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RESEARCH ARTICLE

NASAL ADMINISTRATION OF SILVER NANOPARTICLES AND SILVER IONS REDUCED COGNITIVE PERFORMANCE IN ADULT RATS

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Abstract

Background: Silver nanoparticles have shown adverse effects and a toxic impact on human cell bodies. If a toxic material passed the blood-brain barrier would increase the oxidative stress and can change different parts of the brain such as the hippocampus. Hence, the aim of this study was to examine the potential harmful effects of silver nanoparticles on the nervous system of adult rats.
Method: Rats were exposed to neurotoxicity by being treated with silver nanoparticles at doses of 3 mg/kg and 15 mg/kg and silver acetate intranasally every two days for 20 days. The rats were divided into five groups: control, vehicle, silver nanoparticles at doses of 3 mg/kg and 15 mg/kg, and silver acetate. Cognitive impairment and molecular changes induced by silver were evaluated using behavioral assessments such as the Morris water maze and elevated plus maze, and biomarker analysis such as the malondialdehyde assay.
Result: The findings showed that silver nanoparticles at a dose of 15 mg/kg and silver acetate significantly affected spatial memory. In addition, silver nanoparticles at doses of 3 mg/kg and 15 mg/kg and silver acetate caused an increase in anxiety in the animals. Furthermore, levels of malondialdehyde were significantly raised by silver nanoparticles at doses of 3 mg/kg and 15 mg/kg.
Conclusion: The findings showed that silver nanoparticles and silver acetate, particularly silver nanoparticles with dosage of 15 mg/kg, result in neurotoxicity and behavioral impairments.
Keywords: Anxiety-like behaviors, learning and memory, neurotoxicity, silver nanoparticles, silver acetate.

INTRODUCTION

In recent times, there has been a surge in the use of metal nanoparticles such as silver for various applications including sterilization, anti-bacterial agents, food additives, and medicine. The distinct electronic, optical, mechanical, magnetic, and chemical properties of these nanoparticles differ greatly from their bulk metal counterparts, leading to a wide range of uses[1,2]. As a result, there is growing concern about the proliferation of silver nanoparticles in human habitats[2]. Silver nanoparticles can be introduced into the body through various means such as ingestion and inhalation, potentially resulting in adverse effects[3].
Different biological models have been employed to assess the impact of nanoparticles on living beings. They have been found to negatively impact the reproductive systems and embryonic development of animals such as mice and zebrafish[4,5]. After entering the body, nanoparticles can travel to various organs through the bloodstream. Based on their size, shape, and chemical properties, they may also be able to cross the blood-brain barrier and reach the brain through axonal transport along the olfactory nerve[6].
Numerous recent studies conducted both *in vivo* and *in vitro* have shown that silver nanoparticles can have harmful effects and a poisonous influence on human cells[7,8]. Numerous recent studies conducted both *in vivo* and *in vitro* have shown that silver nanoparticles can have harmful effects and a poisonous influence on human cells[9,10,11]. Research has demonstrated that silver nanoparticles are capable of crossing the blood-brain barrier and accumulating in the brain[12]. These mechanisms may provide pathological conditions that may cause brain damage[12]. Moreover, there is evidence that silver nanoparticles disrupt the transporting neurotransmitters such as dopamine, norepinephrine, and serotonin in neural pathways[13,14], and these changes in neurotransmitters can affect cognitive and

behavioral mechanisms, especially learning and memory[15].

Studies have demonstrated that gradual declines in spatial learning and memory functions are inversely correlated with ROS values in the brain. Moreover, recent evidence suggests a direct correlation between oxidative stress and anxiety behavior in animals[16]. Furthermore, once silver nanoparticles have crossed the blood-brain barrier, they can generate oxidative stress (similar to reactive oxygen species) in various regions of the brain such as the hippocampus, potentially leading to impairments in learning and memory processes[17,18]. Research has demonstrated that exposure to silver nanoparticles during pregnancy can negatively affect cognitive performance in the Morris water maze test, but no differences were observed in anxiety-like behavior in the elevated plus maze test[19]. In addition, while one study has shown that these nanoparticles can negatively impact short-term memory in animals, other research has not found a significant difference in spatial memory in animals exposed to silver nanoparticles[17].

