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Original Research Article

The Effect of Administering Combination of Caffeine and Taurine on Improving Memory in Male Wistar Mice Using The Morris Water Maze Test

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ABSTRACT

Memory is the most important component for humans to receive, store, process and replicate impressions, understanding or responses. Caffeine and taurine possess antioxidant activity with proven efficacy in improving memory. The study aims to evaluate the effect of the combination of caffeine and taurine on memory improvement compared to individual agents. This study used the Morris Water Maze method by looking at the latency time required for test animals to find the platform on the Morris Water Maze apparatus. The study used 21 male Wistar mice with 7 treatment groups of 3 mice each: normal control, inducer, caffeine 0.40 mg/20 g BW, taurine 0.14 mg/20 g BW, caffeine 0.40 mg/20 g BW + taurine 14 mg/20 g BW, caffeine 0.40 mg/20 g BW + taurine 7 mg/20 g BW, and caffeine 0.20 mg/20 g BW + taurine 14 mg/20 g BW. The test involved three stages: acquisition trial, probe trial 1, and probe trial 2. The results of the latency time for the test animals to reach the platform were analyzed using one-way ANOVA. The analysis showed no significant differences (p>0.05) between each test group. However, treatment group 3 showed the highest percentage of memory improvement. The study concluded that there were no significant differences between groups given a combination of caffeine and taurine with a single administration in improving memory and the combination of caffeine and taurine at 0.40 mg /20 g BW + taurine 14 mg/ 20 g BW (group 3) can significantly improve memory in male mice.

Keywords: Memory improvement, Caffeine, Taurine, Morris water maze.

Introduction

Memory is the most important component for humans to receive, store, process and replicate impressions, understanding or responses.[1] Factors that influence memory include age, education, intelligence, self-concept, health, motivation and effort, some psychoactive drugs and alcohol consumption[2]. Alcohol abuse and dependence are associated with a higher risk of memory decline and dementia[3]. Ethyl alcohol can cause severe memory deficiencies. Ethanol is known to selectively influence several functional processes related to learning and memory in the central nervous system[4]. Giving 10% ethanol can reduce the memory function of test animals because the average latency time obtained is greater than the average latency time in the learning phase.[5] Caffeine has been shown to improve memory and improve mood. Caffeine is a presynaptic inhibitory antagonist of adenosine receptors which has a greater ability to bind to adenosine receptors to improve memory.[6] Taurine can regulate calcium release causing potential effects on the brain, heart, and skeletal muscle. Taurine supports the proliferation of neural progenitor cells and the formation of synapses in brain regions necessary for long-term memory.[7]

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One test that can be done to observe changes in memory is using the Morris Water Maze method. The Morris Water Maze Method was used to test spatial memory in rodents, such as mice. The Morris Water Maze method plays an important role in molecular, pathological and research on memory disorders.[8] Therefore, this study, assessed the effect of a combination of caffeine and taurine on the improvement of memory compared to the administration of each agent in male Wistar mice exposed to an ethanol inducer using the Morris Water Maze test method.

Materials and Methods

Acquisition trial

Morris Water Maze method test was used in this study by looking at the percentage increase in memory and its effect on improving memory. Testing using the Morris Water Maze consists of an acquisition trial and a probe trial. This test involves measuring latency time which is calculated based on the time it takes the test animal to find the platform on the Morris Water Maze apparatus. The acquisition trial is a test to see the training phase as a learning process for the formation of spatial memory in test animals. The acquisition trial phase was carried out for 5 days without treatment and was divided into 7 groups, each test animal was made to swim three times. The acquisition trial was carried out for 5 days. Wistar mice were made to swim to find a platform located 2 cm below the water surface in one quadrant three times. The time ended when the mouse reached the platform. However, if the mice did not succeed in finding the platform for 60 seconds, the mice were directed to find the platform, leaving it for 15 seconds before the next stage.

Ethanol (10%) induction

On the 6th day, the mice were given 10% ethanol for 5 days aiming to see 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

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stage was carried out with a hidden platform, where the mice were made to swim in a pool that had been mixed with flour to make it look opaque.

