Original Article

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A Study on the Effects of Orally Administered Copper Sulfate on Learning and Spatial Memory of Wistar Rats

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ABSTRACT

BACKGROUND AND OBJECTIVE: Copper is one of the main micronutrients in the human body. Malfunction in copper homeostasis results in Menkes syndrome and Wilson's disease, which are associated with complications such as seizure and impairments in learning and memory. Use of high copper concentrations can cause permanent damage to the cells and neurons. The aim of this study was to examine the toxic effects of orally administered copper sulfate on rats' learning in Morris water maze.

METHODS: In this experimental study, 39 Wistar rats were divided into male (n=21) and female (n=18) groups. These two groups were each randomly divided into three sub-groups. The control group received distilled water, while the other two groups were administrated 1 and 1.5 mM of copper sulfate, dissolved in distilled water for a period of one month. After this period, the Morris water maze was incorporated to evaluate the spatial memory of rats.

FINDINGS: In male rats, copper sulfate, which was added to drinking water, made no significant changes in the distance traveled to find the platform (24.09%±3.01 in the control group, 26.06%±2.95 in the 1 mm copper sulfate group, and 25.68%±1.82 in the 1.5 mM copper sulfate group), the time spent to find the platform (23.93±2.87 in the control group, 25.54±3.47 in the 1 mM copper sulfate group, and 25.33±1.92 in the 1.5 mM copper sulfate group), or the swimming speed. The comparison of female groups showed that 1 and 1.5 mM concentrations of copper sulfate could not cause any significant impairments in learning of rats.

CONCLUSION: The results showed that the addition of copper sulfate to drinking water have no detrimental impacts on the memory or learning of male and female rats.

KEY WORDS: *Copper Sulfate, Spatial Learning, Morris Water Maze, Rats.*

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Introduction

Copper is one of the main micronutrients in the human body, which plays a key role as a cofactor in the function of metabolic enzymes such as cytochrome c oxidase, superoxide dismutase, metallothionein, dopamine beta-hydroxylase, lysyl oxidase, and coagulation factors V and VIII. Copper is also involved in cellular processes such as energy production in mitochondria, iron homeostasis, detoxification of free radicals, melanin structure, synthesis of neurotransmitters, and reinforcement of connective tissues (1, 2).

Previous studies have revealed that malfunction in copper homeostasis can result in Menkes syndrome and Wilson's disease (1). Moreover, there is evidence on the effective role of copper in the pathogenesis of diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (3).

The neurological complications of Menkes syndrome include mental retardation, seizure, hyperthermia, dysphagia, abnormal myelination, cerebral and cerebellar atrophy, and remarkable loss of neural tissues in the cerebellum (4, 5). On the other hand, the neurological complications of Wilson's disease include degenerative nerve disorders, mental disorders (such as psychosis), obsessive compulsive disorder, seizure, migraine-like headaches, and memory disorders (3-6).

Exposure to high concentrations of copper can induce toxic effects in many organs of the human body. Epidemiological studies have demonstrated that high concentrations of copper in the diet are closely associated with reduced cognitive function (7) and symptoms of Alzheimer's disease (8). Moreover, an inverse relationship has been confirmed between serum concentrations of copper and cognitive abilities (9-11). Overall, contradictory results have been reported regarding the effects of copper sulfate on memory and learning in animal studies. Some studies have shown that copper injection can cause impairments in spatial learning of rats in the Morris water maze (12, 13), while others have revealed that this compound has no effects on spatial memory or learning of rats, despite its effects on long-term potentiation (LTP) inhibition (14). So far, no research has focused on the potential effects of copper on male and female rats. Therefore, the aim of this study was to evaluate the effects of chronic oral administration of copper on learning and spatial memory of rats in the Morris water maze.

Methods

This experimental study was conducted on 21 male and 18 female Wistar rats, with a weight range of 200-250 g. The male and female groups were each randomly divided into three sub-groups. The control group received distilled water as the drinking water supply for a period of one month, while the copper sulfate groups were administered 1 mM and 1.5 mM of copper sulfate, dissolved in distilled water, respectively during one month. All animals had free access to food and water. A month after the start of the experiments, the spatial learning and memory of rats were evaluated in the Morris water maze.

Water maze structure: The water maze was made of a black cylindrical water tank (140 cm in diameter and 60 cm in height), which was filled with water up to a height of 32.5 cm. The water temperature was similar to laboratory temperature, i.e., 22°C. A flexible black 30 cm platform was placed inside the pool in a way that it was 2.5 cm in water. The lower base of the platform (in the bottom of the pool) was 30 cm in diameter, while the upper surface, where the rat was placed, was 10 cm in diameter. The pool was placed in a room where different shapes were hanged on the walls. It should be mentioned that during the experiment, the examiner remained in one single place. Throughout the experiment, animal movements were recorded by a video camera, which was placed above the maze.

At the end of the experiments, the animals' behaviors were evaluated by a software program, which was able to measure the distance traveled by the animal, the average speed of animal's movements, and the time the animal spent in each quadrant of the maze.