This research aimed to examine the negative impact of inhaling silver nanoparticles (AgNPs) and silver acetate (AgAc) particles on behaviors such as learning, memory, and anxiety using MWM and EPM tests. The study also compared the toxicity between bulk- and nano-sized particles by using the bulk size of AgNPs.

MATERIALS AND METHODS

Animals

For this study, we used 50 adult male Wistar rats weighing between 200-230g. These rats were obtained from Shahid Chamran University of Ahwaz's animal facility. They were housed in a room with a temperature of 22±2°C and a 12-hour light/dark cycle. The rats had proper ventilation and access to food and water.

Experimental protocol

The Wistar rats were randomly assigned to one of five groups, with 10 rats in each group. Group 1 served as the control group. Group 2 received sodium citrate (0.12 M) every other day for 20 days. Group 3 received silver acetate (4.6 mg/kg) every other day for 20 days. Groups 4 and 5 received AgNPs at doses of 3 and 15 mg/kg, respectively, every other day for 20 days. All treatments were administered intranasally[20].

Synthesis of nanosized silver particles

Preparation of AgNPs

In this examination, silver nitrate decreased the usage of sodium citrate and hydrazine hydrate to synthesize silver nanoparticles. To synthesize silver nanoparticles, 400 and 600 microliters of 0.12 M sodium citrate (0.35 mg in 10 ml) have been mixed with 1 ml of 0.02 M silver nitrate (0.033 g in 10 ml) in a 2 ml micro tube and diluted to a volume of 2 ml with double-distilled water. Finally, one microliter of hydrazine hydrate changed into delivered and the aggregate turned into left at room temperature for two hours within the darkish. The samples had been then centrifuged at 10,000 rpm for 15 mins. After centrifugation, the supernatant becomes separated from the precipitate and the yellow color of the supernatant indicates the formation of nanoparticles. The pH of the ensuing solution turned into 6.7.

Determination of the concentration

The concentration of nanoparticles was determined using the Beer-Lambert formula and the molar extinction coefficient for nanoparticles with diameters ranging from 10-15 nm or 20-30 nm. The formula OD =E*C*L was applied, where L represents the cuvette length (1 cm), C represents the concentration in mg/ml, and E represents the molar extinction coefficient. Nanoparticles with a diameter of 10-15 nm had a concentration of 20 mg/ml and were carried in a yellow solution. Nanoparticles with a diameter of 20-30 nm had a concentration of 64 mg/ml and were carried in a brown solution. These nanoparticles had a purity of 99.3%[21].

Behavioral tests

Morris water maze

The MWM test evaluated the spatial learning and memory of rats. The test involved a circular pool with four quadrants filled with water at 25°C. A submerged platform was placed in the northeast quadrant. Rats were trained for four days to find the platform. Each day consisted of four trials, with 60 seconds for the rat to find the platform. If unsuccessful, the experimenter placed the rat on the platform[22]. A "probe trial" was performed 24 hours after the last session to evaluate spatial memory. The platform was removed and the rat had 60 seconds to find the target quadrant. A visible platform test was then performed using aluminum foil to cover the platform. A video tracking system recorded the rats' movements[23].

Elevated plus maze

The Elevated Plus Maze (EPM) test is a widely used method for assessing anxiety-like behavior in rodents. The maze consists of two open arms and two closed arms, each measuring 30x5x15 cm. During the test, rats are placed at the center of the maze and their behavior is observed for 5 minutes. Data analysis includes recording the number of entries into the open and closed arms, as well as time spent in each arm[24].

