane (6.41%), respectively.[12] Ethyl-p-methoxycinnamate isolated from *Kaempferia galanga L.* is found to be the vital anti-inflammatory constituent that exerts its anti-inflammatory effect.[13] It might inhibit pro-inflammatory cytokines such as IL-1β.[14] IL-1β is found to have a role in the development of cerebral edema. In IL-1 type 1 receptor (IL-1R)-deficient mice show a reduction in both vasogenic and cellular edema after mild hypoxia-ischemia.[10] IL-1β is also found increased AQP4 expression via activation of pro-inflammatory transcription factor, nuclear factor κB (NFκB).[11] According to those results, following TBI IL-1β possibly promotes cerebral edema by way of the regulation of AQP4. Considering the anti-inflammatory effect of *Kaempferia galanga L.*, our team tried to conduct a whether *Kaempferia galanga L.* extract with its anti-inflammatory effect can reduce traumatic brain edema with an indirect indicator in the form of decreased expression of AQP4. The decrease in AQP4 may occur due to suppression of the proinflammatory cytokine IL-1β by the anti-inflammatory effect of *Kaempferia galanga L.* It has lipophilic properties that helps to penetrate the brain blood barrier.[12] Two studies with similar setting showed administration of *Kaempferia galanga L.* affect in expression of brain-derived neurotrophic factor (BDNF) and malondialdehyde (MDA) following TBI which might indicate its neuroprotective behavior.[15,16]

CONCLUSION

In conclusion, AQP4 expression in rats' brain was lower at 24 and 48 hours after TBI in rats receiving *Kaempferia galanga L.* extract with dose 1200 mg/kg BW compared to the other groups. This finding indicates that *Kaempferia galanga L.* might affect the expression of brain AQP4 in a dose-dependent manner. However, the true mechanism of *Kaempferia galanga L.* extract affect the expression of AQP4 is still a hypothesis and further exploration may be warranted. Measuring cerebro-spinal fluid or blood levels of the active substance contained in the extract of *Kaempferia galanga L.*, measuring gross parameters for the occurrence of brain edema and verifying the main mechanism of the active substance in *Kaempferia galanga L.* extract affect expression AQP4 in vitro are several limitations of our study. Considering all of those limitations before further research might enhance the result.

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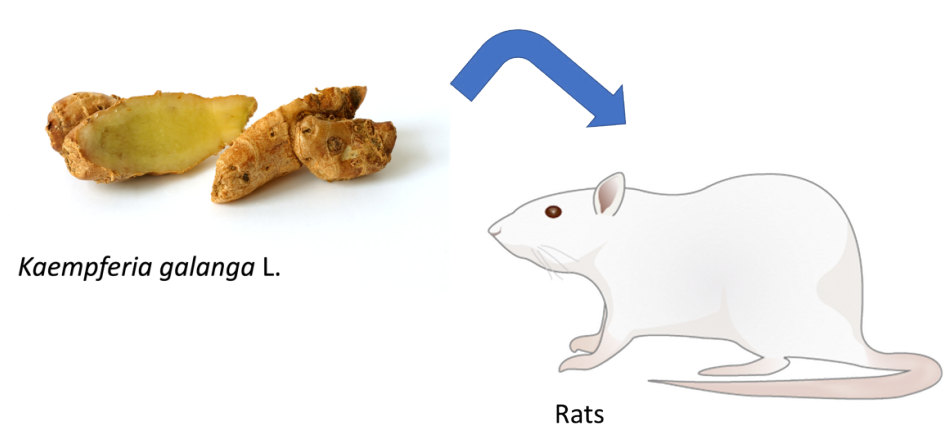
GRAPHICAL ABSTRACT



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