



The Change of BDNF Expression in Traumatic Brain Injury After *Kaempferia galanga* L. Administration: An Experimental Study

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Introduction: BDNF has potent effects on neural synapses and high expression in the brain. Its pathway affects cell survival and other biological processes, namely neurite and axonal growth. *Kaempferia galanga* L. (aromatic ginger) has many active substances with antioxidant and anti-inflammatory effects. Administration of antioxidants can modulate BDNF levels useful for reducing the progression of traumatic brain injury. We analyzed the expression of BDNF in the injured brains of the rats after administration of *Kaempferia galanga* L. extract.

Methods: This experiment analyzes the expression of BDNF in four different group of Wistar rats (*Ratus norvegicus*) based on the presence of TBI using Feeney TBI weight drop model and *Kaempferia galanga* L. extract administration into Groups A (no trauma & extract), B (with trauma & no extract), C (with trauma and 600 mg/kgbw extract), and D (with trauma and 1200 mg/kgbw extract). Groups B, C, and D are further divided into those who had their BDNF measured on 24- (B₂₄, C₂₄, and D₂₄) and 48-hours post-trauma & extract administration using immunohistochemistry evaluation.

Results: BDNF expression were found to be statistically significant between Group A with both B₂₄ ($p = 0,009$) and D₂₄ ($p = 0,009$) on the 24-hour post-trauma decapitation analysis. On the 48 hours after trauma and extract administration, Group B₄₈ ($p = 0,002$), C₄₈ ($p = <0,001$), and D₄₈ ($p <0,001$) were found to be significantly different with Group A. In all the rats receiving trauma & extract administration, there were no statistically significant differences between groups.

Conclusion: There was a significant difference in the results of BDNF expression within 24 and 48 hours in the control group without treatment with the other three groups, namely the control group with treatment, the treatment group with *Kaempferia galanga* L. extract 600 mg/kgbw, and the treatment group with *Kaempferia galanga* L. extract 1200 mg/kgbw. There was no significant difference in the results of BDNF expression both within 24 hours and 48 hours in the control group with the treatment group with 600 mg/kg *Kaempferia galanga* L. extract, and the treatment group with 1200 mg/kg *Kaempferia galanga* L. extract. However, there is an increase in the level of BDNF expression from the graphs of the results of the assessment of BDNF expression at 24 and 48 hours. Therefore, the administration of *Kaempferia galanga* L. extract to patients with brain injury can be considered as an option in increasing brain BDNF levels which are neuroprotective, but larger and specific studies are needed to determine the appropriate dose and duration for the use of *Kaempferia galanga* L. extract as a neuroprotectant agent in brain injuries.

Keyword : BDNF, traumatic brain injury, *Kaempferia galanga* L., Wistar rats

I. INTRODUCTION

Traumatic brain injury (TBI) is still a problem faced by many neurosurgeons and in Indonesia is still a major cause of disability and death which is also costly. Today, along with advances in technology and development, the frequency of TBI is increasing.¹ This is due to the increasing number of motorized vehicles, especially two-wheelers, as well as the undisciplined behavior of motorists on the streets. The study of TBI at Dr. Soetomo General Academic Hospital for 12 years (2002-2013) showed a total of 17,254 patients, and 2,749 patients (16%) were included in the category of severe TBI.²

Brain injury is the cause of almost half of all deaths due to trauma, given that the head is the most common and vulnerable part of being involved in an accident.³ The mortality rate of TBI is quite high, one study reported that the mortality rate reached 23.9% in patients with diffuse injury and 40.4% in patients with focal injury.² Brain injury itself also has a high morbidity rate due to the large number of patients who experience cognitive impairment and disability after the trauma occurs.

