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THE EFFECTIVENESS OF KAFFIR LIME PEEL EXTRACT (*CITRUS HYSTRIX*) ON PLASMA MALONDIALDEHYDE LEVEL IN DEMENTIA MICE

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ABSTRACT

Background: Dementia is a disorder of the central nervous system that results in decreased memory, ways of thinking and one of the main causes of disability and dependence. Antioxidant can catch and neutralize free radicals so that the process of oxidative stress can be stopped and cell damage can be avoided. Kaffir lime peel contains antioxidant compounds that have the potential to be a neuroprotective agent and can protect neurons from free radical-induced damage. **Objective:** To evaluate the effect of kaffir lime peel extract (*Citrus hystrix*) on the plasma malondialdehyde (MDA) level of dementia-induced scopolamine (SCM) mice. **Method:** This research was a true experimental study with a post-test-only controlled group design. Thirty mice were randomly divided into five groups consisting of healthy control (K+), Negative Control (K-), extract 5 mg/20gBW group (P1), 10 mg/20gBW group (P2), 20 mg/20gBW group (P3). SCM was injected intraperitoneally on days 1-7 and kaffir lime peel extract was given orally on days 2-7, the MDA levels testing of mice using the TBARS method on day 8. Data analysis used the One-Way ANOVA test and continued with Post Hoc LSD test. **Results:** The mean MDA levels of K+, K-, P1, P2, P3 were 4,212; 4,644; 3,481; 4,555; 4,733 nmol/mL, respectively. MDA levels of P1 were lower than K-, even though there were no statistically significant differences. **Conclusion:** There was no effect of the administration kaffir lime peel extract (*Citrus hystrix*) on MDA levels in mice with scopolamine-induced dementia.

Keywords: *Citrus hystrix, malondialdehyde, dementia, mice*

INTRODUCTION

Dementia is a disorder of the central nervous system that results in decreased memory and ways of thinking. Dementia is one of the main causes of disability and dependence in the elderly.

About 50 million people are suffering from dementia and each year there are almost 10 million new cases. This number will continue to grow over time, and it is estimated that there will be 82 million cases in 2030 and 152 million cases in 2050, especially in low-income countries.[1]

There are several types of dementia, but Alzheimer is one of the most common dementia disorders (50% - 60% of cases). Alzheimer's is dementia associated with genetic changes and protein changes in the brain. Another type of dementia that also often occurs is vascular dementia due to disturbances in the blood vessels of the brain.[2]

Oxidative stress is important pathogenesis of dementia. Oxidative stress is a disturbance in the balance between the production of free radicals and the body's ability to neutralize them through antioxidants. Free radicals are relatively unstable molecules because they contain elements that have one or more unpaired electrons in their outer orbits. Free radicals are compounds that are very reactive and easily interact with surrounding molecules (lipids, proteins, DNA, and carbohydrates). Free radicals can be contained in various materials such as textile dyes, food preservatives, foods that are processed by frying, grilling, or baking, repeatedly used oils, and others.[3]

Theory of oxidative stress explains the occurrence of memory disorders associated with the aging process**.** The accumulation of oxidative stress in the elderly causes the accumulation of oxidation of lipids, nucleic acids, and proteins that can cause cell dysfunction and can reduce the body's resistance to free radicals from outside.[3] Several organic compounds that cause oxidative stress are grouped as compounds Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS).[4]

ROS including ion Superoxide, Hydrogen peroxide, Hydroxyl radical, and Peroxyl radical.[4] Lipid peroxidation is the result of the reaction when the hydroxyl radical attacks the plural of unsaturated fatty acids or polyunsaturated fatty acids (PUFA). This process begins with the formation of a carbon-

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nucleated lipid radical. These lipid radicals can rapidly react with oxygen to form lipid peroxyl radicals. Peroxyl radicals can abstract hydrogen atoms from other lipid radicals and form lipid hydroperoxides as primary products of lipid peroxidation. Through heating or reactions involving metals, lipid hydroperoxides can easily turn into toxic compounds, namely malondialdehyde (MDA), propanal, hexanal, and 4-hydroxynonenal (4-HNE).[5]