Probe trial

The test compound was given for 5 days to each group, and the mice were made to swim again on the last day with a hidden platform 2 times in 1 day. The test compounds given to each group were as follows:

a. Group 1 (control group, given standard feed/pellets and water)
b. Group 2 (10 % ethanol 0.5 mL/20 g BW)
c. Group 3 (caffeine 0.40 mg/20 g BW)
d. Group 4 (taurine 14 mg/20 g BW)
e. Group 5 (caffeine 0.40 mg/20 g BW + taurine 14 mg/20 g BW)
f. Group 6 (caffeine 0.40 mg/20 g BW + taurine 7 mg/20 g BW)
g. Group 7 (caffeine 0.20 mg/20 g BW + taurine 14 mg/20 g BW)

Statistical analysis

The data were analysed using one-way ANOVA. Subsequently, the significant results were analysed by Duncan's multiple range test (p<0.05) using IBM SPSS Statistics V24.[9]

Results and Discussion

The seven groups of test animals without treatment were given learning for 5 days with 3 repetitions. Table 1 shows that each group of test animals has different latency times for reaching the platform. In each group, there was a decrease in time each day, but on certain days there was a slight increase in latency time. The seven groups showed different improvements in memory, with a significant decrease each day. However, it was observed that the latency time graph in each group does not show a significant decrease each day. On the first day, the test animals needed a relatively long latency to reach the platform. Because on the first day, the test animals were just starting their initial learning process and were getting to know the Morris Water Maze tool for the first time. On the last day of the acquisition trial, the latency time needed for the test animals to reach the platform was faster, because the mice had begun to recognise their environment and were able to remember what they had done previously. The results of the acquisition trial showed some variations which may have been caused by environmental factors such as the temperature of the water used. At the time of the study, the test was carried out outdoors, so it was not certain what the water temperature was, it could be hotter or colder. Water temperature affects the performance of test animals during testing. Colder temperatures can increase stress levels in test animals, and hotter temperatures can cause animals to tend to float rather than swim to escape.[10]

Table 1: Trial acquisition latency time in 5 days

Test Group	Latency Time (seconds)					
	Day 1 ± SD	Day 2 ± SD	Day 3 ± SD	Day 4 ± SD	Day 5 ± SD	mean ± SD
Group 1	30.91 ± 23.52	18.59 ± 11.30	18.70 ± 3.83	16.88 ± 11.66	11.00 ± 2.75	19.21 ± 8.83
Group 2	31.13 ± 22.23	18.58 ± 13.32	18.76 ± 3.19	10.60 ± 12.19	9.55 ± 6.76	17.72 ± 11.39
Group 3	14.01 ± 8.37	13.56 ± 1.79	10.66 ± 5.16	8.81 ± 7.51	9.67 ± 1.50	11.34 ± 3.19
Group 4	14.05 ± 1.85	21.03 ± 12.15	4.66 ± 0.81	13.18 ±4.45	13.62 ± 4.45	13.31 ± 4.62
Group 5	38.44 ± 20.02	19.86 ± 17.95	29.55 ± 26.39	20.85 ± 20.06	14.21 ± 13.90	24.58 ±18.76
Group 6	27.37 ± 12.06	9.92 ± 3.03	9.99 ± 6.05	9.41 ± 5.88	5.42 ± 2.78	12.42 ± 1.41
Group 7	26.95 ± 6.73	35.07 ± 12.63	11.75 ± 5.14	30.51 ± 12.54	18.04 ± 7.58	24.46 ± 4.69