The development of about pathophysiology and management of TBI continues to grow. One of the central concepts based on laboratory, clinical, biomolecular and genetic research is that neurological damage does not only occur at the time of impact injury, but develops over the following hours and days and is influenced by the patient's susceptibility to trauma. The brain damage and dysfunction that occurs after brain injury is mainly due to secondary injury. The mechanisms underlying secondary injury include oxygen free radicals, neuroinflammation, brain edema formation, and brain apoptosis.⁴

In TBI, free radicals are high and can cause oxidative stress to the cells involved. In addition, high levels of these can cause cell death, impaired synaptic plasticity, and cognitive impairment, which is characterized by reduced brain derived neurotrophic factor (BDNF) and its downstream signaling pathway effects.⁵ To overcome these problems, antioxidants are needed to reduce the levels of free radicals. BDNF is the most studied neurotrophin due to its potent effects on synapses and broad expression in the brain. BDNF is secreted by neurons and glial cells, and has an important role in the regulation, function, and survival of neuronal

cells. The BDNF pathway and the tropomyosin receptor kinase B (TrkB) have effects to regulate cell survival and other biological processes. BDNF is important for neurite and axonal growth.⁶

Kaempferia galanga L. (aromatic ginger) has many active substances with therapeutic potentials. *Kaempferia galanga L.* extract has antioxidant and anti-inflammatory effects, which have lipophilic properties that can be assessed based on their partition log. A positive partition log indicates that the compound is lipophilic. The content of flavonoids, saponins, and tannins can have a good effect on disorders of the central nervous system (CNS).⁷ Several studies have also shown that *Kaempferia galanga L.* extract can affect the CNS and conclude that *Kaempferia galanga L.* extract can penetrate the blood brain barrier (BBB).⁸ *Kaempferia galanga L.* extract also has potential as an antioxidant, the total phenolic and flavonoid content which includes luteolin and apigenin can act as antioxidants.⁹ Administration of antioxidants can modulate BDNF levels,¹⁰ and is useful for reducing the progression of TBI. In this experimental study, we will evaluate the expression of BDNF in the brains of injured rats after administration of *Kaempferia galanga L.* extract.

II. RESEARCH METHODOLOGY

This study is experimental research with simple random sampling to ensure that the experimental animals and other research materials are homogeneous. All samples were treated at the same time, and observations were performed utilizing a post-test only control group design after a period of treatment. The research group is considered to be drawn from a population of experimental animals, and the study uses a randomized post-test only control group design. By comparing the experimental to the control group, this design can determine the effect of treatment (intervention) on the experimental group. Healthy, male Wistar rats (*Rattus norvegicus*), aged 2.5–3 months old, weighing 200–250 grams, and procured from the ITD Unair laboratory were utilized in this investigation. Wistar rats are genetically related to humans and have the ability to adapt to the laboratory environment, hence why they were chosen as experimental animals. The study lasted 5 (five) months and included stages including material and tool preparation, treatment, examination, and report compilation. The experimental animals were given a one-day treatment, after which the brain tissue preparations were examined with a 400x light microscope on the first (24 hours) and second (48 hours) days in the form of the number of cells expressing BDNF from brain tissue. Positive cells were counted in 5 (five) field of view (HPF) in each sample. The study's subject was separated into four groups, the first of which was a negative control group that received no therapy for traumatic brain damage and was not given *Kaempferia galanga L.* extract. Three experimental groups (Groups B, C, and D) were given one treatment for traumatic brain damage, and then the study subjects in groups C and D were given *Kaempferia galanga L.* extract (600 mg/ kgbw and 1200 mg/ kgbw, respectively).

Preparation of extracts of *Kaempferia galanga L.*

Kaempferia galanga L. was sliced and dried at 70°C before being mashed into a simplicia at Universitas Airlangga Faculty of Public Health's Laboratory. The simplicia was macerated and then rotary-evaporated before being combined with 70 percent ethanol in a 100 mL solution. Before usage, the *Kaempferia galanga L.* extract in ethanol was evaporated first. This activity took place at Faculty of Pharmacy Universitas Airlangga. The next procedure is to make a 0.5 percent CMC suspension by dissolving the extract *Kaempferia galanga L.* in 100 cc of water.

