

BLACK FRUIT SEED EXTRACT EFFECT ON THE MEMORY OF WISTAR WHITE MOUSE INDUCED $AlCl_3$

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ABSTRAK

Black fruit seed extract (*Haplolobus monticola*) contains flavonoid, alkaloid, and terpenoid compounds. Flavonoids are beneficial to the cognitive function of the human brain. Terpenoid derivatives can be used as potential therapeutic agents for the treatment of dementia. Alkaloids have shown good affinity with the cholinergic enzyme AChE. To find out the effect of black fruit seed extract on the memory of Wistar white mouse induced $AlCl_3$. $AlCl_3$ was used to induce experimental animal models of dementia. Experimental design using Complete Randomized Design and is comparative. The subject of the study used was Wistar White Mouse. The subjects of the study were randomly divided into 4 groups including K1 (aquadest 21 days continued Na-CMC 1% 10 days), K2 ($AlCl_3$ 21 days followed by Na-CMC 1% 10 days), K3 ($AlCl$ induction 3 21 days continued Donepezil), and K4 ($AlCl$ 3 21 days followed by 10 days). Memory evaluation using Morris water maze test data results were analyzed with a t-test to see differences before treatment and after. Then the One-way Anova test was to see the differences in the results of each group and continued with the Least Significant Difference (LSD) test. The mean value of the morris water maze after therapy administration in the K3 and K4 groups showed significant cognitive improvement. There was no meaningful difference between K3 and K4 against the decrease in the average value of the morris water maze test. Black fruit seed extract can improve memory in a Wistar white mouse that has been induced by $AlCl_3$ and is comparable to donepezil.

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INTRODUCTION

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Memory is the store of knowledge gained so that it can be recalled. Memory forms the basis of how individuals adapt to their environment. Memory can experience disorders such as dementia, among others, due to dementia (Sherwood, 2015).

Dementia according to the World Health Organization (WHO) is a symptom that can be chronic or progressive resulting in a decline in cognitive function that is not part of the normal aging process. It affects memory or memory of thought, orientation, comprehension, learning ability, language, and judgment. Based on data from WHO in 2018, Dementia is the seventh leading cause of death among all diseases and one of the leading causes of disability and dependence in the elderly worldwide. About 55 million people worldwide suffer from dementia, more than 60% of whom live in low- and middle-income countries. Dementia is particularly vulnerable in the elderly and the proportion of the elderly in the population is increasing in almost every country, dementia is projected to increase to 78 million by 2030 and 139 million by 2050. In 2019, the cost of treating dementia consumed more than 89 billion per hour with an estimated 5 hours per day per person with dementia. According to (Rao et al., 2006) Indonesia is estimated to have around 1.2 million people with dementia in 2016, which will increase to 2 million in 2030 and 4 million people by 2050. Various studies on the use of herbs have been widely tested to maintain and improve memory function. Research on black pepper extract can be able to improve memory in experimental animals induced by AlCl₃ (Iqbal, Iqbal, Mahboob, M Farhat, & Ahmed, 2016). Black fruit (*Haplolobus monticola*) which is native to Wondama West Papua. This fruit is a type of local plant that is intensively used as an additional food source (Ungirwalu, Awang, Maryudi, & Priyono, 2016). In ethyl acetate extract, seeds from black fruit contain flavonoid compounds, alkaloids, tannins, polyphenols, triterpenoids, and steroids (Toja, Suprayitno, & Yanuhar, 2020). Flavonoids are beneficial for the physiological health and cognitive function of the human brain such as improved memory, and accelerated psychomotor processes (Saputra & Sitepu, 2016). Terpenoid derivatives show high activity and can be used as potential therapeutic agents for the treatment of dementia (Dembitsky, Dzhemileva, Glorizova, & D'yakonov, 2020). Based on Insilco docking interactions and binding affinity studies, alkaloids have shown good binding affinity with AChE cholinergic enzymes that have a mechanism of cooperation with AChEi (Dembitsky et al., 2020). Black fruit seeds have been studied to be used as an antibacterial and there is still little research related to the use of seeds from black fruit for health.

