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THE EFFECT OF KAFFIR LIME PEEL EXTRACT (*Citrus hystrix*) ON SPATIAL MEMORY OF MICE WITH DEMENTIA USING MORRIS WATER MAZE

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ABSTRACK

Background: Antioxidants are a class of chemicals that protect biological systems from the potentially harmful effects of oxidation processes or their by-products. Kaffir lime peel (Citrus hystrix) contains a variety of antioxidants that can protect neurons from free radical damage. **Objective:** This study was a true experimental study with a controlled group design that only included a post-test. Thirty mice were randomly assigned to one of five groups. On days 1-7, SCM was injected intraperitoneally, and kaffir lime peel extract was given orally, with mice's spatial memory tested using the Morris Water Maze on day 8. The One-Way ANOVA test ($\alpha = 0.05$) was used to analyze the data, which was then followed by Post Hoc LSD analysis. **Results:** The mean spatial memory of K+, K-, P1, P2, P3 were 16,33 ± 7,42; 70,00 ± 32,24; 25,66 ± 24,43; 23,00 ± 19,39; 25,00 ± 19,50 seconds respectively. There were significant differences in spatial memory in P1, P2, dan P3 to the negative control group, but there is no significant differences between treatment grups. **Conclusion:** Kaffir lime peel extract (*Citrus hystrix*) can improve the spatial memory of scopolamine-induced dementia (SCM) mice. There is no dose effect relationship between the treatment groups.

Keywords: Citrus hystrix, spatial memory, dementia, Morris Water Maze, mice

INTRODUCTION

Dementia is a neurodegenerative syndrome characterized by a chronic and progressive disorder that causes difficulties with sublime functions such as calculation, learning capacity, language, and decision making.[1] In Indonesia, It is estimated that approximately 1.2 million people in Indonesia suffered from dementia in 2016, with the number expected to rise to 2 million by 2030 and 4 million by 2050.[2]

The most common form of dementia is Alzheimer's disease (AD).[1,3–6] It is caused by the build-up of amyloid-beta (Aβ) plaques and neurofibrillary tangles (NFT), the former in the brain cortex and hippocampus, resulting in nerve damage and neuropathy.[6] Extracellular amyloid-beta (Aβ) plaque deposition, neurofibrillary tangle (NFT) formation, chronic neuroinflammation, and cholinergic neuron loss are the primary neuropathological signs of AD.

These Aβ plaques cause neuronal damage via oxidative stress, caspase activation, and mitochondrial dysfunction.[7] These neuropathological lesions will manifest in specific brain areas involved in learning, memory, language, and other cognitive abilities, such as the hippocampus and cortex.[8]

The pathogenesis and progression of dementia involve neuroinflammatory mechanisms. Aβ stimulates an inflammatory response that activates signaling pathways for the process of neuronal degeneration.[8] The accumulation of Aβ then causes microglia migration in the parenchyma and blood vessels, resulting in acute and chronic inflammatory responses and the production of free radicals such as reactive oxygen species (ROS), pro-inflammatory cytokines, and prostaglandin E2 (PGE2), which cause cell death.[9]

Neuroinflammation is a basic immune response that both protects and compensates for neuronal damage. At the same time, its neurotoxic effects aggravate neuronal damage.[10] Neuronal damage is what will manifest into clinical symptoms shown by dementia patients. One of the most common clinical symptoms encountered in dementia is memory loss. The decline in memory does not occur immediately, but as the patient's neuronal damage develops, there is a progressive decline. The absence of spatial memory will challenge individuals to understand their position, perceive the shape and space of three-dimensional figures, fail to remember the direction or location of an object, and estimate the distance of a place.[8]

Flavonoid antioxidants can prevent inflammatory reactions caused by free radicals by binding to reactive free radical compounds, resulting in more stable and less reactive radicals.[11] Kaffir lime

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peel is considered to have many antioxidant contents such as flavonoids, saponins, hesperidin naringin, etc.; thereby kaffir lime peel extract is envisioned to reduce dementia rates.[12]

The researchers discovered an effect of kaffir lime peel extract (Citrus hystrix) on the spatial memory of scopolamine-induced (SCM) mice with dementia in the current study. In this study, kaffir lime peel extract doses of 5, 10, and 20 mg/20gBB were used.