IV. RESULTS AND DISCUSSION

Rattus norvegicus male rats were used in this study, which were separated into four groups. Five rats were in the first group, and five rats were in each of the second, third, and fourth groups (24 and 48 hours). The expression of BDNF was measured using an immunohistochemistry examination (IHC) approach, with associated monoclonal antibodies tallied per 5 fields of view and the brown color in the cytoplasm of neuronal cells observed under a light microscope at 400x magnification.

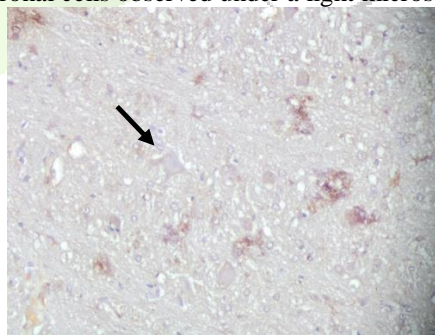


Figure 1. The results of IHC BDNF staining on a cross-section of the brain of rats without brain injury treatment using the "Feeney's weight drop" model and without giving *Kaempferia galanga L.* extract. At a magnification of 400x, the image was viewed under a microscope. BDNF-positive neurons are indicated by black arrows.

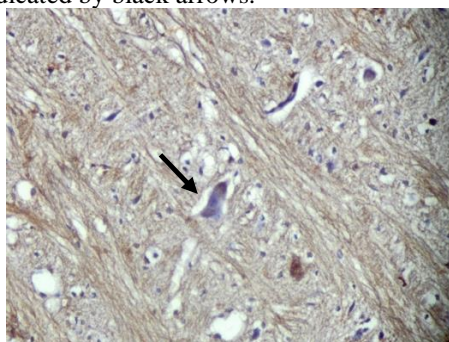


Figure 2. The results of IHC BDNF staining on a cross-section of the brain of rats treated with brain injury using the "Feeney's weight drop" model, without giving *Kaempferia galanga L.* extract and sacrificed after 24 hours. At a magnification of 400x, the image was viewed under a microscope. BDNF-positive neurons are indicated by black arrows.

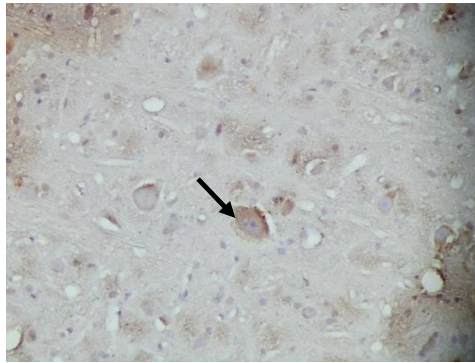


Figure 3. The results of IHC BDNF staining on a cross-section of the brain of rats treated with brain injury using the "Feeney's weight drop" model, without giving *Kaempferia galanga L.* extract and sacrificed after 24 hours. At a magnification of 400x, the image was viewed under a microscope. BDNF-positive neurons are indicated by black arrows.

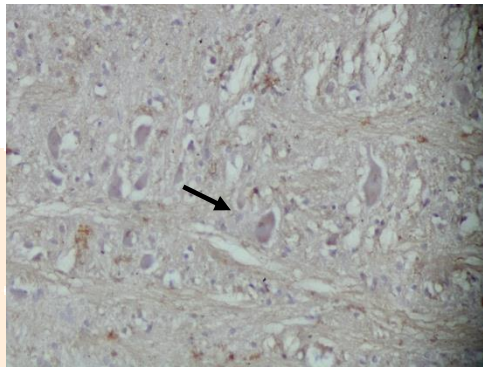


Figure 4. The results of IHC BDNF staining on a cross-section of the brain of rats treated with brain injury using the "Feeney's weight drop" model, with 600mg/kgbw *Kaempferia galanga L.* extract and sacrificed after 24 hours. At a magnification of 400x, the image was viewed under a microscope. BDNF-positive neurons are indicated by black arrows.