METHOD

Experimental design using Complete Randomized Design (RAL) and is comparative. The research was carried out at the Wet Laboratory of the Faculty of Medicine, University of Papua from June to August 2022. The evaporation process is carried out at the STIKES Sorong Pharmaceutical Integrated Laboratory. This research has received ethical approval from the Health Research Ethics Committee of the Health Polytechnic of the Ministry of Health of Sorong. The research Permit Number is DM.03.05/6/030/2022.






The black fruit seed extract is carried out using the method of maceration or soaking using ethyl acetate solvent. Before the extraction process, the seeds are separated from the pulp and then cleaned with water after which they are dried using an oven at a temperature of 40°C. then puree until they become powder. Then soaked 200gr of powder in 800 ml of solvent for 2 days. Then it is filtered using filter paper. The filtrate is then evaporated using a Rotary evaporator at a temperature of 40 °C (Toja et al., 2020). Then proceed with a water bath to obtain a solid extract was carried out at a temperature of 60 °C and continued with phytochemical tests.

The subject of the study used was a Wistar White Mouse 16 male and adult aged 4-6 months with a weight of 200-350 grams. The subjects of the study were randomly divided into 4 groups including group 1 (aquadest 21 days followed by Na-CMC 1% 10 days), Group 2 (AlCl₃ induction 3 21 days followed by Na-CMC 1% 10 days), Group 3 (AlCl₃ induction 3 21 days continued Donepezil), and Group 4 (AlCl₃ induction 3 21 days followed by 10 days). The subjects of the study were acclimatized for 14 days before being given treatment. Given standard feed as well as drinking ad libitum. Room air humidity is 40% with a temperature of 18-26 °C and lighting is set every 12 hours (Agustina & Kulia, 2017). Wistar white mouse memory or memory evaluated with Morris Water Maze Test (MWM) (Lissner, Wartchow, Toniazzo, Gonçalves, & Rodrigues, 2021). The equipment used in the Morris Water Maze Test test is a pool with a round shape (158cm x 60cm) filled with water and a platform that is 2 cm carried by water. The Morris Water Maze Test is assessed by recording the time (seconds) spent by the animal trying to reach the platform positioned in the pool. The evaluation was carried out 3 times before the treatment was given, after 21 days of the first treatment, and after 10 days of the second treatment. Before the memory evaluation was carried out, the subjects were given training for 5 consecutive days by dividing the pool into 4 quadrants and then placing the platform in one of the quadrants then the research subjects were input in the pool in the first quadrant then left for 60 seconds to find the platform and left for 15 seconds above the platform. It then starts in the same way in quadrant 2 to quadrant 4 (Syed, Ikram, Yaqinuddin, & Ahmed, 2015). Mice that do not reach the platform for 60 seconds are directed to get to the platform. After training, subjects induced AlCl₃ 175mg/kg bb orally for 21 days (Liu et al., 2020). Aluminum chloride (AlCl₃) is extensively used for dementia stimulation in many animal models (Shunan, Yu, Guan, & Zhou, 2021). (After induction for 21 days, an evaluation of memory was carried out using the morris water maze test, the results of the evaluation data were analyzed using paired t-tests to analyze whether there was a decrease in rat memory before and after being given AlCl₃. After that groups 2,3, and 4 were treated orally for 10 days each: (1) group 2: Na-CMC 1% (Negative Control); (2) group 3: Donepezil preparation 10 mg (Positive Control); (4) group 4: extract black fruit seeds based on research (Liu et al., 2020). the dose is given 400mg/Kgbb. After memory evaluation, the results of these data are then carried out in pairs t-tests to analyze whether there is an improvement in rat memory before and after being given therapy. Furthermore, the result data were also analyzed with the One-way Anova test and continued with the Least Significant Difference (LSD) test.

RESULTS AND DISCUSSION

The solid extract of black fruit seeds was obtained as much as 16gr obtained from the extraction process of 630gr of black fruit seeds dried using an oven. From the results of phytochemical tests, positive samples were obtained containing several contents including flavonoids, phenolics, terpenoids, and alkaloids.

