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Effects of Moringa Leaf Extract on a Mouse Model of Dementia on Spatial Memory, MDA Levels, and Hippocampal Pyramidal Cell Count (CA1 & CA3)

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ABSTRACT

Antioxidants are substances needed by the body to neutralize free radicals and prevent damage caused by free radicals. Moringa leaves contain flavanoids, alkaloids, saponins and tannins that are antioxidants because of their ability to capture free radicals. This study aims to determine the effect of moringa leaf fractions on spatial memory ability using the Radial Arm Maze method, a decrease in malondialdehyde levels and inhibition of damage to the number of hippocampal pyramidal cells in CA1 & CA3 areas. Moringa leaves were extracted using the maceration method with 70% ethanol solvent. Then the ethanol extract of Moringa leaves was fractionated using the liquid-liquid method with n-hexane, ethyl acetate and water as solvents. Next, the mice were subjected to induction treatment with lead acetate, then observed for 21 days with the Radial Arm Maze, then the mice underwent surgery and their brains were removed for MDA level testing and histopathological observation. Data were analyzed statistically using one-way ANOVA ($p<0.05$) followed by Tukey's test. Based on the results obtained, the ethyl acetate fraction, n-hexane fraction and water fraction can influence the improvement of spatial memory by reducing latency time and type B errors, reducing malondialdehyde levels with TBARS and inhibiting damage to CA1 and CA3 hippocampal pyramidal cells which are characterized by pknotic neuron nuclei (shriveled) dark solid and vacuolized.

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1. Introduction

Dementia is a neurodegenerative syndrome characterized by progressive decline in cognitive function, particularly memory, language, and executive function. Alzheimer's disease (AD) is the most common form of dementia, accounting for approximately 60–70% of cases globally [1]. In Indonesia, the prevalence of dementia is estimated to have reached 1.2 million cases in 2020 and is projected to increase to more than 3 million by 2050, in line with the aging population [2]. This underscores the importance of efforts to prevent cognitive impairment, including through the management of oxidative stress as one of the key mechanisms of neurodegeneration.

One environmental risk factor contributing to cognitive decline is exposure to heavy metals such as lead (Pb). Lead acetate can cause oxidative stress, lipid peroxidation, protein damage, and increased ROS; all of which can lead to neuron death [3]. Due to Pb's ability to accumulate in tissues, including the brain, the WHO classifies Pb as one of the most dangerous environmental toxins [4]. Pb exposure is particularly harmful to the central nervous system, especially during the developmental phase. It can also cause memory impairment and neurogenesis difficulties [5], [6]. The hippocampus, which is an important part of the spatial memory process, is damaged by Pb exposure, according to research [7].

The hippocampus, particularly the CA1 and CA3 regions, is responsible for memory and learning processes. While pyramidal cells in CA1 are highly sensitive to hypoxia, pyramidal cells in CA3 are more sensitive to long-term and physical stress, which can cause memory impairment and a decrease in the number of neurons [8]. In animal models exposed to lead, spatial memory impairment is directly related to damage in both of these areas [9].

Due to their low side effects and wide availability, plant antioxidants have become a major focus. These antioxidants serve to neutralize free radicals and prevent cell damage [10]. Moringa leaves (*Moringa oleifera*), is one of the potential plants that contain flavonoids, alkaloids, tannins, and saponins. More than ninety micronutrients and various bioactivities are found in moringa. Antioxidant, anti-inflammatory, and neuroprotective effects are among them [11]. As natural antioxidants, flavonoids aid in tissue regeneration, improve cerebral perfusion, and increase BDNF levels, which are responsible for memory [12]. Alkaloids and tannins can also capture free radicals and prevent lipid peroxidation [13]. Conversely, saponins function as neuroprotective agents by enhancing cholinergic transmission between neurons and improving vascular function [14].

Previous studies have shown that moringa leaf extract can improve memory deficits in Pb-induced animal models [15]. However, not much research has been done on moringa leaf fractions, which may have higher concentrations of active compounds. This study focuses more on spatial memory parameters, malondialdehyde (MDA) levels, and hippocampal pyramidal cell histopathology. Thus, the purpose of this study is to examine how moringa leaf fractions affect spatial memory ability, MDA levels, and the number of CA1 and CA3 pyramidal cells in the hippocampus of mice induced with lead acetate.

2. Method

2.1. Materials

The sample material used in this study was moringa leaf extract (*Moringa oleifera* Lam.) obtained from the Tawangmangu area, Central Java, and the following reagents: 70% ethanol, pellets (mouse food), Lead (II), 0.5% CMC, Ginkgo biloba, Dragendroff's reagent, Mayer's reagent, Wagner's reagent, and concentrated sulfuric acid.

2.2. Tools

The tools used for maceration are 2L dark glass bottles, flannel cloth, a grinding machine, a number 40 sieve, an Erlenmeyer flask, filter paper, a glass funnel, a test tube, a stirring rod, a measuring cup, an evaporator, an oven, a Sterling-Bidwell, a mouse cage, animal scales, a syringe, a mask, gloves, a stopwatch, and a Radial Arm Maze.

