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The Effect of Administration of *Kaempferia galanga L.* Extract on Levels of Tumor Necrosis Factor Alpha (TNF-α) and Acute Pain Behavior in Post-Injury Wistar Rats

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ABSTRACT

Background: Untreated post-injury pain has negative effects in quality of life. Post-injury pain and inflammation have been treated with medication which have many side effects. The ethanol extract of *Kaempferia galanga L.* is believed have an important role in the control of mediators involved in inflammation.
Methods: This is a randomized post-test only control group experimental design, using male Wistar rats. The samples were divided into 4 groups, control group (P1), treatment group given 150mg/kgBW ethanol extract (P2), treatment group given 300mg/kgBW ethanol extract (P3), and ethanol extract 600mg/kgBW (P4) The variables studied were TNF- α levels and acute pain behavior 4 hours after tissue injury
Conclusion: Administration of *Kaempferia galanga L.* ethanol extract can reduce the inflammatory process by reducing TNF-α levels and reducing acute pain behavior after tissue injury compared to the control group. The highest dose of *Kaempferia galanga L.* extract in this study have a significant clinical response.

Keywords: *Kaempferia galanga L,* post-injury, acute pain

BACKGROUND

Post-injury pain is a common problem in everyday life. Untreated post-injury pain has negative effects such as increased morbidity, physical disorders, quality of life, recovery, as well as sleep disturbances and anxiety.[1] In injured tissue, an inflammatory response will occur which causes peripheral and central sensitization.[2]

The acute inflammatory response is characterized by local oedema, redness, tenderness and pain, increased temperature, and limited function. If extensive leukocyte accumulation has occurred, the tissue can become dense (induration).[3] The acute inflammatory response is often accompanied by fever, an increase in the number of blood leukocytes, and the appearance of acute phase proteins in plasma such as fibrinogen and C-reactive protein.[4]

Until now, post-injury pain and inflammation have been treated with medication which have many side effects such as gastrointestinal irritation, bleeding, respiratory failure, kidney failure and heart infarction.[5] Hence it is necessary to explore new drugs from natural ingredients that have anti-inflammatory and analgesic capabilities to reduce side effects and increase therapeutic effects.

The ethanol extract of *Kaempferia galanga L.* was rich with flavonoids, polyphenols, tannins, quinones and sesquiterpenes. Flavonoids are a group of secondary metabolite compounds that are most

commonly found in plant tissues. Flavonoids have anti-inflammatory properties through different mechanisms such as inhibition of regulatory enzymes and transcription factors that have an important role in the control of mediators involved in inflammation. In addition, flavonoids are able to modulate the activities of various inflammatory mediators so that flavonoids have the potential to be an anti-inflammatory agent.[6]

METHODS

This research was a randomized post-test only control group experimental design, using male Wistar rats aged 8-9 weeks with a body weight of 180-200 grams. The material used in this research was the ethanol extract of *Kaempferia galanga L*. The samples were divided into 4 groups, control group (P1), treatment group given 150mg/kgBW ethanol extract of kencur (P2), treatment group given 300mg/kgBW ethanol extract of kencur (P3), and ethanol extract of galangal 600mg/kgBW (P4) The ethanol extract of *Kaempferia galanga L* was given 1 hour before the plantar incision was carried out as a post-injury wound model. The variables studied were TNF- α levels and acute pain behavior 4 hours after tissue injury. TNF- α levels were examined using an ELISA kit, and post-injury pain behavior was carried out using the Grimas scale. Data was recorded and analyzed using the Windows version of SPSS.

RESULTS

Analysis of treatment effects was tested based on the mean TNF-α between groups after being given treatment. The results of the significance analysis using the One-Way Anova test are presented in Table 1 below.

Variables	Group	n	Mean TNF-α	p
TNF-α	P1	7	69,14	< 0,001
	P2	7	58,65	
	P3	7	48,90	
	P4	7	40,77	

To find out which group is different from the control group, it is necessary to carry out further tests using the Least Significant Difference – test (LSD). The test results are presented in Figure 1.

