

Conference Paper

## Taurine Memory Enhancing Activity Against Phenobarbital Induced Male White Mice by Morris Water Maze Methode

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### ABSTRACT

Memory decline often occurs in every individual. Data in Indonesia in 2010 there were around 7.2% of people with memory decline. Many efforts are made to improve memory. One compound that is beneficial in improving memory is taurine. This compound has several functions including as the development of the central nervous system and as a neuroprotector. Taurine is abundant in humans, mammals, and marine animals. This study aimed to determine whether taurine has memory-enhancing activity in male white mice and to determine the most effective dose (mg/kg BB) of taurine in improving memory in male white mice. The method used in this study is the Morris Water Maze method. Test animals in this study used 25 mice divided into 5 groups. Group 1 is normal, group 2 is a positive control group given encephalon tablets, and the next group is a group given taurine at doses of 10 mg/kgBB, 20 mg/kgBB, and 40 mg/kgBB. Data analysis using One Way Anova followed by Tukey Post Hoc to find out the differences from each test group. The results of this study showed that taurine at a dose of 40 mg/kgBB had a higher percentage of memory enhancers than other doses.

*Keywords: Memory, taurine, morris water maze, phenobarbital*

### Introduction

Many individuals in the world experience memory decline. In 2010 about 36.5 million people in the world suffered from memory decline. 58% of memory loss sufferers live in low- and middle-income countries, expected to increase to 63% by 2030 and 71% by 2050 (Prince et al., 2013). In Indonesia in the same year, there were around 7.2% of sufferers of memory loss (Ministry of Health, 2010). Alzheimer's disease, amnesia, depression, and schizophrenia are diseases that are closely related to memory disorders. Slow memory and poor memory function can occur due to age, stress, and emotions, resulting in premature aging (Mojsola et al., 2012).

One compound that is beneficial in improving memory is taurine. Taurine is a free amino acid that has a biological role and is found in humans (Marcinkiewicz & Kontny, 2014). Taurine has several functions including the development of the central nervous system, maintaining the integrity of membrane structures, regulating calcium and hemostatic transformations, as osmolytes, as neuromodulators, and as neuroprotectors (Wu & Prentice, 2009). Taurine is a compound that has a variety of physiological functions and has an important role in nerve function and memory (Huxtable, 1992).

Morris Water Maze is one of the methods that is widely used in learning and memory experiments carried out on experimental animals. This method has become the "Gold Standard" in neuroscience (Nunez, 2008). Morris Water Maze is a method of trying better memory than other trying methods. This method is very simple and the results can be seen easily (Brandeis et al.,

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1989). The advantage of this method is that experimental animals always want to get out of the water, so they will continue to find a way out of the water (Jelita et al., 2019). Until now there has been no research on the memory-enhancing activity of taurine on male white mice induced phenobarbital by the Morris water maze method.

## Material and Methods

### Material

The sample materials used were taurine, aquadest as a solvent, phenobarbital tablets as memory inducers, and encephabol tablets as a positive control.

### Tool

The tools used are analytical balances (Kenko), beaker glass (Pyrex), measuring cups (Pyrex), stirring rods (Pyrex), drip pipettes (Pyrex), porcelain dishes, syringes, mouse cages, memory tests using Morris Water Maze in the form of a round pool.

### Experimental animals

The experimental animals used in this study were male white mice. Before the study, experimental animals were adapted to the surrounding environment and weighed the body weight of each experimental animal. Each group consists of 5 experimental animals. The number of test animals in this study was divided based on Federer's formula, namely  $(t-1)(n-1) \geq 15$  (Wahyunigrum & Probosari, 2012). Remarks:  $t$  = number of treatments  $n$  = many test animals in each treatment group.

Table 1. Grouping of experimental animals

No	Group	Treatment	Sum
1	Group I	Negative control	5
2	Group II	Positive control	5
3	Group III	Taurine Dosage 10mg	5
4	Group IV	Taurine Dosage 20mg	5
5	Group V	Taurine Dosage 40mg	5

### Dosage calculation

#### 1. Taurine

The dose of taurine in this study refers to previous studies conducted by Bhupinder P S et al. (2000) namely 10mg/kgBB, 20mg/kgBB, and 40mg/kgBB.

#### 2. Phenobarbital

This study used a 30mg phenobarbital tablet dose for humans, converted to mice  $30\text{mg} \times 0.0026$  so that a phenobarbital dose of 0.078mg/kgBB was obtained.

#### 3. Encephabol

This study used a 100 mg dose of encephabol tablets for humans, converted to mice  $100\text{mg} \times 0.0026$  so that the dose of encephabol was obtained which was 0.26mg/kgBB.