2.3. Moringa leaf extraction

Maceration is carried out by placing one part of dry powdered simplisia or 500 grams into a macerator, then adding 10 parts of 70% ethanol solvent, totaling 5000 ml. It is soaked for the first 6 hours while stirring repeatedly, then left to stand for 18 hours before the macerate is filtered. The extraction process is repeated at least once with the same type of solvent and a solvent volume equal to half the volume used in the first extraction, or 2500 ml. The collected macerate is then evaporated using a vacuum evaporator or low-pressure evaporator until a concentrated extract is obtained.

2.4. Fractionation of moringa leaf extract

Twenty grams of ethanol extract from moringa leaves were dissolved in a small amount of hot water, then partitioned with 100 ml of water and 100 ml of n-hexane solvent into a separating funnel and repeated three times. The n-hexane filtrate was separated from the water fraction, collected, and concentrated with a rotary evaporator at a heating temperature of 50ºC. The water fraction remaining from the n-hexane fraction was then refracted with 100 ml of ethyl acetate solvent using a separating funnel. This process was repeated three times. The ethyl fraction is separated from the water fraction and then concentrated with a rotary evaporator at a temperature of 50ºC. The remaining filtrate from fractionation with ethyl acetate is the water fraction, which is then thickened with a water bath until thick.

2.5. Test Animal Grouping

This study used 35 mice divided into 7 test groups, each consisting of 5 mice, as follows group I was the positive control, group II was the negative control with 0.5% CMC, group III: positive control with Ginkgo biloba 9.75 mg/kg mouse body weight, group IV: moringa leaf extract 420 mg/kg mouse body weight, group V: n-hexane fraction, group VI: ethyl acetate fraction, group VII: water fraction.

2.6. Memory Test Procedure using a radial arm maze

The mice were adapted to the food and environment for 7 days. After that, the mice were adapted to the Radial Arm Maze apparatus for 5 consecutive days, where on the first day the mice were placed in the center of the apparatus and adapted for 10 minutes, on the second day the mice were placed in the center of the apparatus and adapted for 10 minutes with bait placed alternately on the four arms at the front of the arms, On the third and fourth days, the mice were placed in the center of the apparatus and adapted for 10 minutes, with food provided at the four arms alternately in the middle and at the ends of the arms. On the fifth day, the mice were placed in the center of the apparatus and food was provided at the four arms alternately at the ends of the arms. After the adaptation process, each test group was given lead (II) acetate induction at 140 mg/kg BW (ip) once for 7 days, followed by observation of latency time and % type B errors in entering the Radial Arm Maze arm. Type B errors were defined as mice entering more than half the length of the arm but not eating the bait. The following day, the mice were treated for 21 days according to the test animal distribution. To measure the memory function of all mice, the latency time and error rate of mice entering the arm (type B error) were observed for 10 minutes.

2.7. Malondialdehyde level test

After conducting memory tests, the cerebrum (large brain) was removed and divided into two parts, namely the left cerebrum and right cerebrum, for malondialdehyde calculation and hippocampal histopathological examination. To measure malondialdehyde levels, the left cerebrum was fixed with 10% formalin to determine the lipid peroxide level. The malondialdehyde level test used in this study was the thiobarbituric acid reactive substance (TBARS) method. A total of 0.1 grams of brain was added to cold 0.9% NaCl. It was centrifuged at a speed of 8000 rpm for 20 minutes. 0.1 ml of the homogenized brain was added to 0.1 TCA, then 0.1 ml of TBA solution was added. The mixture was heated in a water bath at 100°C for 20 minutes, cooled, centrifuged at 500 rpm for 10 minutes, and the supernatant was collected and its absorbance measured. The mixture is then added with water to a volume of 4.0 ml, heated at 95°C for 60 minutes, and cooled with water.

2.8. pyramidal cell calculation

Calculation of damaged hippocampal pyramidal cells in the CA1 and CA3 areas and observation of hippocampal histopathology. The right cerebrum was fixed with 10% formalin, then a histological preparation was made with hematoxylin eosin staining. The finished preparation was then observed under a microscope with the aid of an Optilab device with 400x magnification, which was connected to a computer. After obtaining the pyramidal cells in the CA1 and CA3 areas, the microscope display was photographed. The photos were processed using Adobe Photoshop CS4 until the pyramidal cells in the CA1 and CA3 areas were visible.

2.9. Data analysis

The data obtained were latency time, % error B, and MDA levels. Each data set was statistically analyzed using the Shapiro-Wilk test to determine data normality and the

Levene test to determine data homogeneity. If the data met the requirements, it was then analyzed using a one-way ANOVA to determine whether there were differences in the data. If differences were found, a Tukey Post Hoc test was then used to determine the actual differences. If the data obtained is not normally distributed, the Kruskal-Wallis test, Mann-Whitney test, or Wilcoxon test can be used.

3. Results and Discussion

3.1. Radial Arm Maze Test Results

Memory testing using the Radial Arm Maze method was conducted by calculating latency time and type B error percentage during testing. Latency time was calculated based on the time required for the test animal to find the bait provided. Type B error percentage was calculated based on the test animal's error of entering more than the length of the arm but not eating the bait provided. This study was conducted with 5 days of adaptation, 7 days of lead (II) acetate induction, and 21 days of treatment, followed by observation of latency time and percentage of type B errors [16].