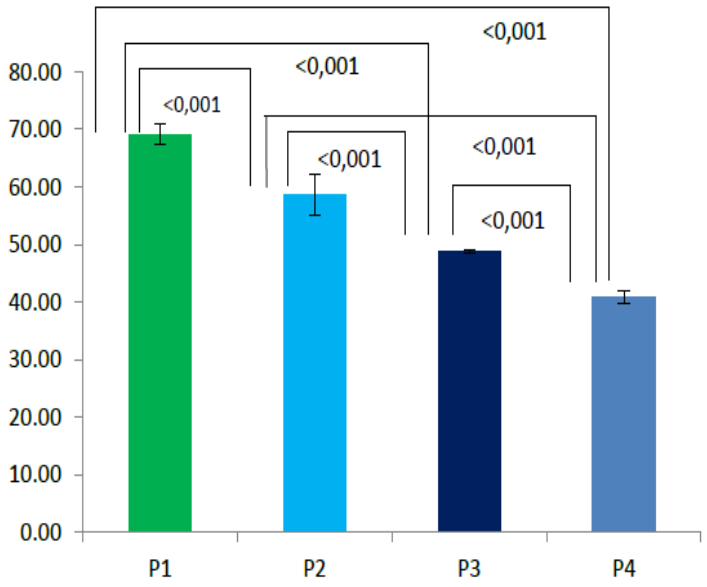

Figure 1. Comparison of TNF-α between Groups

Treatment effect analysis was tested based on the average acute pain behavior between groups after treatment. The results of the significance analysis using the Kruskal Wallis test are presented in Table 2 below.

Variables	Groups	n	Mean Grimase scale	Q1 – Q3	P
Acute pain behaviour	P1	7	1,20	1,20 – 1,20	0,001
	P2	7	0,60	0,60 - 0,60	
	P3	7	0,40	0,40 – 0,40	
	P4	7	0,20	0,20 – 0,20	

To find out which group is different from the control group, it is necessary to carry out further tests using the Least Significant Difference – test (LSD). The test results are presented in Figure 2.

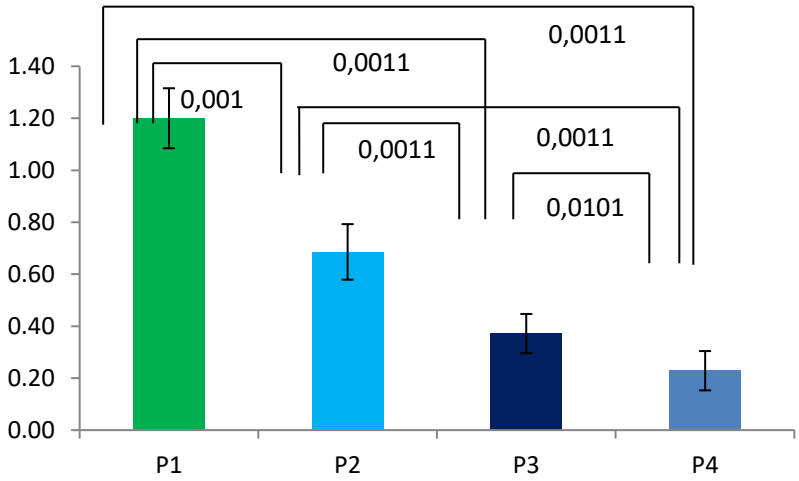

Figure 2. Comparison of Acute Pain Behavior between Groups

DISCUSSION

In this study, the inflammatory process was carried out on the feet of mice using a plantar incision model. When tissue injury occurs, the body will produce pro-inflammatory cytokinin and inflammatory mediators. Tissue damage will cause an inflammatory process which then activates neural and non-neural cells to produce pro-inflammatory cytokines including TNF-α.[7]

TNF-α plays an important role in the formation of tissue oedema, mechanical allodynia and thermal hyperalgesia and neutrophil migration, where part of this mechanism is a new approach in the treatment of pain and inflammation.[8] In this study, serum TNF-α in the blood of mice was taken at the 4th hour after the plantar incision because the 4th hour is the peak of TNF-α levels when inflammation occurs. In a study of plasma levels of TNF-α and expression of the TNFR receptor in multiple trauma patients, it was explained that TNF-α would be detected 2 hours after trauma in the plasma and reached a peak after 24 hours then remained steady until day 3 and gradually decreased until day 5. R also increases with increasing TNF-α in plasma, which is correlated with the severity of trauma.[9]

