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Short Communication

The Effect of Gambir Catechins (*Uncaria gambir* (Hunter) Roxb.) on Improving the Memory of Male Mice Using the Morris Water Maze Test

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ABSTRACT

Memory is a very important component of the cognitive function of every individual. One of the causes of memory decline is the onset of oxidative stress. The Gambir plant (*Uncaria gambir* (Hunter) Roxb) contains catechin compounds that have been proven to be able to repair oxidative damage, so it is thought to improve memory. The purpose of this study was to determine the effect of Gambir catechins on improving the memory of male white mice using the Morris Water Maze test with the parameter of latency time (the time it takes to reach the platform). This study used 25 male white mice (*Mus musculus*) divided into 5 control groups of 5 animals each, namely the negative control group (Na CMC 0.5%) and the positive control group, as well as 3 treatment groups with pure catechin compounds (0.875; 1.75; 3.5 mg/kgBW). Test animals were given 10% ethanol orally as a memory-lowering inductor. Each group was trained on the Morris Water Maze (MWM) test equipment in 2 stages, namely the acquisition trial and probe trial stage. The results obtained which was analyzed using a one-way ANOVA statistical test and then with the Duncan test. The statistical results showed that there was a significant difference between the treatment group and the negative control group with a significant value of $p<0.05$. The results showed that each treatment with a dose of 0.875; 1,75; and 3.5 mg/kgBW of Gambir catechins, provided functional improvements in the memory of male mice.

***Keywords*:** Catechins, Gambir, memory, alcohol, Morris water maze

Introduction

Cognitive function disorders are closely related to brain function as the centre of human memory. Memory plays a very important role in the process of recording, storing and recalling information in the past, both in the form of knowledge, thoughts, desires, behaviours and movements.[1] Memory is influenced by several factors, such as age, gender, physical activity, emotional factors, interests, and also nutritional intake.[2] In addition, memory decline can also be affected by excessive alcohol consumption.[3] Alcohol is one of the drinks that can damage the body and is the most contributor to degenerative diseases. Of the 241,000,000 people in Indonesia, the prevalence of alcohol use is around 0.8%, and there is another 0.7% who have alcohol dependence in both men and women.[4] In the study it was proven that the administration of 10% ethanol orally for three consecutive days with a dose of 0.5 mL has the potential to reduce memory in mice.[5] Alcohol use can cause oxidative stress, which is one of the factors causing impaired cognitive function, such as memory loss. Oxidative stress causes an imbalance between the production of Reactive Oxygen Species (ROS) and the body's antioxidant defence system. To maintain a balance between the production of Reactive Oxygen Species (ROS), it is necessary to take food supplements that are rich in antioxidants.[6] One plant that acts as an antioxidant is Gambir.

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Indonesia is one of the countries with the largest number of Gambir suppliers in the world, reaching 80%. Most of the suppliers come from Lima Puluh Kota and Pesisir Selatan Districts.[7] Gambir is a plant that has high levels of catechin compounds, with a percentage of 73.3% in good quality.[8] Catechins come from the family of oligomeric procyanidin tannins, and have the same pharmacological effects as flavonoids, and are useful as antioxidants.[9] There are various methods that can be used to observe memory improvement in test animals, one of which is the Morris Water Maze. This method is useful for studying spatial memory navigation and long-term spatial memory, which depend on the hippocampus.[10] With the Morris Water Maze, test animals learn to recognise their environment and find a way out of the test.[11]

This study aimed to determine the effect of Gambir catechins on improving the memory of male white mice induced with 10% ethanol using the Morris Water Maze test to determine the latency time, as a measure of memory improvement.

Materials and Methods

Materials

Digital scales (OHAUS®), measuring cups (IWAKI®), *glass beakers* (IWAKI®), stirring rods, mouse sonde, 1 mL syringes, plastic cages, gloves, feed bins, and Morris Water Maze test kits. Pure catechin level 99.99% was purchased from FitoPure® with the number Certificate of Analysis (CoA) 01/RC-FP/2022, distilled water, ethanol 96%, Na CMC (500 g), and standard mouse feed.

Animals

Twenty-five (25) male white mice (*Mus musculus*) acclimatised for 10 days in the Animal House of the Faculty of Pharmacy, Universitas Andalas, with access to sufficient food and water *ad libitum* were used

for this study. Test animals were adapted to the experimental environment during the acclimatisation process. The test animals were declared healthy if their body weight did not fluctuate more than 10% and showed normal behaviour during the acclimatisation process.[12] Ethical approval for the study was obtained from the Faculty of Pharmacy Ethics Committee of Universitas Andalas, with approval number 34/UN.16.10.D.KEPK-FF/2024.