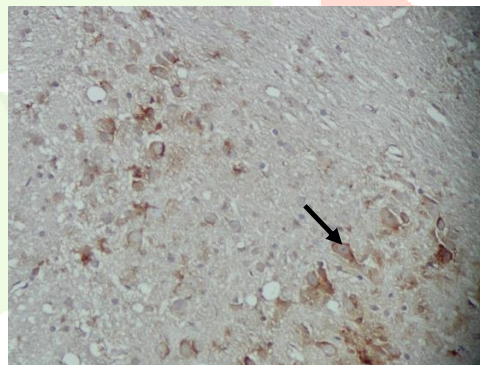


Figure 5 The results of IHC BDNF staining on a cross-section of the brain of rats treated with brain injury using the "Feeney's weight drop" model, with 600mg/kgbw *Kaempferia galanga L.* extract and sacrificed after 48 hours. At a magnification of 400x, the image was viewed under a microscope. BDNF-positive neurons are indicated by black arrows.

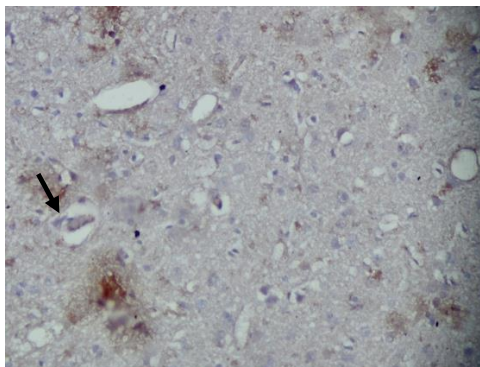


Figure 6. The results of IHC BDNF staining on a cross-section of the brain of rats treated with brain injury using the "Feeney's weight drop" model, with 1200mg/kgbw *Kaempferia galanga L.* extract and sacrificed after 24 hours. At a magnification of 400x, the image was viewed under a microscope. BDNF-positive neurons are indicated by black arrows.

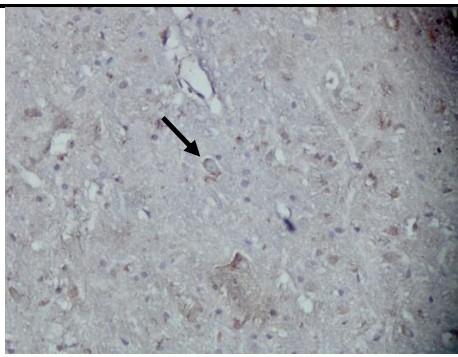


Figure 7. The results of IHC BDNF staining on a cross-section of the brain of rats treated with brain injury using the "Feeney's weight drop" model, with 1200mg/kgbw *Kaempferia galanga L.* extract and sacrificed after 48 hours. At a magnification of 400x, the image was viewed under a microscope. BDNF-positive neurons are indicated by black arrows.

Comparative Analysis of BDNF Expression Results at 24 Hours

Table 1. Descriptive BDNF 24 Hours

Group	n	Min	Max	Mean	Median	SD
Negative Control (without treatment)	5	0,0945	0,1509	0,1190	0,1200	0,2074
Positive Control (with Treatment)	5	0,1600	0,4912	0,2568	0,2105	0,1352
Treatment + Extract <i>Kaempferia galanga L.</i> 600 mg/kgbw	5	0,0555	0,4385	0,1721	0,1294	0,1530
Treatment + Extract <i>Kaempferia galanga L.</i> 1200 mg/kgbw	5	0,2666	0,4225	0,3224	0,3076	0,0587

In this study, the descriptive table is divided into four groups to show the distribution of data from the variables analysis, as measured by the lowest, highest, mean, median, and standard deviation values.