### Test Run of Memory-Enhancing Activities

#### 1. Acquisition trial

The acquisition trial phase was carried out for 5 days. At this stage, mice will be trained to get a platform that is below the surface of the water in one quadrant 4 times a day. The mice were placed in one of the quadrants randomly in the pool. Once the mouse reaches the platform, it is stopped by a timer and it is recorded. If within 60 seconds the mouse has not reached the platform, then the time is recorded for 60 seconds. If the mouse does not find the platform, it is directed to the platform and rested for 15 seconds on the platform (Vorhees & Williams, 2006).

## 2. Probe Trial

The probe trial was conducted on the 6th day after the acquisition trial. At this stage, each mouse was swam for one minute and did not use the platform, then seen at what minute the test animals passed through the place where the platform was previously located (Vorhees & Williams, 2006).

## 3. Spatial reversal

This stage is carried out for 5 days. In this test, the water in the Morris water maze is given flour so that the water becomes opaque. At this stage mice will be trained to get a platform that is below the surface of the water in one quadrant 4 times a day. The mice were placed in one of the quadrants randomly in the pool. Once the mouse reaches the platform, it stops the timer and records the time (Vorhees & Williams, 2006).

### **Data analysis**

The data obtained from each group of mice will be analyzed statistically using the One Way ANOVA method and then continued with Tukey Post Hoc.

### **Results and Discussion**

#### **Exercise stage and phenobarbital induction**

Memory-lowering induction in this study used phenobarbital tablets of 30 mg. Phenobarbital is an old-generation antiepileptic drug that is effective for focal epilepsy. The effects of antiepileptic drugs occur by increasing inhibition. Phenobarbital binding to GABA receptors prolongs the open time of the Cl-channel resulting in hyperpolarization. The use of Phenobarbital causes side effects of sedation and hypnosis resulting in impaired attention and concentration (Mustarsid et al., 2011).

Table 2. Average swimming time of mice at the exercise stage and after phenobarbital induction

Group	Average swimming time	
	Training level+ SD	Phenobarbital induction + SD
I	15,94 second + 4,11	-
II	10,84 second + 1,17	15,09 second + 3,25
III	16,52 second + 6,70	20,40 second + 6,20
IV	11,19 second + 2,11	23,51 second +7,39
V	11,10 second+ 4,24	16,69 second + 8,72

The results of the training stage showed a difference in swimming time in each test group. This is due to the limitations of test animals in remembering environmental factors around the test area. After the exercise stage, groups 2, 3, 4, and 5 were given oral phenobarbital induction. After giving phenobarbital, test animals were again swam to the Morris water maze to determine whether there was a decrease in swimming time or not. Based on Table 2 after oral administration of phenobarbital, the average swimming time of test animals was greater than at the exercise stage.

#### **Acquisition trial phase**

This test consists of measuring latency time, latency time is calculated from the beginning of the mouse swimming until it finds the platform. The first stage of the Morris Water Maze test is the acquisition trial. At this stage, each group was given taurine except group one. Acquisition trial is a learning process to form spatial memory. This stage is carried out for 5 days and mice are swam 4 times a day.

Table 3. Acquisition stage of swimming trial after administration of taurine

Test Group	Average swimming time + SD				
	Day 1	Day 2	Day 3	Day 4	Day 5
I	14,83 second + 1,90	13,55 second + 3,41	10,75 second + 1,71	12,66 second + + 4,00	10,19 second + + 1,32
II	11,52 second + 1,48	9,65 second + 1,03	8,01 second + 0,98	6,53 second + 0,88	6,18 second + 0,91
III	13,42 second + 2,70	10,72 second + 1,96	9,87 second + 0,69	8,98 second + 0,83	7,72 second + 0,91
IV	14,70 second + 2,97	10,86 second + 1,20	9,88 second + 0,86	8,30 second + 0,37	6,93 second + 0,56
V	13,75 second + 1,75	10,01 second + 0,60	9,10 second + 0,60	7,57 second + 0,73	6,31 second + 0,61

From the data in Table 3, it can be seen that the five groups of test animals trained every day experienced improved memory. However, there was a group that experienced a slight increase in swimming time on the fourth day, possibly due to environmental and device factors. But on the fifth day experienced a memory improvement. The first day is the result of the first exercise, so the time to reach the platform is longer, and on the fifth day, the swimming time reaches the platform faster because the mice can already remember the position of the platform.

### **Probe trial phase**

The probe trial is an experimental stage to see the ability of test animals to store memory after conducting the experimental stage of the Acquisition trial. This stage is carried out for one day with 4 swims on each test animal. At this stage, each test group is given taurine except group one which is the normal group.