Table 1. Latency Calculation

	Average latency time (seconds)±SD				
Treatment	T0	T1	T2a	T2b	T2c
I	53.8±7.8	227.2±6.5	220.4±6.6	216.8±6.1	218±6[b]
II	52.2±8	213±6.6	208.4±4	206.8±9.8	203±5.3[b]
III	52±7.8	228±5.7	243±4.8	254±10.1	260.8±12.4[ac]
IV	52.6±9	218±8.8	215±3.3	213.8±3.1	212±2.3[b]
V	53±8.8	221.6±7.5	223.8±8	219±10.8	208.6±6.6[ab]
VI	53.8±5.7	214.8±9.2	215.6±3.3	215.2±3.7	208.6±2[b]
VII	52.6±9	217.6±9.6	220±1.2	217±3	218±3a[b]

Description:
a : significantly different from normal control
b : significantly different from negative control
c : significantly different from positive control
T1 : Day 7 after induction with lead (II) acetate
T2a : Day 7 after administration of moringa leaf ethanol extract
T2b : Day 14 after administration of moringa leaf ethanol extract
T2c : Day 21 after administration of moringa leaf ethanol extract
I : normal control
II : positive control
III : negative control
IV : EEDK 420 mg/kg BW menccit
V : n-Hexane fraction
VI : ethyl acetate fraction
VII : water fraction

There were differences in latency times between each group of test animals (Table 1). All treatment groups experienced an increase in latency time after being induced with lead (II) acetate at a dose of 100 mg/kg BW for 7 days, where lead (II) was intended to damage the brain, thereby impairing memory function [17]. The next step was an SPSS test using

Shapiro-Wilk, which produced a normally distributed value with p > 0.05. The results of the statistical analysis of the T0-T1 data using a paired sample test yielded p<0.05, which means that there was a significant difference in the data after lead (II) induction. This is in line with the research objective, which expected mice to experience an increase in the time taken to find the reward provided due to the damage caused by lead (II) acetate induction.

The memory enhancement phase for the test animals was conducted over 21 days with a latency parameter, which is the time required for mice to find food. On day 7 (T2a), the negative control group showed no significant difference compared to the normal control, positive control, 420 mg/kg BW extract, n-hexane fraction, ethyl acetate, or water fraction. The Shapiro–Wilk test showed that the data were normally distributed (p>0.05), and the One Way ANOVA test also showed no significant differences (p>0.05) after Pb(II) induction. On day 14 (T2b), the negative control began to show significant differences compared to all other treatment groups. The data remained normally distributed (Shapiro–Wilk p>0.05), but the ANOVA results showed significant differences between groups after 14 days of lead exposure.

On day 21, the negative control group differed significantly from the positive control, 420 mg extract, ethyl acetate fraction, and normal control, while the n-hexane fraction and water fraction differed from the positive control according to the Shapiro Wilk test (p > 0.05) and One Way ANOVA (p < 0.05). Exposure to Pb-acetate appeared to reduce learning/memory ability, while positive control treatments (e.g., flavonoids) accelerated the time to find the bait, possibly due to the antioxidant and neuroprotective effects of flavonoids. Environmental factors and stress can also affect memory performance: recent studies show that stress, especially if prolonged, can impair spatial memory in mice [18].

Table 2. Calculation of type B error %

Average % of errors B±SD					
Treatment	**T0**	**T1**	**T2a**	**T2b**	**T2c**
I	12.5±14.2	25±8.8	15±10.4	25±8.8	15±10.4[b]
II	15±10.4	22.5±8.8	25±8.8	25±8.8	27.5±10.4[ac]
III	15±10.4	25±10.4	12.5±8.8	10±6.8	0±0[b]
IV	15±16.2	25±12.5	15±10.4	15±13.6	12.5±12.5[b]
V	15±10.4	22.5±10.4	15±10.4	20±6.8	20±14.2[ac]
VI	10±10.4	22.5±13.6	12.5±8.8	10±10.4	10±10.4[b]
VII	12.5±15.3	17.5±14.2	15±10.4	15±10.4	20±11.1[ac]

Description:
a : significantly different from normal control
b : significantly different from negative control
c : significantly different from positive control
T1 : Day 7 after induction with lead (II) acetate
T2a : Day 7 after administration of moringa leaf ethanol extract
T2b : Day 14 after administration of moringa leaf ethanol extract
T2c : Day 21 after administration of moringa leaf ethanol extract
I : normal control
II : positive control

III : negative control
IV : EEDK 420 mg/kg BW menccit
V : n-Hexane fraction
VI : ethyl acetate fraction
VII : water fraction

Based on Table 2, it can be seen that after administration of Lead(II) acetate, on day 7 the type B error rate in test animals increased. The paired t-test showed a significant difference between the conditions before (T0) and after induction (T1) indicating a negative effect of Pb on memory ability. Pb exposure can trigger neuronal apoptosis and damage to the hippocampus, thereby adversely affecting learning and memory functions. For example, studies show that Pb acetate causes degeneration of hippocampal pyramidal neurons, decreased BDNF, increased apoptosis, and spatial memory deficits in rats [19].