MDA is a compound dialdehyde which is the end product of lipid peroxidation in the body and is often used as a biomarker that can be used to determine the level of oxidative stress on the body. Analysis of MDA levels is an indirect analysis and is very easy to determine the levels of free radicals formed.[6]

Scopolamine is an anticholinergic drug that is often used as a chemical to obtain experimental animal models that cause memory deficits. Scopolamine non-selectively blocks ACh muscarinic receptor adhesion sites in the cerebral cortex and resulting uneven release of ACh, damaging hippocampal neurons and causing learning and memory impairment in mice.[7,8] Cognitive impairment due to scopolamine induced in experimental animals is often associated with changes in oxidative stress levels in the brain. Tang's study said that the administration of scopolamine induced a state of oxidative stress in the rat brain. Scopolamine can damage cellular antioxidant defense mechanisms by suppressing and inhibiting antioxidant activity resulting in an increase in free radicals such as reactive oxygen species (ROS).[9,10]

Antioxidants are substances capable of capturing and neutralizing free radicals so that further reactions that cause oxidative stress can stop and damage cells can be avoided or induction of a disease can be stopped. In biological systems, the body can usually produce its antioxidants in the form of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase.[11-13]

Based on phytochemical tests carried out on the skin of kaffir lime fruit there are many flavonoid compounds. The flavonoids found in kaffir lime include narirutin, naringenin, hesperidin, neohesperidin, nobiletin, and tangeretin. Tangeretin is a flavonoid that has antioxidant properties that can function to lower cholesterol, anti-tumor, and neuroprotector.[14-17]

In this study, we investigated the effect of kaffir lime peel extract (*Citrus hystrix*) on MDA levels in mice with SCM. The doses of kaffir lime peel extract used in this study were 5, 10, and 20 mg/20gBB.

METHOD

This research is true experimental with a post-test only controlled group design. The subjects of this study used experimental male mice aged 2-3 months. The research was conducted from August to October 2021. This research received ethical clearance from the Research Ethics Commission of the Faculty of Medicine, Diponegoro University on August 25, 2021 with the number 101/EC/FK-UNDIP/VIII/2021.

The study was conducted at the Biomedical Laboratory of the Faculty of Medicine, Diponegoro University and the Central Laboratory of RSND/FK UNDIP using 30 mice which were divided into 5 groups, namely healthy control (K+), negative control (K-), Treatment 1 (P1) extract 5 mg/20gBB /day, Treatment 2 (P2) extract 10 mg/20gBW/day, Treatment 3 (P3) extract 20 mg/20gBW/day. Kaffir lime peel extract was administered on days 2-7 and SCM 1 mg/kgBW/day was injected intraperitoneally on days 1-7. The inclusion criteria were healthy male mice weight 20-40 grams and 2-3 months old and the exclusion criteria were dead or sick mice. On day 8, each group was drawn blood and tested for TBARS to measure MDA levels in mice plasma.

The MDA levels obtained were statistically analyzed using the Shapiro-Wilk test. MDA level data results were normally distributed (P>0.05), so it was continued using the test One-Way ANOVA and Post Hoc LSD test to see differences between groups (P <0.05).

Dementia in mice was obtained from induction of scopolamine 1 mg/kg/day for 7 days that can lead to an increase in lipid peroxidation in the hippocampus and memory impairment.[10]

RESULTS

The analysis of MDA levels obtained is a type of numerical ratio data with a sample of less than 50, so the Shapiro-Wilk test and Levene test is used (table 1).