Table 4. Probe trial phase

Test Group	Average swimming time + SD
I	10,16 second + 1,73
II	5,40 second + 0,51
III	7,82 second + 4,24
IV	6,20 second + 0,74
V	4,76 second + 0,79

In Table 4 it can be seen that all five groups of test animals trained daily experienced improved memory. All five test groups had a faster swim time to reach the platform because the mice were used to being swabbed and could remember when retrained. The group given higher doses of taurine had faster swimming times than the other group. At the same time, the normal group had a longer swimming time than the other group.

**Spatial reversal stage**

This test is carried out to find out whether the test animal can find a platform or not, and see the ability of menict in swimming, the ability of the sense of vision of mice in influencing swimming time. The swimming time of the test animals was greater than at the training stage.

Table 5. Spatial Reversal Stage of swimming after taurine administration

Test Group	Average swimming time + SD				
	Day 1	Day 2	Day 3	Day 4	Day 5
I	15,98 second + 6,93	10.86 second + 2.01	6,63 second + 2,37	5,33 second + 1,47	6.09 second + 0,82
II	3,97 second + 0,95	3,76 second + 0,41	3,51 second + 0,55	3,06 second + 0,20	2,56 second + 0,40
III	7,82 second + 4,44	5,47 second + 1,21	4,81 second + 1,03	4,35 second + 0,57	4,52 second + 0,79
IV	5,03 second + 0,70	4,80 second + 1,00	4,19 second + 0,24	3,74 second + 0,32	3,31 second + 0,50
V	4,37 second + 0,35	4,10 second + 35	3,72 second + 0,21	3,22 second + 0,46	2,97 second + 0,47

In Table 5 it can be seen that several test groups experienced increased swimming time. This happens because at this stage the water inside the Morris water maze becomes cloudy. Furthermore, until the last day of the fifth test, the test group had a faster swimming time to reach the platform because the mice remembered where the platform was.

**Percentage increase in memory**

Table 6. Percentage increase in memory

Test Group	Memory Percentage
I	58,9%
II	77.8%
III	66,3%
IV	75%
V	77,8%

The percentage of memory improvement in each group had different results. Group 2 which was a positive control group given encephabol and group 5 which was given taurine at a dose of 40mg/kgBB experienced the highest percentage of memory improvement at 77.8%. The normal group who were only given food, experienced the lowest percentage increase in memory at 57.2%.

Giving taurine at a dose of 40 mg/kg body weight is more effective in improving memory compared to taurine doses of 10 mg/kg body weight and 20 mg/kg body weight. Taurine is a non-essential amino acid that is widely found in mammals, skeletal muscles, liver, and platelets, especially in the heart and brain. Taurine plays a lot of role in physiological activity. In the brain, taurine works through the membranes of brain receptors, including GABA, glycine, opiates, and glutamate. Many parts of the brain function in memory learning, GABA and glutamate are the main neurotransmitters involved in memory learning (Roysommuti & Wyss, 2015). This compound has

a role as a neurotransmitter, neuromodulator, antioxidant, anti-inflammatory, membrane stabilizer, and as protector of the nervous system (El Idrissi, 2008).

The data obtained from this study were analyzed using the oneway ANOVA test because there were two variables in this study, namely the test group and the time of swimming mice. Before the one-way ANOVA test, the Test of Normality and Test of Homogeneity of Variances were carried out. Test of Normality scores using Shapiro-Wilk in this study were for group I 0.584, group II 0.525, group III 0.439, group IV 0.202, group V 0.102. Based on the results of the Test of Normality, it is known that the swimming time of mice is normally distributed with a significance value of  $P > 0.05$ . Furthermore, continued with the Test of Homogeneity of Variances, this research data has a significance value of 0.870. The homogeneity test shows that the data used is homogeneous with a value of  $P > 0.05$ . In the one-way ANOVA test, there is a significant difference between the five test groups which can be seen from the significance value of  $< 0.05$ , which is 0.013. Because there are significant differences in the results of the one-way ANOVA test, it is continued with the Tukey Post Hoc test to find out significant differences from the test data. From the Tukey Post Hoc test, it can be seen that Group One has significant differences with Group Two and Group Five. From the Tukey Post Hoc test it is known that Group I and Group II have significant differences, and group I and Group V have significant differences.

## Conclusion

Taurine (2-aminoethane sulphonic acid) has memory-enhancing activity. Taurine at a dose of 40 mg/kgBB has higher memory-enhancing activity compared to the taurine group of 10 mg/kgBB and 20 mg/kgBB.

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