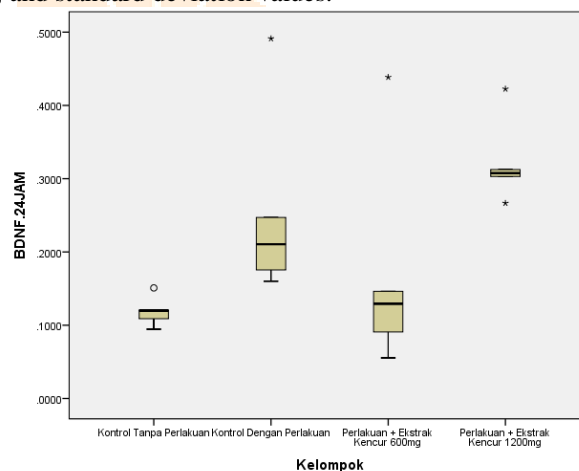


Figure 8. Graph of BDNF expression assessment results at 24 hours

Table 2 Non-parametric comparison test (Kruskal Wallis)

	Group	Median	p
BDNF 24 Hours	Negatif Control (without treatment)	0,1200	0,015
	Positive Control (with Treatment)	0,2105	
	Treatment + Extract <i>Kaempferia galanga L.</i> 600 mg/kgbw	0,1294	
	Treatment + Extract <i>Kaempferia galanga L.</i> 1200 mg/kgbw	0,3076	

In this comparison test, it was found that there was a significant difference in the results for 24-hour BDNF expression between the four study groups because a significance value obtained $p < 0.05$

Table 3. Comparison between groups (Mann Whitney)

Comparison group (BDNF 24 hours)		p
Negative control (without treatment)	Positive control (with treatment)	0,009
	Treatment + Extract <i>Kaempferia galanga L.</i> 600 mg mg/kgbw	0,917
	Treatment + Extract <i>Kaempferia galanga L.</i> 1200 mg mg/kgbw	0,009
Positive control (with treatment)	Treatment + Extract <i>Kaempferia galanga L.</i> 600 mg mg/kgbw	0,076
	Treatment + Extract <i>Kaempferia galanga L.</i> 1200 mg mg/kgbw	0,117
Treatment + Extract <i>Kaempferia galanga L.</i> 600 mg/ mg/kgbw	Treatment + Extract <i>Kaempferia galanga L.</i> 1200 mg mg/kgbw	0,117

Comparisons between groups in 24 hours are shown in the table above. The results of the comparison between the control group without treatment and the control group with treatment were p 0.009 (< 0.05), indicating that the results of BDNF expression in the two groups differed significantly. The results of the comparison between the control group without treatment and the treatment group with *Kaempferia galanga* L. extract 600 mg/kgbw were p 0.917 (> 0.05), indicating that there was no significant difference in the results for BDNF expression between the two groups. Following that, comparisons between the control group without treatment and the treatment group with *Kaempferia galanga* L. extract 1200 mg/kgbw yielded a value of p 0.009 (< 0.05), indicating that the results for BDNF expression in the two groups are significantly different.

The next group comparison was between the control, treatment, and treatment + *Kaempferia galanga* L. extract 600 mg/kgbw, with a value of p 0.076 (> 0.05), indicating that there was no significant change in the results for BDNF expression between the two groups. There was no significant difference in the results for the expression of BDNF in the two groups when comparing the control group with treatment and the treatment group with *Kaempferia galanga* L. extract 1200 mg/kgbw, with a value of p 0.117 (> 0.05). With a value of p 0.117 (> 0.05), there was no significant difference in the findings of BDNF expression between the 600 mg/kgbw *Kaempferia galanga* L. extract group and the 1200 mg/kgbw *Kaempferia galanga* L. extract group.