MDA levels were found to be normally distributed (P>0.05) based on the normality test

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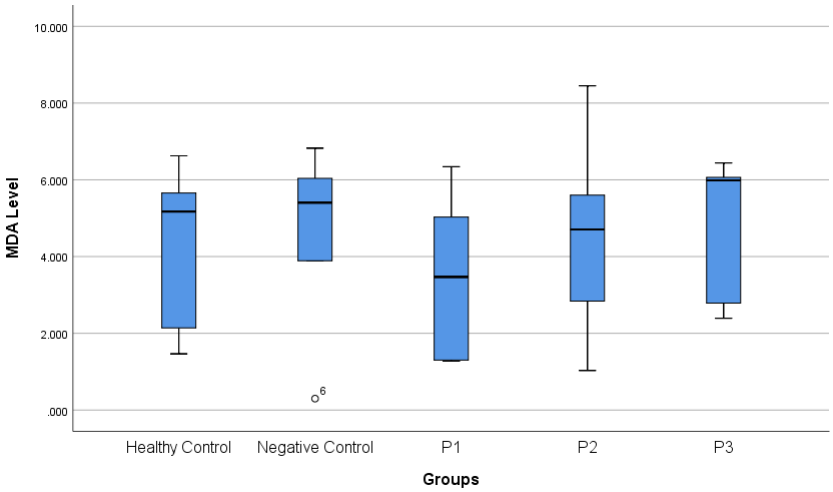
results. The analysis test then was carried out One-Way ANOVA test and the Post Hoc LSD.

Based on the homogeneity test, it was found that the significance based on the mean on the MDA content data was 0.997 (*P*>0.05) so it can be concluded that the variation in the content data in the five groups was homogeneous.

Based on One-Way ANOVA significance of 0.877 (*P*>0.05) was found by so there was no significant difference in the MDA levels of mice, so the Post Hoc test was not performed.

Table 1. Mean Plasma MDA Levels, Shapiro-Wilk test, Levene test, *One-Way* ANOVA test

Group	mean ± SD MDA levels (nmol/mL)	Shapiro-Wilk test (p)	Levene test (p)	One-Way ANOVA test (p)
K+	4,212 ± 2,272	0,335	0,997	0, 877
K-	4,644 ± 2.348	0,180		
P1	3,481 ± 2,094	0,443		
P2	4,555 ± 2,533	0,978		
P3	4,733 ± 1,969	0,056		



Description:
P1 : Group treatment 1 P2 : Group treatment 2 P3 : Group treatment 3

Figure 1. Graph Box Plot of MDA levels between groups.

DISCUSSION

This study is part of a joint study to examine the effect of kaffir lime peel extract on spatial memory function and MDA levels of mice induced by SCM. The total sample of the study was 30 mice. In the treatment period until the blood sample was taken, 1 mice died in the treatment group 3 and 1 mice failed during the blood collection due to red blood cell lysis. The cause of death was probably due to aspiration during oral administration.

In this joint study, it was found that the difference in travel time was significant in the group that was given SCM injection (K-) compared to mice that were only given standard feed (KS). The negative control group had a longer travel time than the healthy control. This indicates that the induction with scopolamine was successful in causing dementia in mice.

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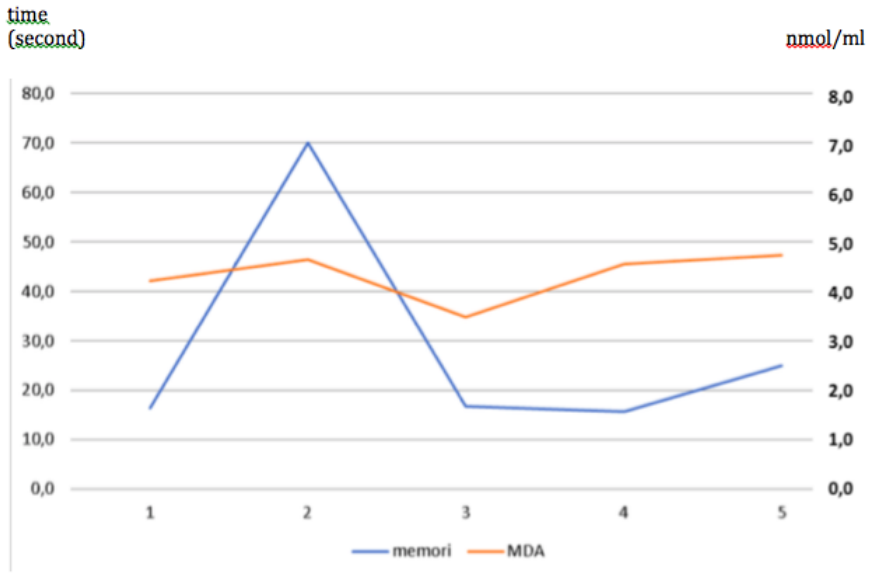