On day 7 (T2a), all treatment groups showed no significant differences compared to the control group, according to the Shapiro–Wilk test (>0.05) and One-Way ANOVA (>0.05). On day 14 (T2b), although the negative control appeared to differ from the other groups, the Shapiro–Wilk test remained >0.05 and the ANOVA >0.05, so the difference was not statistically significant. On day 21, ANOVA showed a significant difference (<0.05). The negative control was significantly different from the positive control, the 420 mg/kg extract, the ethyl acetate fraction, and the normal control. The ethyl acetate fraction showed comparable ability to the positive control, possibly due to its antioxidant flavonoid content. Flavonoids can neutralize free radicals, increase blood flow through vasodilation, and support BDNF regulation. These findings are in line with Susilowati *et al.* [20], who reported that moringa leaf extract improved Pb-acetate-induced spatial memory in mice.

3.2. MDA level test results

Malondialdehyde (MDA) is a marker of oxidative stress as the end result of the lipid peroxidation chain reaction. High MDA concentrations indicate oxidation processes in cell membranes. Erythrocyte and plasma MDA levels have been used as markers of tissue damage caused by free radicals. To date, MDA is the most widely studied marker and is considered a good marker of in vivo lipid peroxidation in both humans and animals, being significantly more accurate and stable than other compounds [21], [22].

Table 3. Mean ± SD of malondialdehyde levels and percentage decrease in MDA

Group	MDA Level	% Reduction in MDA Level
Normal control	4.97±0.04^{bc}	69.4
Positive control	4.81±0.165^{ac}	70.4
Negative control	16.26±0.08^{ab}	0
Extract 420 mg/kg BW mouse	6.84±0.07^{ac}	57.9
n-Hexane fraction	11.34±0.08^{abc}	30.2
Ethyl acetate fraction	4.86±0.03 ^{ac}	70
Water fraction	9.80±0.71^{abc}	39.6

Description:
a. Different meaning from normal control
b. Different meaning from positive control

c. Different meaning from negative control

Based on the results of the SPSS test, the distribution of malondialdehyde (MDA) levels in the brains of mice was normal ($p > 0.05$), and data homogeneity was also met ($p < 0.05$). ANOVA analysis showed significant differences between treatment groups ($p < 0.05$). The graph shows that the average MDA levels in the positive control and ethyl acetate fraction groups were much lower than in the negative control. The 420 mg/kg BW extract, n-hexane fraction, and water fraction also showed a decrease in MDA levels compared to the negative control, although the decrease was not as drastic as in the positive control [23]. The negative control had high MDA levels, which could be caused by the administration of Pb-acetate, which triggers lipid peroxidation [24].

The percentage decrease in MDA levels showed that the positive control and ethyl acetate fraction had the highest effect (≈70%), followed by the 420 mg/kg BW extract (57%), the water fraction (38.9%), and the n-hexane fraction (30.2%). The mechanism of MDA reduction mainly stems from the activity of flavonoids, which are capable of capturing free radicals by donating hydrogen ions, increasing the expression of endogenous antioxidant genes, and inhibiting ROS-forming enzymes such as xanthine oxidase and NADPH oxidase. Flavonoids also chelate Fe^{2+} and Cu^{2+}, thereby preventing free radical-producing redox reactions [25], [26]. Alkaloid compounds also act as free radical scavengers and metal ion chelators, thereby stopping the chain reaction of lipid peroxidation. Several alkaloids, especially the indole group, show effective ability to bind hydroxyl radicals and prevent cell damage due to oxidative stress [27].

Other studies have also shown that alkaloids have strong antioxidant activity by capturing free radicals and neutralizing ROS [28]. Tannin compounds also play an important role, as tannins can stabilize membrane lipids, inhibit lipid peroxidation, and chelate metal ions such as Fe^{2+}, thereby stopping the Fenton reaction and the formation of hydroxyl radicals [29]. These interactions help maintain cellular homeostasis and protect membranes and biomolecules such as lipids and DNA from oxidative damage [30]. In addition, saponins have also been reported to have antioxidant activity and the potential to inhibit enzymes such as α-glucosidase, although their direct mechanism of action on ROS or lipid peroxidation has been less discussed. The presence of saponins as part of secondary metabolites with antioxidant properties supports their protective role against oxidative stress [31]. Thus, the presence of compounds such as alkaloids, tannins, and saponins in plant extracts provides a protective mechanism against ROS through radical scavenging, metal chelation, and inhibition of oxidative reactions, which may explain the decrease in MDA levels and protection against lipid peroxidation and tissue damage.

3.3. Results of Calculations of the Number of Damaged Pyramidal Cells in the Hippocampus in the CA1 and CA3 Areas

Data collection was performed by counting the number of damaged pyramidal cells in the CA1 and CA3 areas. Normal pyramidal cells have large nuclei with clear nucleoli, while damaged pyramidal cells are characterized by pyknotic, dense, darker-colored neuron nuclei and vacuolization [32], [33]. Based on the results of the SPSS test of pyramidal cells in the CA1 and CA3 areas using the Willcoxon test, $P < 0.05$ was found, which means that

the data showed a significant difference. The differences between each group occurred due to the administration of lead II acetate induction.