Table 1. Phytochemicals Result Picture

Phytochemicals	Result	Picture
Phenolic	The Solution is a Greenish-Black (Putra, 2019)	
Flavonoid	Orange Red Color Solution (Hidajati & Triwahyuono, 2019)	
Alkaloid	Reagen Meyer: White Precipitate Wagner: Brown Dragendorff: Red Color Solution (Hidajati & Triwahyuono, 2019)	 
Terpenoid	Brown (Hidajati & Triwahyuono, 2019)	

The results of the study obtained the average time (second) of the Morris Water Maze Test before induction of AlCl₃, of 5.00 in group 2; 5.50 in group 3; and 4.75 in group 4, after being given AlCl₃ for 21 days average score time (second) Morris Water Maze Test of 9.00 in group 2; 3:50 p.m. in group 3; 9.75 in group 4 (Table 2). This shows an increase in a score which means that the average duration of travel time is getting longer after being given AlCl₃ in all three groups.

Table 2. Average Morris Water Maze Test (MWMT)

Group	N	MWMT (sec)	
		Pre-exposure	Post AICl3 21days Induced
K1	4	7.50	7.25
K2	4	5.00	9.00
K3	4	5.50	15.50
K4	4	4.75	9.75

Furthermore, a paired t-test was carried out to determine whether there was a difference in scores before and after the induction of AICl3. The results of the paired T-test were obtained at $p=0.016$ in group 2; $p=0.018$ in group 3; $p=0.034$ in group 4. These results showed a significant difference between before and after the administration of AICl3. It can be concluded that groups 2,3, and 4 have dementia.

After induction of AICl 3 for 21 days, followed by treatment in the form of extra black fruit seeds 400 mg/kg bb for group 4, group 3 was given donepezil preparations of 10 mg, while groups 2 and 1 were given Na-CMC 1%. 10 days later, a memory evaluation was carried out using the Morris Water Maze Test. The lowest average MWMT value was obtained in group 4, which was 5; followed by group 2 which was 7.75, and group 1 by 8. The longest duration was obtained in group 2 of 17.5 (See Figure 1).

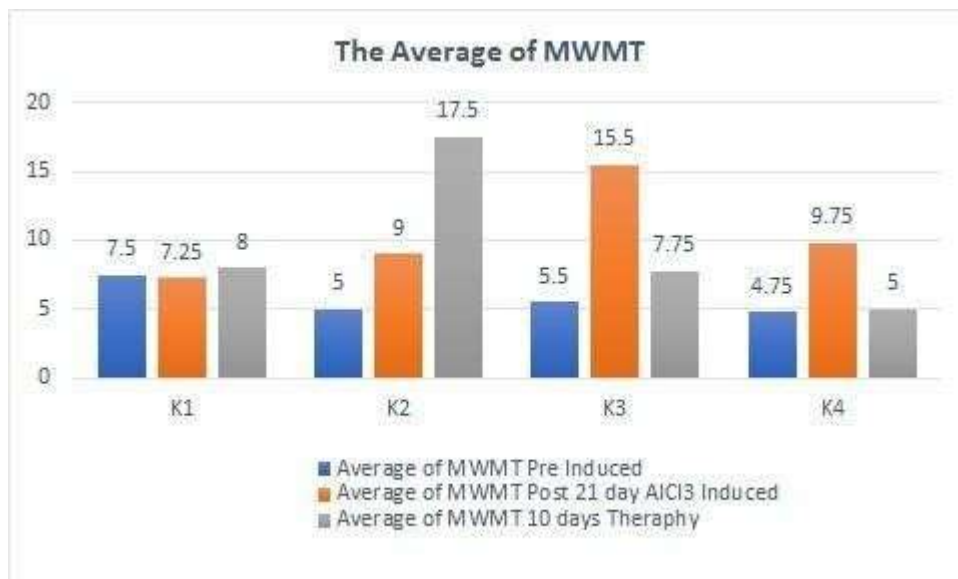


Figure 1. Average of MWMT

Then a paired t-test was carried out to determine whether there was a difference in the average MWMT value after giving AICl3 and after giving treatment. The results of the T-test in each group were obtained $p = 0.761$ in group 1; $p = 0.012$ in group 2; $p= 0.046$ in group 3; $p= 0.023$ in group 4. The results showed that there were significant differences before and after the administration of therapy (extract and donepezil). It can

