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Expression Malondialdehyde (MDA) of Brain after Injury with the Extract of Kencur (*Kaempferia Galanga L*) (Experimental Study Wistar Rats)

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Abstract---Neurological damage in brain injury occurs due to secondary brain injury. Kencur extract has antioxidant potential with total phenolic and flavonoid content including luteolin apigenin and is expected to reduce MDA expression to prevent secondary injury. This study is an experimental laboratory. The treatment of all samples was carried out simultaneously using a post-test-only control group design. Based on the ANOVA test, the significance value of the Kencur extract treatment group was 0.000 ($p < 0.05$) indicating that there was a difference in MDA expression in brain-injured rats without kencur extract with brain-injured rats and given kencur extract. In the 24-hour and 48-hour time groups, a significance value of 0.488 ($p > 0.05$) showed no significant difference in MDA expression. Then the Kencur extract treatment group with a time group of 0.117 ($p > 0.05$) showed no significant difference in MDA expression. There was a significant difference in the expression of MDA in brain-injured rats without kencur extract with brain-injured rats and given kencur extract. There were no significant differences in the MDA expression in the 24-hour and 48-hour time groups and the Kencur extract treatment group and the 24-hour and 48-hour time groups.

Keywords---expression malondialdehyde (MDA), brain after injury, extract of kencur, experimental study Wistar rats, *Kaempferia Galanga L*.

Introduction

Brain injury is still a problem faced by many neurosurgeons and in Indonesia, it is still the main cause of disability, death and requires high costs to treat. Today, along with advances in technology and development as well as activities and the increasing number of human populations, the frequency of brain injury is not decreasing but tends to increase (Roozenbeek et al., 2013). This is due to the increasing number of motorized vehicles, especially two-wheelers, as well as the undisciplined behavior of motorized vehicle drivers on the streets.

One of the factors that influence the pathophysiology of TBI is a secondary brain injury. Secondary brain injury includes oxidative stress which can cause extensive brain tissue damage (Cornelius et al., 2013). TBI results in increased ROS and causes lipid peroxidation. *Malondialdehyde* (MDA) is the end product formed during lipid peroxidation caused by the degradation of cell membrane phospholipids (Lorente et al., 2015). MDA expression can also be used as a TBI biomarker and high expression is also found in patients, especially in patients who are unable to survive as a result of TBI. The lipid peroxidation process is divided into 3 phases. The first phase is the initiation phase where free radicals interact with polyionic fatty acids to form lipid radicals. Next is the propagation phase which is characterized by the reaction of peroxy radicals with *unsaturated fatty acids* to form hydroxy peroxides and new lipid radicals. Then the last is the termination phase when 2 radicals combine to form non-radical compounds or are terminated by antioxidants (Hall et al., 2010). The increase in MDA expression was caused by hyper oxidative conditions, namely an imbalance between antioxidants and pro-oxidants. So antioxidants are needed to overcome this imbalance (Rodrigo et al., 2013). Kencur extract can react with free radicals to produce a more stable product and stop the radical chain reaction. The ABTS cation radical reacts with hydrogen donating antioxidants and therefore the solution is colorless. This assay is commonly used to measure the radical activity of hydrogen donors and chain-breaking antioxidants in plant extracts (Pisoschi & Negulescu, 2011). According to Umar et al. (2012), Kencur extract (*Kaempferia galangal L.*) has anti-inflammatory, analgesic, nematocidal, mosquito repellent, larvicidal, vasorelaxant, sedative, antineoplastic, antimicrobial, antioxidant, and antiallergic effects.

The above study shows that Kencur extract has the potential as an alternative to antioxidant therapy in reducing oxidant reactions in secondary brain injury (Suh et al., 2000; Başkaya et al., 1997). Therefore, the researchers wanted to analyze the expression of *malondialdehyde* brain (MDA) after injury by giving Kencur extract (*Kaempferia galanga L.*).

Methods

This research is a laboratory experiment with *simple random sampling*. Therefore, experimental animals, experimental sites, and other research materials can be said to be homogeneous. All samples were treated simultaneously and after a long treatment, observations were made using the *Posttest Only Control Group Design* (Notoatmodjo, 2003). The research design used a *posttest-only control group*. This design, it allows researchers to measure the effect of treatment (intervention) on the experimental group by comparing the experimental group with the control group. Experimental animals used in this study were male Wistar rats, aged 2.5 – 3 months, bodyweight 280-320 grams, healthy, and obtained from the Bogor Agricultural Institute. The selection of rats as experimental animals was based on the consideration that Wistar rats are genetically similar to humans and can adapt to the laboratory environment (Weber et al., 2019) Sample allocation (grouping) used *simple random* by first numbering each rat.