Table 4. Mean±SD and percentage of inhibition of the number of damaged hippocampal pyramidal cells in the CA1 and CA3 areas

Group	Pyramidal cells of the CA1 area	Pyramidal cells of the CA3 area	% Inhibition of CA1 pyramidal cell damage	% Inhibition of CA3 pyramidal cell damage
Normal control	18±3,09[bc]	17±3,09[bc]	46,8	48,04
Positive control	13±0,5[ac]	10±0,47[ac]	60,24	69
Negative control	33±6,12[ab]	33±8,65[ab]	0	0
Extract 420 mg/kg BW mouse	19±1,88[ac]	17±1,24[ac]	42,8	49,8
n-Hexane fraction	26±6,64[abc]	20±1,24[ac]	21,4	39
Ethyl acetate fraction	14±2,05[ac]	12±0,94[abc]	58,1	64,8
Water fraction	18±2,05[abc]	17±2,16[abc]	39,7	48,9

Description:
a. Different meaning from normal control
b. Different meaning from positive control
c. Different meaning from negative control

Lead acetate (Pb) can disrupt brain function, including learning and memory processes, through several mechanisms. Exposure to Pb reduces the activity of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and other defense enzymes in the brain (hippocampus, cortex, cerebellum), thereby triggering oxidative stress, apoptosis, and synaptic transmission disorders [34]. This condition can cause neuron death, including pyramidal cells in the hippocampus (CA1 & CA3) area, which ultimately impairs spatial memory and learning ability [19].

Based on the CA3 results in table 4, the positive control group, ethyl acetate fraction, n-hexane fraction, water fraction, and 420 mg moringa leaf extract showed a significant decrease in the percentage of damaged cells compared to the negative control. Exposure to Pb-acetate is known to impair memory and learning, not only through oxidative stress but also by reducing antioxidant enzymes, thereby damaging neurons in the hippocampus, frontal cortex, and striatum [35]. Several recent studies have shown that Pb exposure increases lipid peroxidation and reduces endogenous antioxidant capacity, accelerating cell death and neuronal degeneration [36]. Then, based on the results in CA1 and CA3, each treatment group had different abilities to repair hippocampal neurons, possibly due to differences in the content of compounds such as flavonoids, alkaloids, tannins, and saponins. Flavonoids, for example, have been widely reported to have neuroprotective effects that help maintain synaptic plasticity, support neurogenesis, and reduce oxidative stress and inflammation in the brain [37].

The main flavonoids found in moringa leaves are quercetin, kaempferol, and myrecetin [38]. Quercetin has neuroprotective effects by inhibiting NO release through suppression of iNOS activity and reducing excess glutamate in hippocampal neurons, thereby

preventing cytotoxicity and apoptosis [39]. Quercetin also inhibits acetylcholinesterase (AChE) activity, improves cognitive function, and reduces free radicals through its catechol group activity, thereby suppressing nerve inflammation, oxidative stress, and DNA damage. Kaempferol works by modulating neuroinflammation through inhibition of the TLR4 and NF-κB pathways and suppressing the release of NO and TNF-α while enhancing SIRT regulation, which plays a role in neuron protection. However, flavonoids are lipophilic with limited BBB permeability; chemical modification or nanoparticle technology has been shown to increase the bioavailability of flavonoids in neurodegenerative brains [40].

Alkaloids also play a neuroprotective role. One of their components, niazimicin, has been shown to reduce Aβ burden in APP/PS1 mouse models and decrease MDA, cholinesterase, NO, caspase-3, and iNOS [41]. Aβ accumulation is known to trigger synaptic dysfunction and cognitive changes in dementia. Niazimicin even shows effects comparable to donepezil in reducing Aβ, brain MDA, ChE activity, and increasing GSH [42].

Tannins act as antioxidants through the activation of the Nrf2 pathway, which is important in controlling oxidative stress and regulating transcription when cells experience stress [43]. Inhibition of Nrf2 has been shown to improve cognitive impairment. Nrf2 also plays a role in regulating apoptosis by increasing Bcl-2 expression, thereby providing antiapoptotic and cytoprotective effects [44]. In addition, saponins are known to have nerve stimulation effects and various bioactivities, including reducing Aβ deposition, inhibiting tau phosphorylation, antioxidants, antiapoptosis, and anti-inflammation. This mechanism involves a decrease in APP formation, a decrease in ROS, inhibition of the apoptosis-inflammation pathway, and an increase in neurotransmitters [45], [46], [47]. These activities enable saponins to repair cell damage caused by exposure to lead acetate.

Moringa leaves also contain β-sitosterol, which increases antioxidant enzymes through the estrogen/PI3K pathway and increases glutathione levels as a free radical scavenger. β-sitosterol can inhibit lipid peroxidation by integrating into cell membranes, making it beneficial in neurodegenerative disorders including dementia. This compound also exhibits inhibitory activity against ChE, AChE, and BChE and improves working memory and motor coordination. Additionally, β-sitosterol enhances neural stem cell proliferation in the SVZ zone, supporting the neurogenesis process [48], [49], [50].

4. Conclusion

The conclusions drawn from this study include that moringa leaf fraction has the ability to improve spatial memory, reduce brain MDA levels in lead acetate-induced dementia model mice, and can influence the reduction of damage to hippocampal pyramidal cells in CA1 and CA3 pyramidal cells.

REFERENCES

[1] WHO (World Health Organization), "Dementia fact sheet," 2020, *Geneva: WHO Press*.

[2] Alzheimer's Disease International (ADI), *World Alzheimer Report 2021: Journey through the diagnosis of dementia*. London: ADI, 2021.