Preparation of 10% ethanol
In this study, 10% ethanol was used to induce brain damage resulting in memory loss. The 10% ethanol was prepared by diluting 0.10 L of 96% alcohol, then dissolving it in 0.9 L of distilled water, and 0.5 mL/20 gBW of the test animal was used for this study.

Preparation of 0.5% Na CMC solution
Na CMC (500 mg) was weighed and dissolved in 10 mL of hot distilled water. The mixture was left to stand for ± 15 minutes until it turned clear and formed a stable gel, and stirred until homogeneous. The mixture was then diluted with distilled water to a volume of 100 mL.

Memory test procedures
This study was conducted for 25 days. The experimental mice were adapted for the first 10 days and were then treated for the next 15 days using the Morris Water Maze method, which consisted of 2 stages of testing, namely an acquisition and probe trials. The test pool was divided into 4 quadrants (north, south, east, and west). Repetition of each stage of testing was carried out at the same starting point, namely the south quadrant, and the platform as the endpoint was located in the north quadrant. The data obtained is the average latency time of the test animal reaching the platform on each repetition calculated using a stopwatch. Latency time is the time needed for the test animal to reach the platform on the testing device. The treatment in this study was as follows:

Acquisition trial.
Test animals were trained to find a platform located 2 cm below the water surface in one of the quadrants 3 times a day for 5 days. The time ends if the test animal successfully reaches the platform or after swimming for 60 seconds. However, if the test animal did not succeed in finding the platform for 60 seconds, then the test animal was directed and placed on the platform for 15 seconds before the next exercise.[13]

10% ethanol induction
On the 6th day, the test animals were given 10% ethanol induction for 5 days aimed at a decrease in memory function. The first probe trial stage was carried out on the last day after induction and was repeated 2 times. This stage is carried out with a hidden platform, where the test animals swim in a pool that has been mixed with coconut milk or flour to make it look opaque.[13]

Trial probes
The test compound was given for 5 days to each group, and the test animals were made to swim again on the last day with a hidden platform 2 times in 1 day.[13] The test compound doses given to each group were as follows:
Negative control group (0.2 mL/20g BW of 0.5% Na-CMC)
Positive control group (catechin 0.875 mg/kg BW without ethanol inducer)
Group P1 (catechin 0.875 mg/kg BW+ ethanol inducer)
Group P2 (catechin 1.75 mg/kg BW+ ethanol inducer)
Group P3 (catechin 3.5 mg/kg BW+ ethanol inducer)

Statistical Analysis
The data were analysed by one-way ANOVA. Subsequently, significant results were analysed by Duncan's multiple range test (p<0.05) using IBM SPSS Statistics V24.

Results and Discussion

Morris Water Maze is a memory test used to study spatial memory navigation and long-term spatial memory. In this test, the test animal learns to recognise its environment and find a way out of the test chamber. The test uses the Morris Water Maze which consists of 2 stages, namely the acquisition trial and the probe trial. The pool is divided into 4 quadrants with the platform located 2 cm below the water level on one of the quadrants. The parameters observed in this test are measurements of the latency time required by test animals to find a platform on the Morris Water Maze.[14]
In the acquisition trial phase, the test animals without treatment were allowed for 5 consecutive days with three repetitions every day. This phase is also referred to as the learning phase because it aims to be a learning process for test animals in the formation of spatial memory. Table 1 shows that each group of test animals has different latency times for reaching the platform. On the first day, the test animals were still adapting to the pool environment, so the latency time needed to reach the platform was longer. It is evident that on the last day, the ability of the test animals to reach the platform increased as presented by the latency time (Table 1) which was faster compared to the first day. Supposedly, in the learning phase, the test animal experienced a decrease in latency time every day. However, several groups of test animals experience an increase in latency time. The increase in latency time was most noticeable on the second and fourth days (Table 1). Several factors can affect the learning performance of test animals in the acquisition trial phase, such as stress and environmental factors in the test pool. Colder pool temperatures can cause test animals to develop hypothermia. Meanwhile, the temperature of the pool > 28 ± 2°C resulted in the test animals not being motivated to recognise the environment around the pool, so they tended to float rather than swim to escape to reach the platform. This factor can affect the spatial learning phase and cause stress in test animals to increase. One of the indications that the test animal was exposed to stress during the test was the occurrence of tigmotaxis. Tigmotaxis is the tendency of animals to move along the edges of their environment, this is a common pattern of behaviour related to anxiety or fear. This event causes the test animal to have difficulty finding the location of the platform, so the latency time will be extended.[15]