Malondialdehyde assay

The thiobarbituric acid (TBA) method is commonly used to measure malondialdehyde (MDA) levels in blood samples. The process involves combining the samples with 1% potassium iodide and 0.1% butylated hydroxytoluene, followed by incubation at 50°C for 20 minutes. Next, 0.4% TBA is added and the samples are incubated at 60°C for 60 minutes. Finally, high-performance liquid chromatography with fluorescence detection is performed using isobutyl alcohol to assess the samples.

Statistical analysis

The data is presented as the mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used to analyze the Elevated Plus Maze (EPM) and probe test data in the Morris Water Maze (MWM). Two-way ANOVA was used to compare groups during the learning phase of the MWM. A *p*-value of less than 0.05 was considered statistically significant. Statistical analysis was performed using GraphPad Prism 8.0 software. This study was approved

by the Ethics Committee of Shahid Chamran University in Ahvaz, Iran.

RESULTS

Effects of AgNPs on learning and memory in MWM

The Morris Water Maze (MWM) test was used to evaluate spatial learning and memory in all groups. Over the course of four days, both the path length and escape latency to find the hidden platform were reduced. Two-way analysis of variance (ANOVA) showed no significant differences in total traveled distance or escape latency between the control, vehicle, NP3, NP15, and AgAc groups of rats (Figure 1(a) and Figure 1(b)). Twenty-four hours after the last session, a probe test was conducted to assess spatial memory by measuring the mean percentage of distance traveled and time spent in the target quadrant. The results showed significant differences in the percentage of distance traveled in the target quadrant for the NP15 (p<0.01) and AgAc (p<0.05) groups compared to the control group. Additionally, the percentage of time spent in the target quadrant was significantly lower for the NP15 group (p<0.01) compared to the control group (Figure 2(a) and Figure 2(b)).

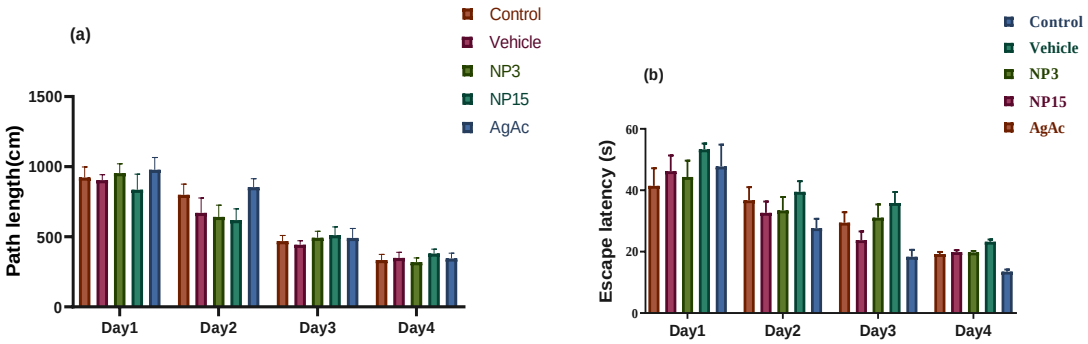

Figure 1: Morris water maze.

The total distance traveled and escape latency by rats showed no significant difference among groups (a, b).

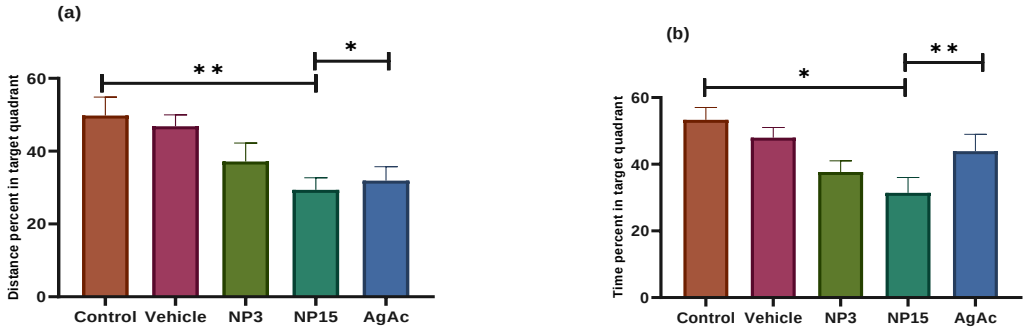

Figure 2: Probe test.