be concluded that group 2 experienced a decrease in memory which was indicated by a significant increase in the average value of MWMT, and groups 3 and 4 experienced memory improvements which were shown by a significant decrease in the average value of MWMT after administration of therapy. An ANOVA test was carried out to determine whether there was a difference in the average MWMT value between treatment groups. The ANOVA test results were obtained significantly with a p-value = 0.002. Furthermore, an LSD test was carried out to find out which treatment groups had significantly different results. The LSD test results showed significant differences between group 1, group 3, and group 4 with group 2. If you compare group 3 and group 4 with group 1 there is no significant difference. Then group 3 compared to group 4 found no significant difference. So, from the results of LSD, it can be concluded that the extract of black fruit seeds and donepezil influences improving memory and the results of improving the extracted memory of black fruit seeds are comparable to donepezil.

Table 3. LSD test of MWMT

Group	Group	P value
K1	K 2	.003
	K 3	.779
	K 4	.273
K 2	K 3	.002
	K4	.000
K3	K4	.406

Aluminum is a powerful neurotoxin involved in the development of various cognitive disorders. Chronic aluminum exposure induces oxidative stress and prolongs cognition disorders (Liu et al., 2020). It has been reported that Aluminium chloride (AlCl₃) produces a marked decrease in cognition based on memory evaluation using the Morris Water Maze Test (Syed et al., 2015) (Liu et al., 2020). Increased oxidative stress caused by increased accumulation of free radicals can cause damage to cellular lipid proteins and DNA, resulting in nerve cell damage. Nerve cell damage results in a decrease in acetylcholine (ACh) neurotransmitter levels, as evidenced by previous studies that exposure to AlCl₃ can lower Ach levels (Liu et al., 2020). ACh is an important neurotransmitter in the memory process (Sherwood, 2015). Exposure to aluminum can also result in microglia producing inflammatory mediators (NO, TNF- α , IL-1 β) (Liu et al., 2020). Activated microglia will activate a series of cascade pathways, namely mitogen- activated proteinkinases (MAPKs) pathways and nuclear factor- κ B (NF- κ B) signaling (Yan et al., 2017). Activation of the I κ B kinase/NF κ B signaling pathway leads to the development of various pathological conditions in humans, such as neurodegenerative, inflammatory disorders, autoimmune diseases, and cancer (Sharma, Kumar, & Singh, 2019). Other studies have shown that memory impairment in the administration of AlCl₃ not only increases oxidative stress and pro-inflammatory cytokines but can also reduce BDNF (Brain-derived nerve factor) levels (Shaji, Sivakumar, Rao, & Paul, 2018). Exposure to AlCl₃ lowers intracellular Ca²⁺ and cAMP levels causes TORC1 nuclear translocation, disrupts the interaction between SIRT1, TORC1, and

CREB, and eventually affects. BDNF gene transcription can affect memory (Yan et al., 2017). So that the possible memory decline that occurs in groups 2, 3, and 4 is caused by an increase in oxidative stress, pro-inflammatory cytokines, a decrease in BDNF, and a decrease. The extract of black fruit seeds contains flavonoids, terpenoids, phenolics, and alkaloids. flavonoids work to reduce inflammation by regulating the performance of microglia and modulating the MAPK pathway and NF- κ B signaling pathway. Based on research in rats induced by AlCl₃, alkaloids can reduce oxidative stress and neurotoxin which shows potential as antioxidants. Terpenoids can suppress NF- κ B, reducing ROS (reactive oxygen species). The memory process is very dependent on BDNF, which in previous studies found a meaningful relationship to increasing BDNF levels and improving memory in animals in neurodegeneration models (Adu & Mabandla, 2019). So that the possibility of improving memory in the group given an extract of black fruit seeds because they contain compounds that can trigger BDNF expression. BDNF plays a role in central nervous system neurons to support the presence of neurons, help the growth and differentiation of new neurons, increase synaptogenesis, play a role in neurogenesis, and can protect Neural Stem Cells (NSC) and Neural Precursor Cells (NPC) (Pansri et al., 2021). Quercetin is the most abundant flavonoid found in almost all plants able to increase BDNF expression in the hippocampus of rats (Rahvar, Owji, & Mashayekhi, 2018). Flavonoids are also polyphenol compounds that cause CREB phosphorylation in the hippocampus, followed by an increase in the BDNF protein (Sharma et al., 2019).