The research was carried out for 5 (five) months, covering the stages of preparation of materials and tools, treatment, examination, and preparation of reports. The treatment of experimental animals was carried out for 1-2 days, then the brain tissue preparations were examined on day 3 in the form of the number of cells expressing MDA from injured ipsilateral brain tissue in male Wistar rats (Bhattacharya & Muruganandam, 2003; Fogacci et al., 2016), with a 400x light microscope, positive cells were counted in 5 fields of view (high power field/HPF) in each sample (Ali et al., 2005; Liu et al., 2016).

The treatment of research subjects in the form of traumatic brain injury / TBI was given to 3 experimental groups (groups B, C, and D) one treatment, and after that, the research subjects of groups C and D were given Kencur extract (600 mg /kgBW and 1200 mg/kgBW). The experimental animals were healthy male rats, looked active, aged 2.5-3 months, bodyweight 280-320 grams as many as 36 tails (Yao et al., 2018; Jagadish et al., 2016). All these mice were marked with a number on their fur, which would be used in the process of the *randomization sample*. The research data that showed the effect of Kencur extract (*Kaempferia galanga L.*) were then tested for significance with a significance level of 5% ($p = 0.05$) and analyzed with SPSS version 20.

Results and Discussion

ANOVA tests were used to determine whether there were differences in MDA expression in rats were treated with injury without giving Kencur extract, injured with 600 mg Kencur extract, and injured with 1200 mg Kencur extract.

Table 1
Table test results Two-Way ANOVA

Two-Way ANOVA Test	<i>p</i>
Treatment	0,000
Time	0,488
Treatment*Time	0,117

In table 1, the results of the ANOVA test show that the significant value in the treatment group is 0.000 ($p < 0.05$), which means that there is a difference in MDA expression. in injured rats without giving Kencur extract with injured rats and given Kencur extract. In the 24-hour and 48-hour time groups, a significance value of 0.488 ($p > 0.05$) showed that there was no significant difference in MDA expression. Then in the treatment group, Kencur extract with a time group of 24 hours and 48 hours obtained a significance value of 0.117 ($p > 0.05$) indicating no significant difference in MDA expression.

Table 2
Table of test results the Post Hoc Tukey

	Treatment Group	n	<i>p</i>
Negative Control	Positive Control	5	0,000
	Treatment + Kencur Extract 600 mgs	5	0,727
	Treatment + Kencur Extract 1200 mgs	5	0,408
Positive Control	Treatment + Kencur Extract 600 mgs	5	0,000
	Treatment + Kencur Extract 1200 mgs	5	0,000
Treatment + Kencur Extract 600 mgs	Treatment + Kencur Extract 1200 mgs	5	0,951

Results of the *Post Hoc* Tukey test are a follow-up test from the test *Two-Way* ANOVA, wherein the treatment group the significance value is 0.000 ($p < 0.05$), which means that there is a significant difference in the group. The test was *Post Hoc* Tukey's used to test the differences between the 2 groups with a significance value of $p < 0.05$. Based on the table of test results *Post Hoc* Tukey:

- The negative control group against the positive control group obtained a significance value of 0.000 ($p < 0.05$), which means that there is a significant difference in the 2 groups;
- The positive control group to the treatment group with the administration of kencur extract at doses of 600 mg and 1200 mg had a significance value of $p < 0.05$ so that it could be concluded that there was a significant difference;
- The negative control group to the treatment group with the administration of 600 mg or 1200 mg of Kencur extract had a significance value of $p > 0.05$ so it can be concluded that there was no significant difference;
- The treatment group with 600 mg Kencur extract against the treatment group with 1200 mg Kencur extract had a significance value of $p > 0.05$, which means that there was no significant difference between the two groups.