METHODOLOGY

This was a true experimental study with a control group that was only used for the post-test. The experimental male mice used in this study were aged 2-3 months. The study took place from July to October 2021 and was approved by the Faculty of Medicine's Research Ethics Commission (Indonesian: Komisi Etik Penelitian Fakultas) of Diponegoro University on May 24, 2021, with permit number 79/EC/FK-UNDIP/VII/2021.

The study was conducted at the Animal Laboratory, Faculty of Medicine, Diponegoro University on 30 mice which were divided into 5 groups: healthy control (KS), negative control (K(-)), Treatment 1 (P1) by administering 5 mg/20gBW/day of kaffir lime peel extract, Treatment 2 (P2) with 10 mg/20gBW/day of peel extract, and Treatment 3 (P3) with peel extract as much as 20 mg/20gBW/day. Kaffir lime peel extract was administered on days 2-7, while SCM of 1 mg/kgBW/day was injected intraperitoneally on days 1-7. Inclusion criteria in this study included healthy male mice weighing 20-40 grams and 2-3 months old. On the other hand, the exclusion criteria comprised dead or ill mice. On day 8, each group was tested with the Morris Water Maze to measure their spatial memory.

Then, the spatial memory data obtained were statistically analyzed using the Shapiro-Wilk test. Because the spatial memory data were found to be normally distributed (p>0.05), the One-Way ANOVA and Post Hoc LSD tests were used to detect group differences (p<0.05).

RESULTS

Analysis of the obtained data indicated that spatial memory was a numerical ratio data with a sample of less than 50. As a result, the Shapiro-Wilk and homogeneity tests were carried out by employing the Levene test.

Since the data normality test showed that the spatial memory was normally distributed (p>0.05), One-Way ANOVA and Post Hoc LSD analysis tests were performed.

Furthermore, a homogeneity test using Levene's test was carried out to reveal the differences between the two data groups with different variations. The results demonstrated significance by considering the mean in spatial memory data of 0.053 (> 0.05). It can be concluded that the variation of the level data in the five groups was homogeneous.

The One-Way ANOVA test conveyed a significance of 0.002 (p<0.05). Accordingly, there were significant differences in the spatial memory of mice. The Post Hoc spatial memory test results are presented in Figure 1.

DISCUSSION

In this study, there was a significant difference in travel time in the group injected with SCM (K(-)) compared to mice that were only given standard and ad libithum feed (KS). Statistically, the negative control group had a longer travel time than the positive control one. It occurred when scopolamine nonselectively blocked ACh muscarinic receptor adhesion sites in the cerebral cortex and resulted in uneven ACh release, destroying hippocampal neurons and inducing learning and memory impairment in mice.[13]

Table 1. Analysis of Spatial Memory

Group	Mean ± SD
	Spatial Memory (second)
KS	16.33 ± 7.42
K(-)	70.00 ± 32.24
P1	25.66 ± 24.43
P2	23.00 ± 19.39
P3	25.00 ± 19.50
p	*0.002**[A]