After carrying out an acquisition trial for 5 consecutive days, to determine the effect of the combination of caffeine and taurine in improving memory in the male Wistar mice, each group of animals was tested first using 10% ethanol, except for the control group which was not given any treatment. Administration of ethanol 10% was intended to reduce the memory of the test animals so that the effect of giving a combination of caffeine and taurine can be known to improve memory. Table 2 shows that giving 10% ethanol to the test animals for five consecutive days, reduced the memory of the test animals because the average latency time obtained was higher than the latency time during the acquisition trial stage. These results show that giving 10% ethanol to test animals reduced memory in all treatment groups. Of all the treatment groups, treatment group 3, given caffeine 0.40 mg/20 g BW + taurine 14 mg/20 g BW required the longest latency time. Furthermore, the probe trial stage was used to investigate the test animals' ability to store memory after undergoing the acquisition trial phase and 10% ethanol induction. This stage was carried out for two repetitions by checking the time it took for the test animal to reach the hidden platform. In Table 3, the results of probe trial 2 showed that the control group (standard feed) and the inducer group (10% ethanol) were the groups that took the longest time to reach the hidden platform. The control group needed an average latency of 10.39 seconds. And the induction group required an average latency time of 19.19 seconds. Meanwhile, in the treatment groups, the test animals needed a faster latency time to reach the hidden platform compared to the normal control group and the induction group. Treatment group 1 (caffeine) and treatment 2 (taurine) showed an influence on their ability to improve memory, but the taurine group required a faster latency time than the caffeine group. The combination of caffeine and taurine group also showed an influence on its ability to improve memory. Table 4 shows that the percentage of improvement in memory after being induced with 10% ethanol and given treatment was different in each group (Figure 1).

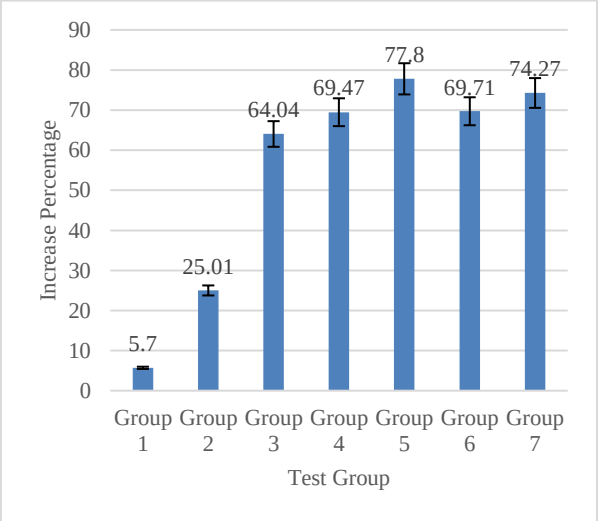

Figure 1: Percentage of memory improvement

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Table 2: Latency time after 10% ethanol induction

Test Group	10% ethanol induction		
	Swimming 1 ± SD	Swimming 2 ± SD	Mean ± SD
Group 1	8.75 ± 6.36	10.90 ± 4.68	9.83 ± 3.39
Group 2	41.28 ± 22.53	9.90 ± 4.95	25.59 ± 8.80
Group 3	33.95 ± 1.84	6.21 ± 1.98	20.08 ± 1.33
Group 4	29.61 ± 1.60	10.75 ± 4.18	20.18 ± 2.49
Group 5	56.26 ± 48.15	17.43 ± 17.62	36.84 ± 19.45
Group 6	29.09 ± 0.87	13.43 ± 4.10	21.26 ± 2.47
Group 7	31.36 ± 6.45	18.37 ± 14.55	24.87 ± 5.08

Table 3: Latency time probe trial

Test Group	Probe Trial		
	Swimming 1 ± SD	Swimming 2 ± SD	Mean ± SD
Group 1	15.50 ± 11.06	5.28 ± 2.01	10.39 ± 5.55
Group 2	28.30 ± 9.43	10.08 ± 5.74	19.19 ± 5.26
Group 3	9.01 ± 7.39	5.42 ± 4.24	7.22 ± 3.54
Group 4	8.11 ± 6.65	4.22 ± 1.16	6.16 ± 3.30
Group 5	6.76 ± 2.22	9.60 ± 11.19	8.18 ± 6.70
Group 6	8.14 ± 7.80	4.75 ± 1.53	6.44 ± 4.51
Group 7	8.63 ± 10.66	4.18 ± 1.91	6.40 ± 5.68

The seven groups had different percentages of improvement in memory, the lowest percentage of improvement was in the normal control group because they were only given food and drink water. The percentage of memory improvement in the group given the combination of caffeine and taurine showed a higher percentage compared to those given caffeine or taurine alone. The percentage of memory improvement from the combination of treatment group 3 which was given caffeine 0.40 mg /20 g BW + taurine 14 mg/20 g BW was 77.80%. Hence, administering a combination of caffeine and taurine can improve memory better than administering a single compound. Apart from that, the combination of caffeine and taurine with a caffeine dose of 0.40 mg/20 g BW + taurine 14 mg/20 g BW improved memory optimally compared to other combinations.