The distance percentages in target quadrant was decreased significantly in NP15 rats (p<0.01) and AgAc rats (p<0.05)compared to the control group. The data is presented as mean ± SEM.**p<0.01 vs. NP15. *p<0.001 vs. AgAc (a).The time percentage spent in the target quadrant was significantly less in the NP15 group (p<0.01) compared to the control group. The data is presented as mean ± SEM. **p<0.01 vs. NP15(b).

Effects of AgNPs on anxiety in EPM

One-way analysis of variance (ANOVA) revealed a significant difference in the average percentage of time spent in the open arms between the control group and the NP3 (p<0.05), NP15 (p<0.01), and AgAc (p<0.05) groups. However, no significant difference was observed among the groups in the average percentage of entries into the open arms (Figure 3(a) and Figure 3(b)). The average percentage of time spent in both the open and closed arms showed significant differences between the NP15 (p<0.001) and AgAc (p<0.01) groups compared to the control group (Figure 3(c)).

Effects of AgNPs on malondialdehyde level in MDA assay

One-way analysis of variance (ANOVA) revealed a significant difference in malondialdehyde (MDA) levels between the control group and the NP3 (p<0.05) and NP15 (p<0.01) groups (Figure 4).

DISCUSSION

The results of this study indicate that AgNPs and AgAc have negative effects on certain aspects of memory and anxiety. The Elevated Plus Maze (EPM) test showed that the percentage of open arm time (OAT) was significantly lower in the NP15, NP3, and AgAc groups compared to the control group. Additionally, total arm entries were lower in the NP15 and AgAc groups. These results suggest an increase in anxiety-like behavior and locomotion problems. The Morris Water Maze (MWM) test was used to assess spatial learning and memory in rats. The results showed no significant differences in traveled distance among the experimental groups, indicating that learning may not be affected by AgNPs and AgAc. Increases in swimming speed were observed in the NP15 and AgAc groups, suggesting a dose-dependent toxic effect of nanoparticles. Furthermore, a decrease in the percentage of travel in the target quadrant during probe

trials was observed in the NP15 and AgAc groups, indicating a negative effect on memory processes. These results suggest that AgAc may have a greater toxicological potential compared to nano-sized particles at equal dosages. These results are consistent with a study by *Kvitek et al.*, which showed that AgNPs are toxic to mammalian cells at high concentrations (60 mg/l), while AgAc causes toxicity at a much lower concentration (1 mg/l)[25]. A study by *Loeschner et al.*, showed that after oral administration of AgAc and AgNPs, the concentrations of AgAc in the brain and plasma were significantly higher than those of nanoparticles[26]. Together, these results showed that AgAc have more toxicological effects compared to AgNPs.