Note :*significant *(p<0,05)*
[A] : One-Way ANOVA Test

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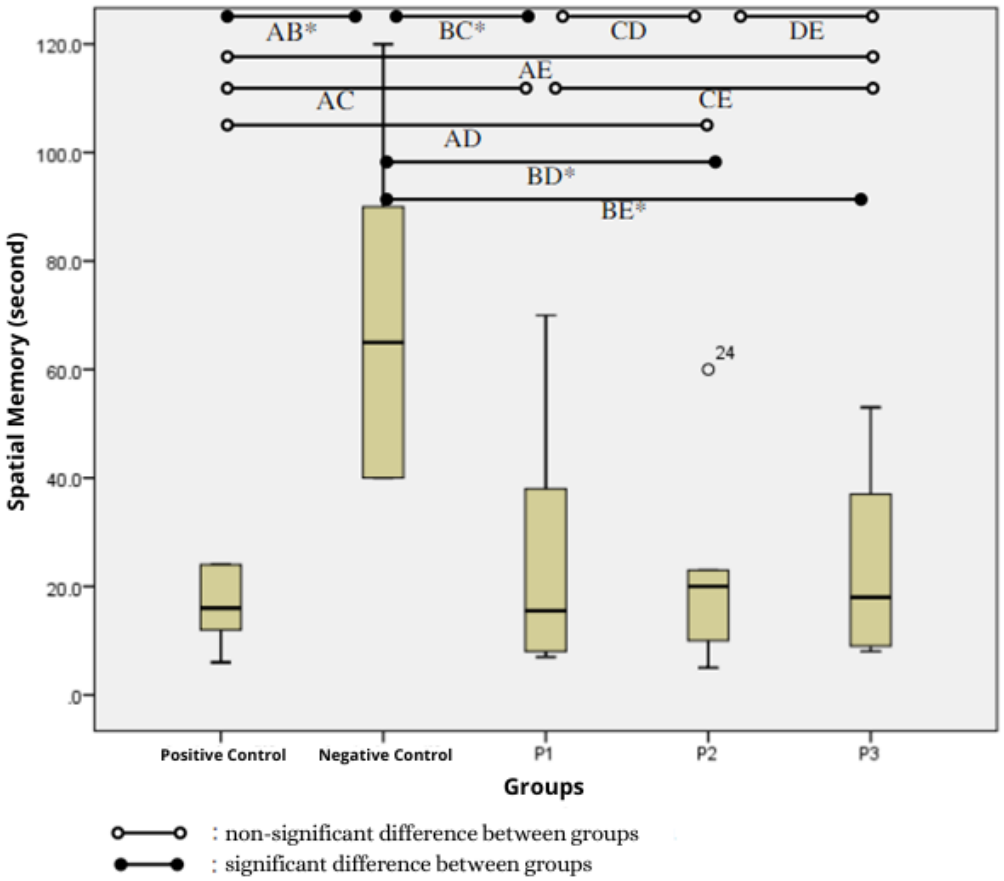

Figure 1. Intergroup Spatial Memory Box Plot Graph

The administration of kaffir lime peel extract in graded doses of 5, 10, and 20 mg/gBW to mice injected with SCM compared to the group only injected with SCM (K(-)) showed significant contrasts in spatial memory. Previous studies revealed that kaffir lime peel extract has been shown to contain antioxidant compounds such as flavonoids, saponins, hesperidin, and others that function to reduce the number of free radicals in the body including the central nervous system.[14] Flavonoids have been shown to improve memory, learning, and cognition in animals.[13,15]

Other studies have shown that flavonoid antioxidants can prevent inflammatory reactions caused by free radicals by binding to reactive free radical compounds, resulting in more stable and unreactive radicals. Flavonoids stabilize reactive oxygen species through reactions with reactive radicals.[11]

For the 5, 10, and 20 mg/20gBW/day doses, there was no significant difference in the healthy control group (KS) or any of the extract groups. This confirms that the administration of kaffir lime peel extract (*Citrus hystrix*) can improve spatial memory in SCM-induced mice with dementia in the presence of flavonoids, which are efficacious as antioxidants to prevent neurodegeneration. Flavonoids in kaffir lime peel extract may protect against oxidative stress caused by SCM induction by lowering the formation of reactive oxygen and nitrogen species (ROS and RNS) and preventing lipid peroxidation.[13,15,16]

Meanwhile, the highest average spatial memory was found in treatment group 2 (P2), 23.00 seconds However, the average spatial memory in treatment group 1 (P1) was 25.667 seconds, while in treatment group 3 (P3) it was 25.00 seconds. Statistically, the spatial memory test data in the treatment groups P1, P2, and P3 did not reveal a significant difference. These findings implied no dose-response relationship in the three treatment groups.

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The limitations of this study were that there was no examination of ROS and RNS levels to prove the effect of giving kaffir lime peel extract on oxidative stress and histopathological examination of the brain of mice to determine the level of organ damage or anxiety due to the development of dementia in mice.

CONCLUSION

At doses of 5, 10, and 20 mg/gBW, kaffir lime peel extract (Citrus hystrix) improved spatial memory levels in scopolamine-induced (SCM) mice with dementia. In the three treatment groups, however, no dose-response relationship is evident.

More research should be done to determine the effect of kaffir lime peel extract on oxidative stress by examining ROS and RNS levels. Also, it is necessary to run a histopathological analysis of the brain of mice to determine the level of organ damage or anxiety due to the development of dementia in mice.

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