Table 1: The trial acquisition latency time is 5 days

Test Group	Latency Time (seconds)					
	Day 1 ± SD	Day 2 ± SD	Day 3 ± SD	Day 4 ± SD	Day 5 ± SD	mean ± SD
k-	27.49 ±12.15	30.25 ±17.65	14.45 ±10.47	18.55 ±7.61	8.20 ±5.96	19.79 ±7.12
k+	24.59 ±10.77	13.09 ±5.12	22.12 ±12.31	19.74 ±10.56	9.00 ±4.27	17.71 ±2.51
p1	35.30 ±15.14	23.61 ±8.02	14.92 ±4.61	16.49 ±8.85	15.96 ±6.33	21.26 ±6.23
p2	12.78 ±6.69	19.33 ±10.57	9.52 ±5.37	15.81 ±14.62	9.88 ±5.28	13.47 ±6.80
p3	26.15 ±15.51	16.39 ±17.54	10.65 ±4.27	7.67 ±2.49	11.45 ±6.64	14.46 ±7.06

The values are expressed as means ± SD (n=5)

Test animals that had passed the learning phase in the acquisition trial for 5 consecutive days were then subjected to induction using 10%

ethanol in each group, except the positive control group. 10% ethanol induction was carried out orally at a dose of 0.5 mL/20g BW for 5 consecutive days. On the last day after ethanol administration, the test animals were made to swim again 2 times and the latency time to reach the hidden platform was calculated (Table 2). Giving ethanol aims to reduce the memory function of the test animals after the learning phase. This test was aimed at determining the effect of the test compound in improving memory. From the test average, if the latency time after administration of 10% ethanol induction was greater (Table 2), compared to the latency time for the acquisition phase of the trial (Table 1) except for the positive control, the compound is inferred to have caused memory improvement. The positive control was not given induction because it was to ensure whether the compound given had an effect on improving memory and was not influenced by other factors. Ethanol with a concentration of 10% can become a free radical which can damage the brain organ so that the memory ability of the test animals will decrease.[16] Ethanol is a small water-soluble molecule that can be quickly absorbed in the digestive tract. Food in the stomach can delay the absorption of ethanol, and in the fasting state, ethanol concentrations can reach peak blood levels within 30 minutes.[17] Therefore, before giving 10% ethanol, the test animals were fasted to achieve optimal absorption of ethanol induction.

Similarly, the probe trial is a test that aims to determine the memory ability of test animals in storing spatial memory after the learning phase at the acquisition trial stage and 10% ethanol induction. At this stage, the test animals were made to swim twice at a time (Table 3). Treatment with the test compound was carried out after giving 10% ethanol induction for five consecutive days. There were differences in latency times for each treatment group (Table 3). Table 3 shows that the negative control group had a greater latency time than the other groups. This indicates that the negative control group took longer to reach the platform. Thus, it proves that the negative control, namely the administration of 0.5% Na CMC, does not affect improving memory function. Treatment with the test compound (Table 3) provided a significant reduction in latency time compared to when given 10% ethanol induction (Table 2). Treatment group 2 with a catechin dose of 1.75 mg/kg BW of test animals had the highest effect compared to the other two dose groups. The groups with doses 1 and 3 (catechin 0.875 mg/kg BW and catechin 3.5 mg/kg BW) also showed an effect in improving memory, but not as significant as the treatment group 2 (1.75 mg/kg BW). A decrease in latency time also occurred in the positive control group with doses of catechin 0.875 mg/kg BW without alcohol induction. This suggests that catechin from Gambir (*Uncaria Gambir* (Hunter) Roxb.) does affect improving memory. Table 4 shows the differences in the memory percentage which shows that the P2 group with a catechin dose of 1.75 mg/kg BW of test animals has the highest percentage increase in latency time with a value of 70.58%. So it can be concluded that the dose variation in treatment group 2 is the most optimal and effective dose in improving memory.