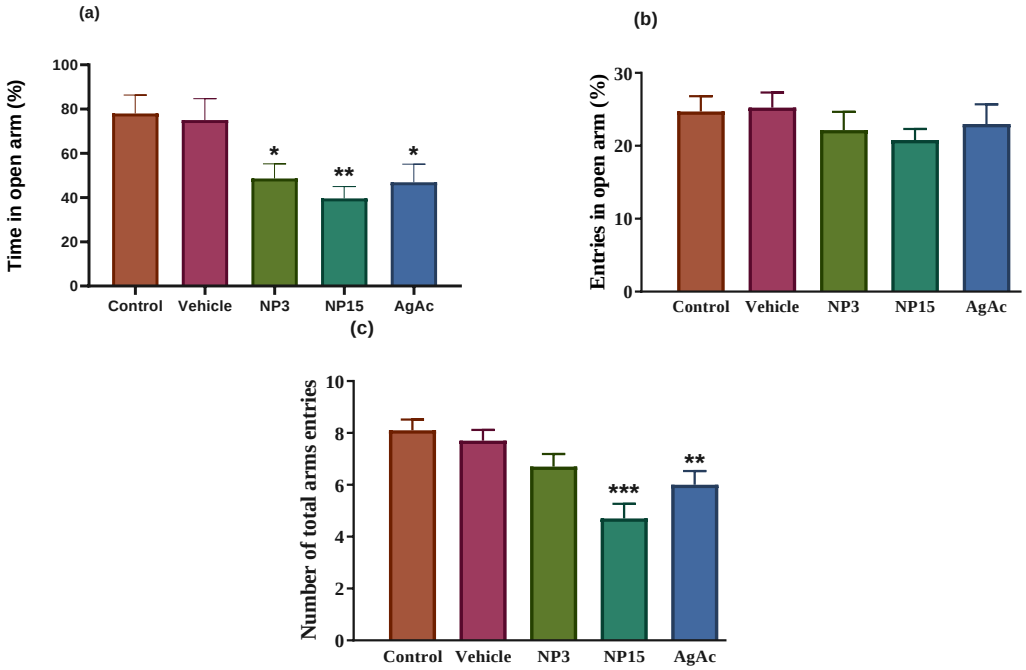

Figure 3: Elevated plus maze.

There was a significant difference between the control group and NP3, NP15, and AgAc groups regarding the average percentages of time spent in the open arm. The data is presented as mean ± SEM. **p*<0.05 vs. NP3. ***p*<0.01 vs. NP15.**p*<0.05 vs. AgAc (a). The average percentages in entries into the open arm demonstrated no significant differences between groups (b). The average percentages of time spent in both open and closed arms showed no significant differences among groups(c).

These findings are consistent with other studies that have shown that exposing zebrafish to AgNPs during development can result in increased anxiety and negative effects on cognitive function and behavior[27]. A study by *Hritcu et al.*, showed that administering AgNPs intranasally can result in spatial memory problems, which may be related to an increase in reactive oxygen species (ROS) in the hippocampus[28]. In contrast to this study, *Liu et al.*, did not find a significant difference in memory after exposure to these particles compared with the control group. These conflicting results may be attributed to differences in the shape, surface coating, and size of the particles, or to variations in the methods of administration[17].

Research has shown that administering AgNPs intranasally can result in their accumulation in the olfactory bulb and ventricles, causing inflammation and an increase in tissue glutathione (GSH) levels. These findings may provide evidence for the transport of nanoparticles from the nose to the brain[29]. Several mechanisms have been proposed to explain how nanoparticles can enter the brain through the nasal passages, including the olfactory and trigeminal neural pathways, as well as paracellular transport[30]. Furthermore, oral administration of nanoparticles has been shown to increase levels of interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and reactive oxygen species (ROS) in brain tissues. This can lead to apoptosis and changes in brain gene expression[31]. A study found that AgNPs of various shapes can cause histopathological changes in brain regions such as the amygdala and hippocampus, which play crucial roles in regulating anxiety, stress behaviors, and memory[32]. Studies have shown that when AgNPs accumulate within cells, they can cause inflammation and oxidative stress, leading to activation of the cells' antioxidant defense mechanism[33].

The brain has a lower capacity for oxidation compared to other organs, making it more vulnerable to oxidative stress. When oxidative stress levels in the brain increase, it can result in damage to areas such as the striatum and amygdala[34]. AgAc can be transported to various organs, including the liver, kidneys, and central nervous system (CNS), via the lymphatic system. It can also be transferred within the CNS through neural axons[35]. Like AgNPs, AgAc can have toxic effects on brain cells, but through different mechanisms. This may be due to variations in the regulation of gene expression and mRNA synthesis related to oxidative stress[10]. The toxicity of silver may result from AgAc binding to important functional proteins. In contrast, AgNPs attach to vesicles and organelles such as lysosomes and collagen, which have little to no effect. Studies have shown that AgAc and AgNPs can have

toxic effects on the central nervous system through different mechanisms that play crucial roles in cognitive functions. These results suggest that AgAc has a greater impact than AgNPs at similar doses.