[3] A. O. S. Yousef, A. A. Fahad, A. E. A. Moneim, D. M. Metwally, M. F. El-khadragy, and R. B. Kassab, "The Neuroprotective Role of Coenzyme Q10 Against Lead Acetate-Induced

Neurotoxicity Is Mediated by Antioxidant , Anti-Inflammatory and Anti-Apoptotic Activities," *International Journal of Environmental Research and Public Health Article*, vol. 16, no. 2895, pp. 1–17, 2019.
[4] WHO (World Health Organization), "Lead Poisoning and Health," 2021, *Geneva: WHO Press*.
[5] A. K. Engstrom and Z. Xia, "Lead Exposure in Late Adolescence through Adulthood Impairs Short-term Spatial Memory and the Neuronal Differentiation of Adult-born Cells in C57BL/6 Male Mice," *Neurosci Lett*, vol. 661, pp. 108–113, 2018, doi: 10.1016/j.neulet.2017.09.060.Lead.
[6] T. Wang *et al.*, "Lead Exposure Impairs Hippocampus Related Learning and Memory by Altering Synaptic Plasticity and Morphology During Juvenile Period," *Molecular Neurobiology*, vol. 53, no. 6, pp. 3740–3752, 2016, doi: 10.1007/s12035-015-9312-1.
[7] C.-W. Lee *et al.*, "Exposure to Polystyrene Microplastics Impairs Hippocampus-dependent Learning and Memory in Mice," *Journal of Hazardous Materials*, vol. 430, p. 128431, 2022, doi: https://doi.org/10.1016/j.jhazmat.2022.128431.
[8] N. Gulyaeva, "Stress-Associated Molecular and Cellular Hippocampal Mechanisms Common for Epilepsy and Comorbid Depressive Disorders," *Biochemistry (Moscow)*, May 2021.
[9] D. R. Ortega *et al.*, "Cognitive Impairment Induced by Lead Exposure during Lifespan : Mechanisms of Lead Neurotoxicity," *Toxics*, vol. 9, no. 23, pp. 1–30, 2021.
[10] Z. Liu, T. Zhou, A. C. Ziegler, P. Dimitrion, and L. Zuo, "Review Article Oxidative Stress in Neurodegenerative Diseases : From Molecular Mechanisms to Clinical Applications," *Oxidative Medicine and Cellular Longevity*, vol. 2017, pp. 1–11, 2017, doi: 10.1155/2017/2525967.
[11] M. Kamran *et al.*, "Phytochemical of Moringa oleifera its Nutritional and Pharmacological Importance," *Postepy Biologii Komorki*, vol. 47, pp. 321–334, Sep. 2020.
[12] S. Tang *et al.*, "Structural Insights and Biological Activities of Flavonoids : Implications for Novel Applications," *Food Frontiers*, no. August 2024, pp. 218–247, 2024, doi: 10.1002/fft2.494.
[13] O. O. Oguntibeju, G. Y. Aboua, and E. I. Omodanisi, "Effects of Moringa oleifera on Oxidative Stress, Apoptotic and Inflammatory Biomarkers in Streptozotocin-induced Diabetic Animal Model," *South African Journal of Botany*, vol. 129, pp. 354–365, 2020, doi: https://doi.org/10.1016/j.sajb.2019.08.039.
[14] M. Zeng *et al.*, "Pharmacological Therapy to Cerebral Ischemia-reperfusion Injury: Focus on Saponins," *Biomedicine & Pharmacotherapy*, vol. 155, p. 113696, 2022, doi: https://doi.org/10.1016/j.biopha.2022.113696.
[15] W. Arozal, M. S. Safutra, A. J. Barinda, H. Hardi, N. C. Dwita, and H. J. Lee, "Comparative Neuroprotective Effects of Moringa oleifera Seed Oil and Aqueous Extract on Cognitive Functions on a High-Fat , High-Fructose Diet Mice : Focus on Senescence Markers," *Te Scientifc World Journal*, vol. 2024, pp. 1–9, 2024, doi: 10.1155/2024/8034401.
[16] T. Shimizu, S. G. Nayar, B. R. Ransom, and W. D. Richardson, "An Appetitive Spatial Working Memory Task for Mice in a Semi-Automated 8-Arm Radial Maze, Reducing Fearful Memory Association in the Maze," *J. Vis. Exp*, no. 221, pp. 1–24, 2025, doi: 10.3791/66456.
[17] A. B. Enogieru and B. T. Williams, "Cognitive- and Memory-enhancing Activity of Cinnamon (Cinnamomum zeylanicum) Aqueous Extract in Lead Acetate-Exposed Rats," *Journal of Trace Elements and Minerals*, vol. 9, no. August, p. 100189, 2024, doi: 10.1016/j.jtemin.2024.100189.
[18] D. Juananda and R. V. Astari, "Stres Imobilisasi Kronik Mengganggu Memori Spasial Mencit Putih (Mus musculus) Galur Swiss Webster Jantan," *Anatomica Medical Journal*, vol. 1, no. 1, pp. 27–33, 2018.
[19] B. Ben-Azu *et al.*, "Lead Acetate Induces Hippocampal Pyramidal Neuron Degeneration in Mice Via Up-regulation of Executioner Caspase-3, Oxido-inflammatory Stress Expression

and Decreased BDNF and Cholinergic Activity: Reversal Effects of Gingko biloba Supplement," *Journal of Trace Elements in Medicine and Biology*, vol. 71, p. 126919, 2022, doi: https://doi.org/10.1016/j.jtemb.2021.126919.