Figure 2. Spatial memory chart vs MDA serum level

The results of the Post Hoc LSD spatial memory test showed a significant difference (P<0.05) between the K(-) group and the P1 group. P2 and P3. The travel time in the P1, P2, or P3 water maze test was significantly faster than the K(-) group. This shows that the administration of kaffir lime peel extract (*Citrus hystrix*) can improve spatial memory in SCM-induced dementia mice. There was no significant difference in travel time between groups P1, P2, and P3, indicating that the effect of kaffir lime peel extract on dementia had no dose effect relationship.

In this study, the measurement of MDA levels on the 8th day showed that the MDA levels of the K(-) group were relatively slightly higher than the K(S) group, but were not statistically significant (figure 2). These results indicate that the induction using scopolamine did not increase MDA levels (P>0.05), this is not in accordance with previous studies which stated that administration scopolamine could induce oxidative stress in the rat brain. Scopolamine can damage cellular antioxidant defense mechanisms by suppressing and inhibiting antioxidant activity resulting in an increase in free radicals such as reactive oxygen species (ROS).[7]

The MDA levels in the 5, 10, and 20 mg/gBW/day groups did not statistically differ significantly. This is not in accordance with previous research.[18] Kaffir lime peel extract contains 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. This study is not in accordance with previous studies which showed that flavonoids have been shown to have an effect on improving memory, learning, and cognition in experimental animals.[16,18]

Orange peel contains Diosmin and Naringenin, flavonoid compounds that have been shown to have antioxidant properties. Research conducted by Shabani examined the effect of Diosmin doses of 50 and 100 mg/kgBW on dementia rats induced by scopolamine. It showed a neuroprotective effect and decreased levels of antioxidants including the pro-inflammatory cytokine TNF-alpha.[19] This study is not in accordance with Shabani's research, possibly because the levels of Diosmin contained in kaffir lime peel extract at doses of 5, 10, 20 mg/gBW/day (5, 10, 20 gram/kgBW) are not high enough to have an antioxidant effect. Diosmin levels contained in orange juice are 13 g/mL.[20]

Naringenin has been shown to have a neuroprotective effect in scopolamine-induced dementia rats.[16] Induction using scopolamine caused

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memory impairment, increased acetylcholinesterase activity and oxidative stress levels in the rat brain. Naringenin doses of 50 and 100 mg/kg for 7 days improved memory function and decreased acetylcholinesterase and oxidative stress levels.[21] This study is not in accordance with previous study, possibly because the levels of Naringenin contained in kaffir lime peel extract at doses of 5, 10,20 mg/gBW/day (equivalent to 5, 10, 20 grams/kgBW) were insufficient.

CONCLUSIONS

There was no significant difference between the treatment group and the control group. There was no effect of giving kaffir lime peel extract on plasma MDA levels in mice with dementia. There was no difference in the effect of stratified dose between the study treatment groups.

SUGGESTIONS

This study needs further research on the examination of ROS and RNS levels to prove the effect of giving kaffir lime peel extract on oxidative stress, examination of histopathological analysis of the brain of mice to determine the level of tissue damage due to induction scopolamine, and examination analysis of MDA levels using a sample of mice brain tissue.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

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AUTHOR CONTRIBUTIONS

Conceptualization, MI, MM, and YN; methodology MM, YN, and EM; formal analysis, MI, MM, and YN; resources, writing—original draft preparation, MI and YN.; writing—review and editing MM AND YN, project administration, MI.

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