The use of Donepezil in this study was able to improve memory. Donepezil is an AChEi class drug that can treat the neurotransmitter acetylcholine. According to the Dementia Australia organization, Cholinesterase inhibitor drugs stop or inhibit enzymes to break down acetylcholine when it passes from one cell to another. Cholinesterase inhibitors produce higher concentrations of acetylcholine, which causes improved communication between nerve cells to improve or stabilize the symptoms of dementia. It has been studied that extracts from blueberries containing flavonoids and phenols can increase Ach levels to improve memory in dementia model mice. The extract of black fruit seeds contains polyphenol compounds in the form of flavonoids and phenolics. Polyphenols may inhibit the enzyme acetylcholinesterase. So, it is likely to cause an increase in communication between nerve cells to improve or stabilize the symptoms of dementia so that there is a memory improvement.

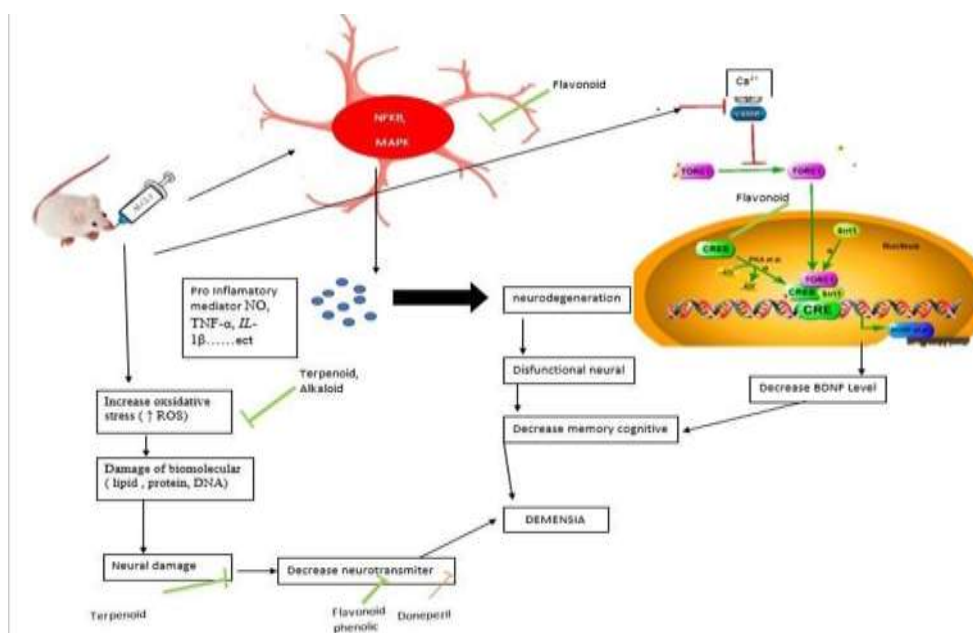


Figure 2. The mechanism of ALCL3 results in a decrease in memory and the mechanism of action of phytochemical compounds of black fruit extract.

CONCLUSION

Black Fruit Black Fruit (*Haplolobus monticola*) Wondama seed extract has the potential to improve memory and learning function, especially in people with dementia. However, this research is limited to the identification of specific compounds from phytochemical compound derivatives, analysis of ACh levels, and also BDNF levels.

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