Water maze test procedure: In the present study, the whole experiment consisted of four days of training and one day of assessment. During the training, the rats were released into the water maze four times. For this purpose, the maze was divided into four sections, i.e., north, south, west, and east (A, B, C, and D); the rats were released into the water maze from one of these quadrants. These quadrants were randomly selected, and every day, A, B, C, and D points were used to free the animals into the pool. At the beginning of the experiment, the rats were placed on the platform for 10 seconds, and then, they were moved away from the pool.

Within one minute, the animals were each released into the pool from one of the designated quadrants. Overall, the rats were given 60 seconds to find the

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platform. If the animal was able to find the platform within 60 seconds, it was allowed to remain on the platform for 10 more seconds. On the other hand, if the rat was unable to reach the platform within this interval, it was slowly guided towards the platform by the hand.

Afterwards, the animal was allowed to remain on the platform for 10 seconds, and then, it was moved away from the pool. At the end of the experiment, the animal was gently dried by a towel and returned to the cage. On the fifth day, the probe test was conducted by removing the platform from the pool and placing the animals inside the pool from quadrant B. As the animal was placed inside the pool for 60 seconds, We measured the amount of time spent in the target quadrant (one-fourth of the pool where the platform was placed) compared with the other quadrants (15, 16).

Statistical analysis: For statistical analysis, the analysis of variance and Tukey's test were performed. $p<0.05$ was considered statistically significant.

Results

Swimming speed: Examination of swimming speed showed no significant difference between female and male rats in the control and copper sulfate groups. Therefore, copper sulfate had no significant effects on animals' movements as a significant factor for the evaluation of spatial memory and learning in Morris water maze.

Comparison of the average distance traveled by rats to find the platform: During the training period, evaluation of the average distance traveled by male rats to find the platform showed a decline in the traveled distance as the training proceeded; however, there was no significant difference among male rats in different groups (fig 1). Similarly, evaluation of the travelled distance to find the platform in female rats showed that the average traveled distance reduced as the training progressed. Comparison of this variable among different groups showed no significant difference among them (fig 2).

Comparison of the average amount of time required to find the platform: The experiments indicated that the average amount of time required to find the platform for male rats reduced as the training proceeded. Comparison of this variable among different groups also yielded no significant differences (fig 1).

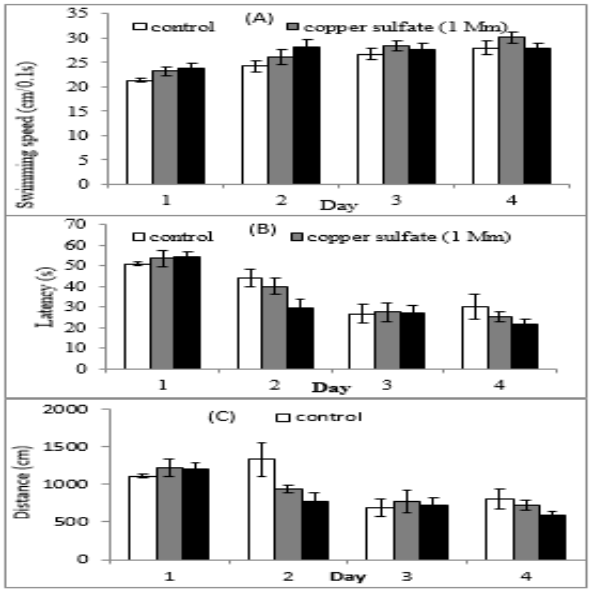

Figure 1. Comparison of (a) swimming speed, (b) elapsed time, and (c) the travelled distance to find the platform during four days of training in the Morris water maze in the male control group, 1 mM copper sulfate group, and 1.5 mM copper sulfate group (Mean±SEM); shorter time is indicative of better learning.

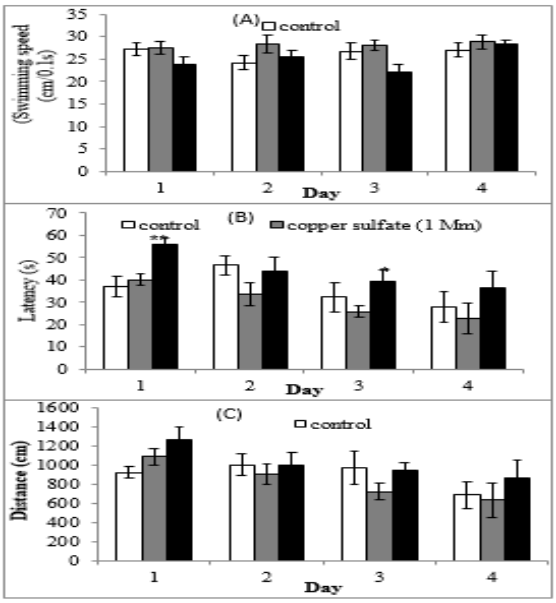

Figure 2. Comparison of (a) swimming speed, (b) the elapsed time, and (c) the travelled distance to find the platform within four days of training in the Morris water maze among female rats in the control group, 1 mM copper sulfate group, and 1.5 mM copper sulfate group (Mean±SEM); shorter time is indicative of better learning.