Comparative Analysis of BDNF Expression Results at 48 Hours

Table 4. Descriptive BDNF 48 Hours

Group	N	Min	Max	Mean	Median	SD
Negative control (without treatment)	5	0,0945	0,1509	0,1190	0,1200	0,2074
Positive control (with treatment)	5	0,2115	0,3225	0,2775	0,2878	0,4664
Treatment + Extract <i>Kaempferia galanga</i> L. 600 mg/kgbw	5	0,2682	0,4074	0,3224	0,3116	0,5326
Treatment + Extract <i>Kaempferia galanga</i> L. 1200mg/kgbw	5	0,2432	0,4492	0,3757	0,3913	0,8447

The descriptive table in this study, consists of four groups, to see the distribution of data from the variables studied which are seen from the lowest, highest, mean, median, and standard deviation values.

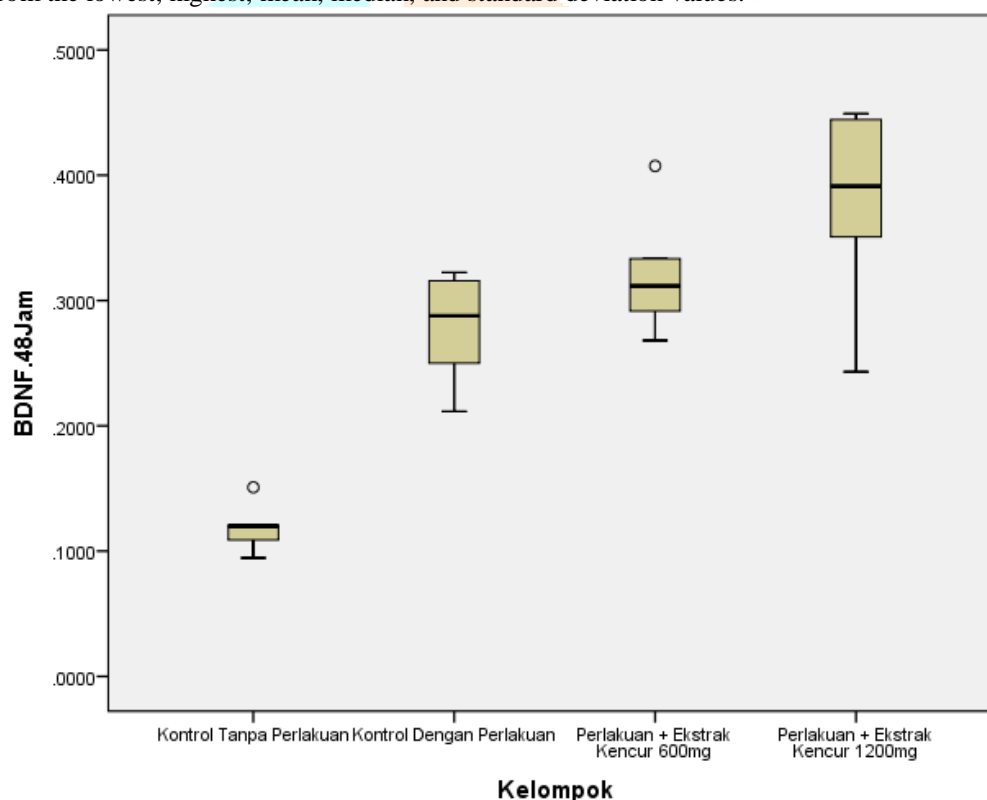


Figure 9. Graph of BDNF expression assessment results at 48 hours

Table 5 Parametric comparison test (One-way ANOVA)

	Group	<i>p</i>
BDNF 48 Hours	Negatif control (without treatment)	< 0,001
	Positive control (with treatment)	
	Treatment + Extract <i>Kaempferia galanga</i> L. 600 mg/kgbw	
	Treatment + Extract <i>Kaempferia galanga</i> L. 1200 mg/kgbw	

In this comparison test, it was found that there was a significant difference in the results of 48-hour BDNF expression between the four study groups because a significance value of obtained was $p < 0.05$.