[20] A. A. Susilowati, G. P. Widodo, and J. Merari, "Efek Ekstrak Etanol Daun Kelor (Moringa oleifera, Lamk.) pada Mencit Model Demensia: Kajian Memori Spasial, Kadar Malondialdehid Dan Jumlah Sel Piramidal Hipokampus Area CA1 Dan CA2-CA3," *Jurnal Farmasi Indonesia*, vol. 2, pp. 64–78, Nov. 2019.

[21] K. Mohideen, K. Chandrasekar, S. Ramsridhar, C. Rajkumar, S. Ghosh, and S. Dhungel, "Review Article Assessment of Oxidative Stress by the Estimation of Lipid Peroxidation Marker Malondialdehyde (MDA) in Patients with Chronic Periodontitis : A Systematic Review and Meta-Analysis," *International Journal of Dentistry*, vol. 2023, pp. 1–16, 2023.

[22] A. G. Sulaiman *et al.*, "Malondialdehyde (MDA) sebagai Marker Stres Oksidatif Berbagai Penyakit," *Medula*, vol. 14, pp. 321–325, 2024.

[23] A. Catalá and M. Díaz, "Editorial : Impact of Lipid Peroxidation on the Physiology and Pathophysiology of Cell Membranes," *Front. Physiol*, vol. 7, p. 423, 2016, doi: 10.3389/fphys.2016.00423.

[24] F. E. Hasan and R. Yunus, "Fungsi Antioksidan dalam Menghambat Peroksidasi Lipid dan Meningkatkan Ketahanan Membran Eritrosit pada Penderita Diabetes Melitus," *Health Information : Jurnal Penelitian*, vol. 15, no. 2, pp. 1–12, 2023.

[25] X.-Q. Lu, P. Ren, S. Qin, J. Li, and X.-N. Zhao, "Antioxidant Potential of Four Flavonoids from Scutellaria baicalensis: a Computational Investigation on Radical Scavenging Activity and Enzyme Inhibition," *New Journal of Chemistry*, no. 26, 2025.

[26] R. Haider, "Flavonoids as Antioxidants," *Clinical Research and Clinical Trials*, vol. 8, no. 3, pp. 1–9, 2023, doi: 10.31579/2693-4779/149.

[27] F. W. Aljanah, S. Oktavia, and F. Noviyanto, "Formulasi dan Evaluasi Sediaan Hand Body Lotion Ekstrak Etanol Daun Semangka (Citrullus lanatus) sebagai Antioksidan," *Formosa Journal of Applied Sciences (FJAS)*, vol. 1, no. 5, pp. 799–818, 2022.

[28] P. R. Sarjono, N. D. Laksmitasari, M. Asy, and E. Fachriyah, "Aktivitas Antioksidan dan Antibakteri dari Alkaloid Bakteri Endofi t Pseudomonas hibiscicola Wk Asal Daun Waru (Hibiscus tiliaceus)," *Jurnal Penelitian Saintek*, vol. 30, no. 1, pp. 10–26, 2025.

[29] T. Basu, S. Panja, A. K. Shendge, A. Das, and N. Mandal, "A Natural Antioxidant, Tannic Acid Mitigates Iron-overload Induced Hepatotoxicity in Swiss Albino Mice through ROS Regulation," *Environmental toxicology*, vol. 33, no. 5, pp. 603–618, May 2018, doi: 10.1002/tox.22549.

[30] S. Ouahhoud *et al.*, "Antioxidant Activity, Metal Chelating Ability and DNA Protective Effect of the Hydroethanolic Extracts of Crocus sativus Stigmas, Tepals and Leaves," *Antioxidants*, vol. 11, no. 932, pp. 1–21, 2022.

[31] Y. Han *et al.*, "Characterization of Saponins and Phenolic Compounds: Antioxidant Activity and Inhibitory Effects on α-glucosidase in Different Varieties of Colored Quinoa (Chenopodium quinoa Willd)," *Bioscience, Biotechnology, and Biochemistry*, vol. 83, no. 11, pp. 2128–2139, Nov. 2019, doi: 10.1080/09168451.2019.1638756.

[32] H. Fikry, L. A. Saleh, F. A. Mahmoud, S. A. Gawad, and H. A. Abd-Alkhalek, "CoQ10 Targeted Hippocampal Ferroptosis in a Status Epilepticus Rat Model," *Cell and Tissue Research*, vol. 396, no. 3, pp. 371–397, 2024, doi: 10.1007/s00441-024-03880-z.

[33] S. Matar, R. A. Gomaa, A. El Wakil, and A. Essawy, "Human Placental Extract Rescues Hippocampal Damage Associated with Cognitive Impairment in Diabetic Male Rats through Antioxidative, Anti-inflammatory , and Neuromodulatory Activities," *Metabolic Brain Disease*, vol. 40, no. 225, pp. 1–18, 2025.

[34] M. H. S. H. Ferrazza *et al.*, "Sub-chronic Administration of Lead Alters Markers of Oxidative Stress, Acetylcholinesterase and Na+K+-ATPase Activities in Rat Brain," *Acta Neurobiol Exp*, vol. 83, pp. 216–225, 2023, doi: 10.55782/ane.