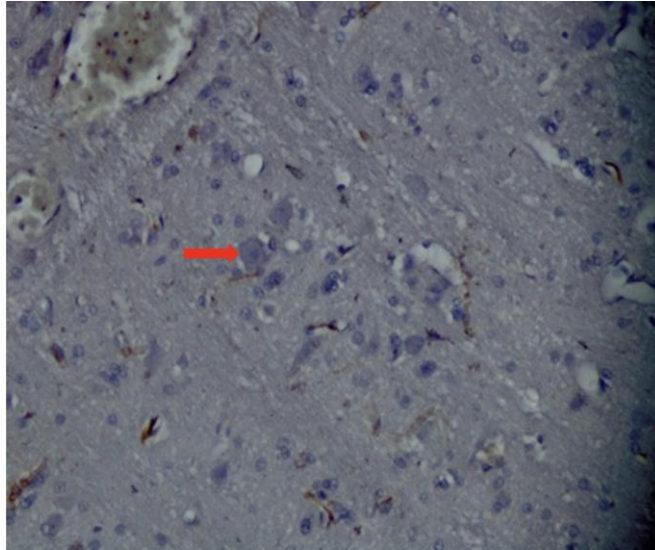


Figure 1. Positive control (Injury (+) & kencur (-)), Rat neuron cell stained brown positive for MDA (arrow) in 400x magnification

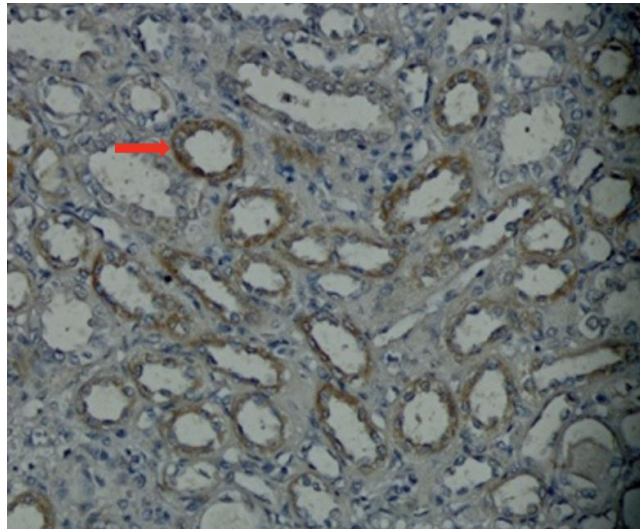


Figure 2. Negative control (Injury (-) & kencur (-)), Rat neuron cell stained brown positive for MDA (arrow) in 400x magnification

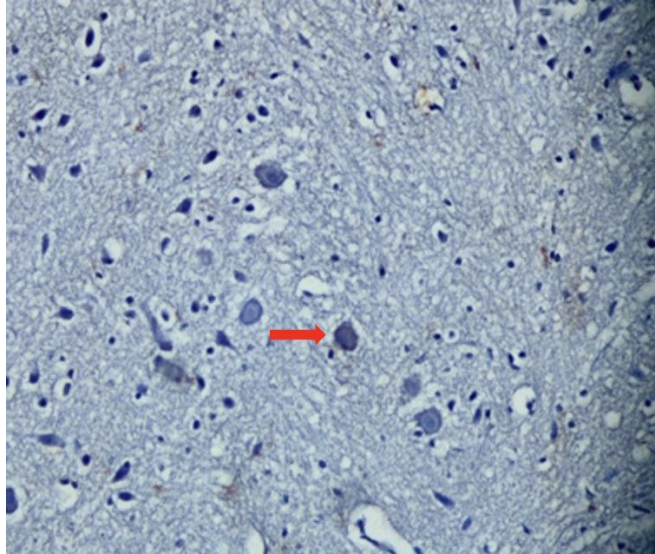


Figure 3. Brain Injury + Kencur Extract 600 mg, rat neuron cell stained brown positive for MDA (arrow) in 400x magnification

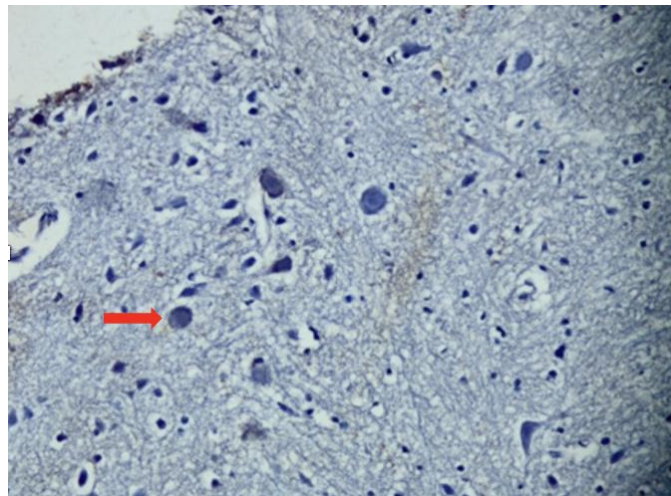


Figure 4. Brain Injury + Kencur Extract 1200 mg, rat neuron cell stained brown positive for MDA (arrow) in 400x magnification

The figures of the 4 research groups above using IHC PA staining and observed with a serial magnification microscope 100x, 200x, and 400x in each group shows MDA expression in neurons where cells are stained brown (Figure 4). Positive expression of molecules with primary antibodies will appear brown under a 400x light microscope. The counted brain cells were located in the subgranular zone (SGZ) of the hippocampal gyrus dentate, positive cells were counted in 5 fields of view (HPF) in each sample (Gobe et al., 1999).