Table 4: Percentage of memory improvement

Test Group	Latency time		Percentage of memory improvement (%)
	10% ethanol induction	Probe trial	
Group 1	9.83 ± 3.39	10.39 ± 5.55	5.70%
Group 2	25.59 ± 8.80	19.19 ± 5.26	25.01%
Group 3	20.08 ± 1.33	7.22 ± 3.54	64.04%
Group 4	20.18 ± 2.49	6.16 ± 3.30	69.47%
Group 5	36.84 ± 19.45	8.18 ± 6.70	77.80%
Group 6	21.26 ± 2.47	6.44 ± 4.51	69.71%
Group 7	24.87 ± 5.08	6.40 ± 5.68	74.27%

The results of the study were subjected to statistical analysis to determine the average difference between two or more groups of samples or data, using a One-way analysis of variance. The Shapiro-Wilk test was carried out to find out whether the data was normally distributed or not. From the Shapiro-Wilk test, a significance value > 0.05 was obtained and it was concluded that the research data was normally distributed. Thus homogeneity and ANOVA tests were carried out simultaneously. From the homogeneity results, a significance value < 0.05 was obtained, so it was concluded that the data was homogeneous. The results of the One Way ANOVA test showed a

significance value of 0.70 > 0.05, indicating that there were no significant differences between groups. However, judging from the percentage of memory improvement, the most optimal group in improving memory was treatment group 3 which was given caffeine 0.40 mg /20 g BW + taurine 14 mg/20 g BW. *In vivo* studies have shown neurological effects after chronic ethanol exposure with reductions in memory, motor function, cognition.[11] All of these effects can be caused by neurotoxicity or neurodegeneration, and there is evidence that oxidative stress associated with ethanol metabolism is involved.[11] Taurine has antioxidant properties that are known to have a protective effect against oxidative stress caused by cellular stress and can scavenge free radicals in various cells and tissues against the toxicity of oxidation. In addition, taurine can reduce lipid peroxidation and increase the activity of antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase.[12] In recent years, several studies have attempted to establish a link between caffeine and oxidative status, as many conditions in which caffeine has been shown to have a beneficial influence can be associated with reduced oxidative stress (OS). The effectiveness of caffeine is also explained by its ability to reduce lipid peroxidation and increase the ability of the antioxidant system in the brain to reduce ROS levels.[13] In this study, the influence of caffeine and taurine, either alone or in combination, both produce a good effect in improving memory in mice. However, the results with the combination of caffeine and taurine (0.40 mg/20 g BW + taurine 14 mg/20 g BW) showed the highest percentage increase in memory. The combination of caffeine and taurine resulted in improved short-term memory during the Morris Water Maze test. These results are consistent with previous research showing that test animals given a combination of caffeine and taurine, both of which are contained in energy drinks in high doses, showed improved memory and attention.[14] Caffeine and taurine play a major role in tasks requiring faster attention, memory and performance in a short period.[15] In this study, the combination of caffeine and taurine caused a decrease in oxidative imbalance in the brain. This may be due to increased antioxidant enzyme activity and free radical scavenging.

Conclusion

In this study, the influence of caffeine and taurine, either alone or in combination, both produce a good effect in improving memory in mice. There was no significant difference (p >0.05) between groups given a combination of caffeine and taurine and a single administration in improving memory in male mice. The optimum dose of the combination of caffeine and taurine that significantly improves memory in male mice was caffeine 0.40 mg /20 g BW + taurine 14 mg/20 g BW.

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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