Table 6 Comparison between groups (Post-Hoc Test)

Comparison group (BDNF 48 hours)		<i>p</i>
Negative control (without treatment)	Positive control (with treatment)	0,002
	Treatment + Extract <i>Kaempferia galanga</i> L. 600 mg/kgbw	0,000
	Treatment + Extract <i>Kaempferia galanga</i> L. 1200 mg/kgbw	0,000
Positive control (with treatment)	Treatment + Extract <i>Kaempferia galanga</i> L. 600 mg/kgbw	0,596
	Treatment + Extract <i>Kaempferia galanga</i> L. 1200 mg/kgbw	0,059
Treatment + Extract <i>Kaempferia galanga</i> L. 600 mg/kgbw	Treatment + Extract <i>Kaempferia galanga</i> L. 1200 mg/kgbw	0,458

Comparisons between groups are made in the table above within 48 hours. The results of the comparison between the control group without treatment and the control group with treatment were p 0.002 (< 0.05), indicating that the outcomes of BDNF expression in the two groups were significantly different. The results of the comparison between the control group without treatment and the treatment + *Kaempferia galanga* L. extract 600 mg/kgbw, with a value of p 0.000 (< 0.05), indicating that the results for BDNF expression in the two groups are significantly different. Following that, comparisons between the control group without treatment and the treatment + *Kaempferia galanga* L. extract 1200 mg/kgbw yielded a value of p 0.000 (< 0.05), indicating that the results for BDNF expression in the two groups are significantly different.

The next group comparison was between the control group and the treatment and treatment + *Kaempferia galanga* L. extract 600 mg/kgbw, with a value of p 0.596 (> 0.05), indicating that the results for BDNF expression in the two groups were not significantly different. Comparison of the control group with treatment and the treatment + *Kaempferia galanga* L. extract 1200 mg/kgbw, with a value of p 0.059 (> 0.05), indicating that the results for BDNF expression in the two groups are not significantly different.

The difference in BDNF expression between the 600 mg/kgbw *Kaempferia galanga* L. extract group and the 1200 mg/kgbw *Kaempferia galanga* L. extract group was p 0.4588 (> 0.05), indicating that there was no significant difference between the two groups.

Comparative Analysis of BDNF Expression Results at 24 and 48 Hours

Table 7 Comparison test of 2 non-parametric treatments (Wilcoxon)

Group Comparison	Time	Median	<i>p</i>
Positive control (with treatment)	24 h	0,2105	0,500
	48 h	0,2878	
Treatment + extract <i>Kaempferia galanga</i> L. 600 mg/kgbw	24 h	0,1294	0,080
	48 h	0,3116	

There was no significant difference in the results of BDNF expression between the control group and the treatment group at 24 and 48 hours, with a value of p 0.500 (> 0.05). The comparison of BDNF expression between 24 and 48 hours in the treatment group + 600 mg/kg *Kaempferia galanga* L. extract revealed no significant difference in BDNF expression with a value of p 0.080 (> 0.05).

Table 8 Comparison test of 2 parametric treatments (paired T test)

Group Comparison	Time	Mean	<i>p</i>
Treatment + Extract <i>Kaempferia galanga</i> L. 1200 mg/kgbw	24 h	0,3224	0,281
	48 h	0,3757	

Table 8 showed that there was no significant difference in BDNF expression between 24 and 48 hours in the treatment group with *Kaempferia galanga* L. extract 1200 mg/kgbw, with a value of p 0.281 (> 0.05).