****p=0.005 for the control and 1.5 mM copper sulfate groups,**

***p=0.440 for 1 and 1.5 mM copper sulfate groups**

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Moreover, the average amount of time required to find the platform for female rats reduced as the training progressed. Comparison of this variable among different female groups showed that the time required to find the platform was significantly longer in the group receiving copper sulfate at a concentration of 1.5 mM on the third day, compared to the control group (p=0.005) and 1 mM copper sulfate group (p=0.044) (fig 2).

The results of probe trial test on the fifth day of the experiment: Comparison of the percentage of elapsed time in the target quadrant during the probe trial yielded no significant differences on the fifth day of the experiment between male rats (23.93%±2.87 in the control group, 25.54%±3.47 in 1 mM copper sulfate group, and 25.33%±1.92 in the 1.5 mM copper sulfate group) and female rats (24.08%±1.95 in the control group, 23.16%± 2.49 in the 1 mM copper sulfate group, and 21.89%±3.99 in the 1.5 mM copper sulfate group) (table 1). Comparison of the travelled distance in the target quadrant in probe trial showed a significant difference between the male groups (24.09%±3.01 in the control group, 26.06%±2.95 in the 1 mM copper sulfate group, and 25.68%±1.82 in the 1.5 mM copper sulfate group) and female groups (25.68%±1.90 in the control group, 23.21%±2.23 in the 1 mM copper sulfate group, and 22.37%±3.23 in the 1.5 mM copper sulfate group) (table 1).

Table 1. Comparison of the percentage of travelled distance and elapsed time in the target quadrant on the fifth day of the experiment (probe examination) in Morris water maze among female and male rats in the control group, 1 mM copper sulfate group, and 1.5 mM copper sulfate groups

Variables / Groups	Traveled distance	Elapsed time
Male		
Control	24.09±3.01	23.93±2.87
1 mM	26.06±2.95	25.54±3.47
1.5 mM	25.68±1.82	25.33±1.92
Female		
Control	25.68±1.90	24.08±1.95
1 mM	23.21±2.23	23.16±2.49
1.5 mM	22.37±3.23	21.89±3.99

Discussion

The results of the present study showed that chronic exposure to copper sulfate through drinking water does not cause impaired spatial learning in either female or male rats. This suggests that copper sulfate has no significant effects on learning or spatial memory of male rats. Conflicting findings have been reported on the effects of chronic exposure to copper sulfate on memory and learning. Epidemiological studies in different countries have shown that copper toxicity can lead to impaired cognitive abilities in humans (17). Animal studies have shown that adding copper sulfate to drinking water for a period of five months can result in impaired spatial learning in mice (18). In line with the present findings, Levia et al. reported that intraperitoneal injection of copper sulfate for 30 consecutive days would not impair spatial memory or learning in rats (14).

It appears that two factors contribute to the discrepancy between the reported findings. Firstly, exposure to copper for less than a month has no adverse effects on memory or learning. Secondly, the response of different animal species to copper sulfate significantly varies, as confirmed by several studies. In fact, research studies on rats have reported no adverse effects induced by copper sulfate, while studies on mice have demonstrated the adverse effects of this compound on memory.

Another finding of this study was the increased time required to find the platform in female rats, which were administrated 1.5 mM of copper sulfate on the third day of the experiment, compared to the control and 1 mM copper sulfate groups. With regard to the increased elapsed time in the group receiving 1.5 mM of copper sulfate, we cannot conclude that copper sulfate has caused impaired learning in this group, since no significant difference was observed between this group and others before or after the third day of the experiment. Therefore, it seems that similar to male rats, addition of copper sulfate to drinking water for one month does not impair spatial memory of female rats. Another important point is the contradiction in the results obtained in Morris water maze and field potential. Previous studies have shown that in slices, obtained from rats receiving copper in water for a month, tetanic stimulation was not able to induce LTP in the hippocampal CA1 area (14, 19).

It seems that since copper can inhibit LTP induction in the hippocampal CA1 area, it should be able to interfere with spatial learning in rats in the Morris water maze. The results of the present study showed that adding copper to drinking water did not impair the spatial memory of rats in the Morris water

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maze. In other words, we can conclude that copper inhibits LTP induction in the hippocampal CA1 area, without influencing animals' learning. In line with the present study, previous research has not indicated any relationship between LTP in the hippocampus, NMDA receptors, and spatial learning (20-24). Under normal conditions, NMDA receptor-dependent LTP plays no role in spatial learning of hippocampus. However, none of these studies have provided useful evidence to describe the mechanisms involved in spatial learning and the possible causes of these findings. The obtained findings substantiate the hypothesis that LTP is not the molecular basis of memory and learning. However, to prove this hypothesis, further research is required. In conclusion, the results of the present study showed that adding copper sulfate to drinking water for one month has no significant influence on rats' learning ability or spatial memory in the Morris water maze.

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