[35] G. Sharma, M. Singh, A. Tiwari, S. K. Bunkar, and P. J. John, "Acute Co-Exposure of Lead and Cadmium Induce Pronounced Neurotoxicity in Wistar Rat," *Journal of Chemical Health Risks*, vol. 15, no. 3, pp. 19–30, 2025.

[36] M. G. Paulis, O. A. Hassan, M. F. Abbass, and M. A.-A. H. Mohammad, "Structural and Lipid Peroxidation Effects of Lead on Rat Hippocampus and Its Attenuation by Hydrogen Rich Water," *Journal of Chemical Neuroanatomy*, vol. 91, pp. 55–62, 2018, doi: https://doi.org/10.1016/j.jchemneu.2018.04.004.

[37] N. Cichon, J. Saluk-bijak, L. Gorniak, L. Przyslo, and M. Bijak, "Flavonoids as a Natural Enhancer of Neuroplasticity – An Overview of the Mechanism of Neurorestorative Action," *Antioxidants*, vol. 9, no. 1035, pp. 1–19, 2020.

[38] M. L. Vergara-Jimenez, M., Almatrafi, M. M., dan Fernandez, "Bioactive Components in Moringa Oleifera Leaves Protect against Chronic Disease," *Antioxidants*, vol. 6, no. 91, pp. 1–13, 2017.

[39] C. Alexander, A. Parsaee, and M. Vasefi, "Polyherbal and Multimodal Treatments: Kaempferol- and Quercetin-Rich Herbs Alleviate Symptoms of Alzheimer's Disease," *Biology*, vol. 12, p. 1453, 2023, doi: https:// doi.org/10.3390/biology12111453.

[40] L. Chen, Y. Guan, S. Wang, X. Han, F. Guo, and Y. Wang, "Engineered Nanoplatforms for Brain-targeted Co-delivery of Phytochemicals in Alzheimer's Disease: Rational Design, Blood-brain Barrier Penetration, and Multi-target Therapeutic Synergy," *Neurotherapeutics*, vol. 22, no. 6, 2025, doi: https://doi.org/10.1016/j.neurot.2025.e00722.

[41] Y. A. R. Mahaman *et al.*, "Moringa Oleifera Alleviates A β Burden and Improves Synaptic Plasticity and Cognitive Impairments in APP/PS1 Mice," *Nutrients*, vol. 14, no. 4284, pp. 1–24, 2022.

[42] E. Abdelsayed, D. Medhat, Y. Mandour, R. Hanafi, and A. Abdel Motaal, "Niazimicin: A thiocarbamate Glycoside from Moringa oleifera Lam. Seeds with a Novel Neuroprotective Activity," *Journal of Food Biochemistry*, vol. 45, Nov. 2021, doi: 10.1111/jfbc.13992.

[43] F. Sehati *et al.*, "Tannic Acid Protects Aged Brain Against Cerebral Hypoperfusion Via Modulation of Nrf2 and Inflammatory Pathways," *Neuroscience Letters*, vol. 765, p. 136263, 2021, doi: https://doi.org/10.1016/j.neulet.2021.136263.

[44] M. Li, P. Liu, Y. Xue, Y. Liang, and J. Shi, "Tannic Acid Attenuates Hepatic Oxidative Stress, Apoptosis and Inflammation by Activating the Keap1 - Nrf2/ARE Signaling Pathway in Arsenic Trioxide - toxicated Rats," *Oncology Reports*, vol. 44, pp. 2306–2316, 2020, doi: 10.3892/or.2020.7764.

[45] A. A. Abduljawad *et al.*, "Alzheimer ' s Disease as a Major Public Health Concern : Role of Dietary Saponins in Mitigating Neurodegenerative Disorders and Their Underlying Mechanisms," *Molecules*, vol. 27, no. 6804, pp. 1–23, 2022.

[46] M. I. Khan *et al.*, "Neuroprotective Effects of Green Tea Seed Isolated Saponin Due to the Amelioration of Tauopathy and Alleviation of Neuroinflammation : A Therapeutic Approach to Alzheimer ' s Disease," *Molecules*, vol. 27, no. 2079, pp. 1–15, 2022.

[47] R. Zhang *et al.*, "Therapeutic Candidates for Alzheimer ' s Disease : Saponins," *International Journal of Molecular Sciences Review*, vol. 24, no. 10505, pp. 1–34, 2023.

[48] M. Ayaz *et al.*, "Anti-Alzheimer ' s Studies on β -Sitosterol Isolated from Polygonum hydropiper L .," *Front. Pharmacol*, vol. 8, no. October, p. 697, 2017, doi: 10.3389/fphar.2017.00697.

[49] N. Dolrahman and W. Thong-asa, "Beta-sitosterol Mitigates Cognitive Deficit and Hippocampal Neurodegeneration in Mice with Trimethyltin-induced Toxicity," *Experimental Animals*, vol. 73, no. 4, pp. 433–445, 2024, doi: 10.1538/expanim.24-0021.

[50] M. Sajad *et al.*, "β-Sitosterol Ameliorates the Cognitive Deficits and Neuropathological Hallmarks in an Alzheimer′ s Disease Model," *Arabian Journal of Chemistry*, vol. 18, no. 1, p. 106072, 2025, doi: https://doi.org/10.1016/j.arabjc.2024.106072.