Table 2: Latency time after 10% ethanol induction

Test Group	Probe latency time 1		
	Swimming 1 ± SD	Swimming 2 ± SD	Mean ± SD
k-	25.96 ± 11.33	15.95 ± 9.30	20.96 ± 9.03
k+	14.26 ± 15.00	12.28 ± 10.58	13.27 ± 7.31
P1	23.93 ± 5.61	17.80 ± 4.50	20.87 ± 2.32
P2	16.76 ± 4.45	36.63 ± 29.17	26.70 ± 15.26
P3	21.73 ± 5.81	11.22 ± 4.44	16.48 ± 2.85

The values are expressed as means ± SD (n=5)

Table 3: Latency time of experimental mice after treatment

Test Group	Probe latency time 2		
	Swimming 1 ± SD	Swimming 2 ± SD	Mean ± SD
k-	13.49 ± 3.72	28.98 ± 14.31	21.23 ± 7.42
k+	7.08 ± 2.55	10.67 ± 2.88	8.88 ± 0.92
P1	13.22 ± 7.03	10.04 ± 3.88	11.63 ± 2.07
P2	6.27 ± 4.21	9.43 ± 7.85	7.85 ± 5.53
P3	6.08 ± 2.62	8.01 ± 5.07	7.05 ± 2.75

The values are expressed as means ± SD (n=5)

Statistical analysis of the latency time data using a one-way ANOVA evaluate differences between two or more groups by considering only one factor. Before the ANOVA test, data normality was first carried out using the Saphiro-Wilk test because the analyzed samples amounted to <100. The results of the data normality showed that the sample was distributed normally, with a significance value of p>0.05. There were significant differences in 5 groups of test animals, with significant values of p (0.00) < (0.05). After further testing using the Duncan test, a significant difference was produced by the negative control group. We, therefore, conclude that the negative control group does not affect improving memory function compared to the positive control and treatment groups. The brain is a very important organ in the centre of human memory, but it is also susceptible to damage. The hippocampus is one of the nerve cells in the brain that is very sensitive to damage due to oxidative stress. The hippocampus is a limbic system that has a very important role in the memory process so that the stored information remains in the long-term memory bank.[18] Excessive exposure to oxidative stress can cause an imbalance between the production of Reactive Oxygen Species (ROS) and the antioxidant defence system in the body.[6] There are three main types of oxidants in ROS, namely superoxide (O_2*), hydrogen peroxide (H_2O_2), and hydroxy radicals (OH*).[19] It is known that ethanol is one of the causes of oxidative stress. Ethanol can be oxidized through cytochrome P450-2E1 (CYP2E1) to produce hydrogen peroxide (H_2O_2) which is the cause of the occurrence of Reactive Oxygen Species (ROS) when interacting with copper or iron.[20] In addition, animal studies have proven that ethanol can increase N-Methyl-D-Aspartate receptor-mediated neurotoxicity, resulting in excess neuroexcitation and eventually nerve damage. In addition, there is a shrinkage in the hippocampus and a larger volume deficit.[2]

In this study, pure catechin (99.99%) purchased from FitoPure®, previously isolated from Gambir was shown to improve memory in test animals. Gambir is one of the plants that has high catechin compounds with a percentage of 73.3%.[8] Catechins are compounds that have two aromatic rings and several hydroxyl groups, so catechins are referred to as polyphenolic compounds. Polyphenols have activity as antioxidants that can inhibit oxidation reactions in oxidative stress.[22] The antioxidants work by scavenging free radicals and highly reactive molecules so that they function as the body's defence system against the

presence of free radicals.[23] Thus, antioxidants can reduce the occurrence of oxidative stress which contributes to memory loss.

Table 4: Percentage of memory improvement in the experimental mice

Test Group	Latency time		Percentage of memory improvement (%)
	10% ethanol induction	Trial probes	
k-	23.94 ± 9.07	21.23 ± 7.42	11.31
k+	13.27 ± 7.31	8.88 ± 0.92	33.10
P1	24.63 ± 4.57	11.63 ± 2.07	52.79
P2	26.70 ± 15.26	7.85 ± 5.53	70.58
P3	16.48 ± 2.85	7.05 ± 2.75	57.23

The values are expressed as means ± SD (n=5)

Conclusion

In this study, the effect of catechin in improving memory in experimental mice induced with 10% ethanol using the Morris Water Maze method was significant at a dose of 1.75 mg/kg BW, possibly due to its antioxidant activity in reducing oxidative stress caused by alcohol induction. Further studies on dose optimization and toxicity are encouraged, especially toward the development of a pharmaceutical product that can be used for the enhancement of impaired memory patients.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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