The picture of the positive control study group (brain injury (+) and Kencur extract (-)) showed MDA expression in the microscope field of view with an average cell count of 0-5 positive cells per 100 neuron cells. The picture of the negative control study group (brain injury (-) and Kencur extract (-)) showed MDA expression painted brown with an average cell count of 10-20 positive cells per 100 neuron cells in the microscope field of view. The picture of the brain injury research group with a dose of 600 mg Kencur extract showed brown-colored MDA expression with an average count of 0-4 positive cells per 100 neuron cells in the microscope field of view. The picture of the brain injury research group with a dose of 1200 mg Kencur extract showed brown-colored MDA expression with an average cell count of 0-3 positive cells per 100 neuron cells in the microscope field of view (Wido et al., 2022).

The results of this study are in line with the research of Mustafa *et al* which showed that Kencur extract also has antioxidant potential and can reduce MDA expression. The content of total phenolic and flavonoid which includes luteolin and apigenin can act as antioxidant properties (Mustafa *et al.*, 2010). The antioxidant effect of Kencur extract as measured by DPPH assay in this study showed that the antioxidants present in Kencur extract were thought to act as hydrogen donors and were responsible for the reduction of DPPH (Kurniawan *et al.*, 2022). DPPH activity of Kencur extract showed a strong and positive correlation with its total phenolic ($R^2 = 0.932$, $p < 0.05$) and flavonoid content ($R^2 = 0.955$, $p < 0.05$) indicating a possible reduction in DPPH supported by high total phenolic content and flavonoids (Islam *et al.*, 2013). Kencur extract can react with free radicals to produce a more stable product, stopping the radical chain reaction (Kanjapothi *et al.*, 2004; Othman *et al.*, 2006). The ABTS cation radical reacts with hydrogen donating antioxidants and therefore the solution is colorless (Pisoschi & Negulescu, 2011).

The results of this study are also in line with research by Aroonrerk & Kamkaen (2009), which showed *Kaempferia galanga L* had inhibitory effects on IL-6 and anti-PGE2 and inhibited COX-2. Research Noro *et al.* (1983), stated *Kaempferia galangal L* as an MAO (inhibitor *Monoamine Oxidase*).

Research by Vittalrao *et al.* (2011), stated that *Kaempferia galangal L* in two doses, 600 mg/kg and 1200 mg/kg, contained anti-inflammatory properties. Two doses of extract *Kaempferia galangal L* were significantly different in containing analgesic activity when compared to the control group. The antioxidant content of Kencur from the rhizome is Total Phenolic Content (TPC) 57 mg gallic acid equivalent (GAE)/100 g. The antioxidant content in Kencur rhizome is 17 mg ascorbic acid (AA)/100 g (Chan *et al.*, 2009).

MDA expression can also be used as a TBI biomarker and high expression is also found in patients, especially in patients who are unable to survive as a result of TBI (Lorente *et al.*, 2015). Research on rats exposed to streptozotocin (STZ) showed that a mixture of bee pollen, Kencur rhizome, turmeric, and areca nut, could reduce MDA expression (Sutaryono *et al.*, 2016). The stability of phenoxyl radicals is reported to reduce the rate of propagation reaction in the lipid autoxidation process which will reduce the final product, namely MDA. In addition, the *in vitro* test of Kencur extract which measures the level of inhibition of lipid peroxidation showed that Kencur extract can inhibit lipid peroxidation so that it will reduce the final product, namely MDA (Mohanty *et al.*, 2008).

Conclusion

Research on expression *malondialdehyde* brain(MDA)after injury by giving Kencur extract (*Kaempferia galanga L*) to experimental animals Wistar rats at FK UNAIR/Dr. Soetomo Hospital Surabaya concluded that there were differences in MDA expression in injured rats without Kencur extract and injured rats and given Kencur extract. There was no significant difference in MDA expression in the MDA expression in the 24-hour and 48-hour time groups and the Kencur extract treatment group and the 24-hour and 48-hour time groups.

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