In this study, it was found that there were no significant differences between the control group and the treatment without *Kaempferia galanga* L. extract and the co-treatment with 600 mg/kgbw of *Kaempferia galanga* L. extract and 1200 mg/kgbw of *Kaempferia galanga* L. There was no significant difference between the administration of *Kaempferia galanga* L. extract 600 mg/kgbw and 1200 mg/kgbw. The same was not reported in the study conducted by Tonsomboon et al. (2021) in their study which reported an improvement in BDNF levels in the administration of *Kaempferia* extract for cases of brain injury.¹¹ However, this study did not report the impact of *Kaempferia* on cases of TBI. Another possible explanation is that severe brain tissue damage interferes with the metabolism of BDNF itself, so that the positive effect of *Kaempferia* on BDNF, which has been described in the previous literature, cannot work optimally under these conditions.¹¹

Failla et al. (2016) conducted a study to see how the influence of serum and CSF levels of BDNF on mortality in acute (0–7 days after brain injury) and post-acute (8–365 days after brain injury).¹² The results showed a significant increase in the CSF of brain-injured patients relative to the control group and conversely found a decrease in serum levels of brain-injured patients relative to the control group. This can be explained by the premise that, apart from in the brain, BDNF is also synthesized and secreted by vascular endothelial cells,¹³ BDNF is also stored and released from platelets,¹⁴ mainly in response against injured tissue. *Kaempferia* is hypothesized to help increase BDNF levels in the brain through one of these mechanisms, but in this study no increase was reported in cases of TBI in animals. The basis of why a negative correlation was found between serum and CSF levels is because it was caused by a disruption of the BBB which caused platelets to secrete excessively BDNF in the brain and there was an acute transfer of serum BDNF to the CNS after TBI.¹⁵ Failla et al. also reported that increased levels of BDNF in CSF had a positive effect on acute mortality.¹² However, Simon et al. instead found a significant difference in mean serum BDNF levels in patients with severe TBI (GCS 3–8) who died and survived.¹⁶ Correspondingly, Chiaretti et al. also showed that no significant association

was found between CSF BDNF levels and neurologic outcome in 27 children, either 2 or 48 hours after moderate and severe TBI.¹⁷ Similarly, this study observed no correlation between baseline plasma BDNF levels and short-term fatal outcome.

Simon et al. explained that there was no significant difference in age between the groups who died and survived in terms of BDNF levels (32.7 ± 12.3 vs. 36.6 ± 13.8 , $p=0.155$), as well as in the study of Chiaretti et al. whose research focused on pediatric patients with TBI.^{16,17} Meanwhile, in the study by Failla et al., a significant difference was found between the groups who died and those who survived after TBI (47 ± 16.6 vs. 32.9 ± 13.6 , $p < 0.001$). This is associated with higher BDNF signalling in older patients due to changes in the expression of the BDNF target receptor (p75) and triggering the apoptotic pathway, which effectively renders BDNF exposure detrimental, especially early after injury. From these findings, it can be concluded that basically BDNF has a neuroprotective role and regulation, but at an older age excessive BDNF secretion can trigger signalling at the target receptor (p75) and trigger neuronal cell apoptosis.^{12,16,17}

Conclusion

The results of this experimental laboratory study that discussed the expression of BDNF in the brains of injured rats after administration of *Kaempferia galanga* L. extract showed that, there was a significant difference in the results of BDNF expression within 24 and 48 hours in the control group without treatment with the other three groups, namely the control group with treatment, the treatment group with *Kaempferia galanga* L. extract 600 mg/kgbw, and the treatment group with *Kaempferia galanga* L. extract 1200 mg/kgbw. There was no significant difference in the results of BDNF expression both within 24 hours and 48 hours in the control group with the treatment group with 600 mg/kg *Kaempferia galanga* L. extract, and the treatment group with 1200 mg/kg *Kaempferia galanga* L. extract. However, when conclude from the graphs of the results of the assessment of BDNF expression at 24 and 48 hours, it can be seen that there is an increase in the level of BDNF expression. Therefore, the administration of *Kaempferia galanga* L. extract to patients with brain injury can be considered as an option in increasing brain BDNF levels which are neuroprotective, but larger and specific studies are needed to determine the appropriate dose and duration for the use of *Kaempferia galanga* L. extract as a neuroprotectant agent in brain injuries.

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