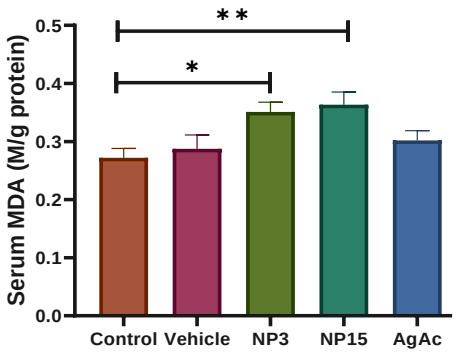

Figure 4: Malondialdehyde assay.

There was a significant difference between the control group and NP3 and NP15 groups regarding the malondialdehyde level. The data is presented as mean ± SEM. *p<0.05 vs. NP3. **p<0.01 vs. NP15.

Alterations in neurotransmitter levels may be another mechanism that contributes to behavioral issues following exposure to these substances. Both silver nanoparticles and AgAc can hinder the differentiation of nerve cells and their ability to produce dopamine [13,14]. According to a previous study by *Hadrup et al.*, exposure to both AgAc and AgNPs increased dopamine levels but had varying effects on other neurotransmitters. AgAc significantly impacted noradrenaline levels, while AgNPs had a greater effect on 5-HT. These neurotransmitters play critical roles in cognitive processes[36]. Research has shown that an imbalance between serotonin and norepinephrine can result in anxiety and hypersensitivity. Additionally, an imbalance between norepinephrine and dopamine can lead to impulsive behavior and issues with reward processing[37]. Behavioral response to anxiety and stress is mediated by many neurotransmitters including dopamine, norepinephrine, serotonin, and acetyl-choline[38]. Research on memory has revealed that dopamine and norepinephrine play crucial roles in various aspects of memory. For instance, a reduction in norepinephrine can lead to minor impairments in working memory during delayed tasks, while a decrease in dopamine in the substantial area can result in memory deficits[39].

These studies aimed to assess the impact of AgNPs on anxiety-like behaviors and learning and memory. The results indicated that treatment with AgAc and AgNPs led to deficiencies in memory and increased anxiety-like behavior. According to other research, these effects may be due to changes in serotonin, dopamine, and norepinephrine neurotransmitters and the toxicological effects of these substances.

Limitations and future studies

The findings of this study are limited by the use of only male animals and these effects can also be studied on female rats in future studies. Moreover, chronic toxicological effects of these materials could be examined as well.

CONCLUSIONS

This study found that AgNPs and AgAc have negative effects on certain aspects of memory and anxiety. Results from the EPM test showed that the OAT percentages in the NP15, NP3, and AgAc groups were significantly lower than in the control group, and the total number of arm entries decreased in the NP15 and AgAc groups. These findings suggest an increase in anxiety-like behavior and locomotion problems. Additionally, the MWM test was used to assess spatial learning and memory in rats. The results showed no significant differences in traveled distance between the experimental groups, indicating that learning may not be affected by AgNPs and AgAc. However, an increase in swimming speed was observed in the NP15 and AgAc groups, suggesting a dose-dependent toxic effect of NP. Furthermore, a decrease in target quadrant travel percentages was observed in the NP15 and AgAc groups during probe trials, indicating a negative impact of these substances on memory processes in animals.

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AUTHOR'S CONTRIBUTION

Farimaneh J: writing, review, and editing. **Tabandeh MR:** acquired the data. **Tabatabaei SRF:** formal analysis, editing. All authors revised the article and approved the final version.

DATA AVAILABILITY

The data and material are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

None to declare.

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