The administration of *Kaempferia galanga L* extract in this study caused TNF-α levels to decrease in the treatment group compared to the control group and was statistically analyzed as significantly different. This shows that the *Kaempferia galanga L* extract has proven effective in reducing TNF-α levels. The results of this study are in line with research conducted by Khajuria 2018 which proved that the *Kaempferia galanga L* was reported to inhibit the production of nitric oxide induced by LPS in J774 cells and RAW264.7 cells, thereby reducing the inflammatory response.[10] Research conducted by Yao et al (2018) using *Kaempferia galanga L* extract showed significant results in reducing inflammation by suppressing the production of NO (Nitric Oxide) in RAW 264.7 cells which was induced by lipopolysaccharide.[11]

Administration of *Kaempferia galanga L* extract at a dose of 150 gr/KgBW, 300

gr/KgBW and 600 gr/KgBW orally reduced the production of TNF-α compared to the control group which did not receive galangal extract. In this study, it was proven that oral *Kaempferia galanga L* extract suppressed serum TNF-α production thereby reducing the inflammatory process. Most of this process is likely through the action of several dominant secondary metabolites contained therein, namely inhibiting the phosphorylation and translocation of NF-kB into the nucleus. This *Kaempferia galanga L* extract is a potential candidate in treating acute post-injury pain associated with acute inflammatory pathways.

Spontaneous pain and pain with stimulation are based on peripheral sensitization and central sensitization mechanisms. Based on research conducted by Kang and Brenan 2016, it was found that there was a mutually supportive relationship between pain and the spontaneous activity of the nociceptive pathway, where spontaneous activity in the dorsal horn of the spinal cord was higher in skin and deep tissue incisions compared to skin and control incisions. This proves that strong spontaneous activity in the dorsal horn of the spinal cord is caused by large peripheral input.[12]

This study shows that administration of *Kaempferia galanga L* extract can cause acute pain behavior in mice as measured by the Grimas scale better than the control group and this has been analyzed as statistically significantly different. So it is concluded that the *Kaempferia galanga L* extract has proven effective in causing better pain behaviour in Wistar rats. The results of this study are in line with research which states that *Kaempferia galanga L* is a pain-relieving agent. According to Sulaiman et al. (2008) In vivo anti-nociceptive activity of water extract of *Kaempferia galanga L* leaves showed significant central anti-nociceptive activity at doses of 100 mg/kg and 300 mg/kg after 2 hours and 1 hour after administration, compared with morphine sulphate which showed significant anti-nociceptive activity after 1 hour of administration. In the formalin test, *Kaempferia galanga L* extract showed significant anti-nociceptive activity ($p < 0.05$) in the early and late phases of formalin-induced nociception.[13] This is supported by other research, the in vivo anti-nociceptive activity of methanol extract of *Kaempferia galanga L* in male Swiss albino mice and Wistar rats determined by Ridtitid et al. (2008) stated that in formalin tests on mice, methanol extract showed significant anti-nociceptive activity at doses of 50, 100, and 200 mg/kg compared to controls in the early and late phases.[14]

TNF-α levels correlate with pain behaviour. Decreased levels of TNF-α can cause pain behaviour as measured by the grimace scale showing lower levels of pain. Several clinical studies have used plasma levels of TNF-α as an indicator of pain.[15] This research is also supported by previous research conducted by Zhao et al 2021 which stated that the level of acute pain after injury was associated with the expression of TNF-α in plasma, hippocampus and prefrontal cortex in experimental research.[16]

In this study, the analgesic effect of *Kaempferia galanga L* has a significant effect due to the possibility of blocking COX 2. This is in line with previous research on the analgesic effect of *Kaempferia galanga L* in mice, finding that *Kaempferia galanga L* extract suppresses pro-inflammatory cytokines which inhibit the induction of genes that encodes COX-2 which is required for the synthesis of prostaglandins from arachidonic acid. This research is also supported by Ihtisam et al 2012 that Kaemferia extract inhibits the enzymatic active site on COX-1 and COX-2.[17]

CONCLUSION

Administration of *Kaempferia galanga L.* ethanol extract can reduce the inflammatory process by reducing TNF- α levels and reducing acute pain behavior after tissue injury compared to the control group. The highest dose of *Kaempferia galanga L.* extract in this study have a significant clinical response.

Declaration by Authors
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