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Mice Exposed to Silver Nanoparticles during Pregnancy Show Depression-Related Reactions

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ABS TRAC T

Even while research on silver nanoparticles is growing, it is still unclear how they work, particularly in terms of their potential toxicity to the process of reproduction, development, and behavior of offspring. Thus, the current research evaluated the impact of exposure to silver nanoparticles during pregnancy on the depressive behavior of children. Thirty virgin female mice were split into three groups (n = 10 for each group): two experimental groups and one control group. Each group received an equivalent volume (0.2 ml) of suspension containing 0, 0.2, and 2 mg/kg of silver nanoparticles, respectively. The suspension was administered upon mating and repeated every three days till accouchement. Tail suspension and forced swimming tests were used to measure depression behaviors in 45-day-old male and female offspring (6 groups, n=10). In the forced swimming test, both doses of silver nanoparticles (0.2 and 2 mg/kg) reduced mobility and increased immobility time in men ($P<0.05$), while no effects were seen in females. In the tail suspension test, 2 mg/kg of silver nanoparticles caused the female offspring's mobility time to decrease ($P<0.05$) and their immobility time to rise ($P<0.05$), but there was no discernible impact on the male offspring's mobility or immobility time. We may infer that gender-specific depression-like behaviors in children are likely caused by prenatal exposure to silver nanoparticles, presumably due to neurotoxic effects during neuronal development.

Keywords: *Silver Nanoparticles, Prenatal Exposure, Depression Behavior, Forced Swimming Test, Tail Suspension Test.*

INTRODUCTION

Because they range in size from 1 to 100 nanometers (nm), nanomaterials are referred to as nanoobjects or nanostructured materials [1, 2]. Silver nanoparticles, or Ag-NPs, are used in a wide range of fields, including industry, biology, medicine, and pharmaceuticals [3–5]. Exposure to Ag-NPs increases when Ag-NPs are used more often in various materials. Certain neurotoxicities, such as diminished learning capacity and short-term memory, are brought on by nanomaterials [6]. Additionally, it has been observed that exposure to nanoparticles during pregnancy might impact neurobehavioral development [7] by increasing ROS duration in the brain compared to other tissues [11]. Additionally, Ag-NPs were retained in the mouse body for longer than four months [12]. These elements provide Ag-NPs adequate time to affect the physiology and development of brain cells. Additionally, the placenta, a crucial barrier, permits Ag-NPs to get through, potentially causing neurotoxicity in the fetus [13, 14]. According to some research, Ag-NPs pass the placenta and concentrate in the tissues of the fetus [15]. However, there is currently a dearth of knowledge about the relationship accumulating in the hippocampus [8]. Females are particularly vulnerable to the various impacts of nanoparticles because their toxicity may disrupt embryonic development and fertility [9]. Additionally, Ag-NPs may accumulate in the brain because the blood brain barrier (BBB) permits their passage [10]. However, it was shown that silver has a lengthy maintenance period even at low concentrations between prenatal Ag-NPs exposure and following behavioral performance.

The concern of whether exposure to Ag-NPs during pregnancy might harm neuronal development and result in neurodisorders in the offspring is raised by the widespread use of Ag-NPs in medicine, their passage through the placenta and BBB, and their extended maintenance and retention times. Low mood, avoidance of activities, or a decrease in biological function are all signs of depression. As a result, assessing behavioral activities became a helpful method for diagnosing depression. With rising rates in both boys and females, it is one of the leading causes of morbidity worldwide [16]. Stress-related mental illnesses include anxiety and depression. Variations in emotionality are the basis for a number of animal models of anxiety and depression. These include elevated plus maze (EPM), light-dark box, and open field behavior [17]. Additionally, the sucrose preference test is a sign of anhedonia, a condition linked to depression [18]. One of the most popular methods for assessing depressed mood among animal models of depression is the mouse forced swimming test (FST), which possesses predictive and reliability validity [19]. Another popular technique for assessing antidepressant-like action in mice is the tail suspension test (TST) [20]. Both of these techniques see falling into water and hanging by the tail as stressful situations that encourage animals to struggle harder. In order to identify potential depression and likely impairment of neurobehavioral development in mouse offspring, the toxic impact of prenatal exposure to Ag-NPs was evaluated in the current investigation using FST and TST.

MATERIALS AND METHODS

The used protocol and animals' ethics were approved by the Research Council of Veterinary Medicine Faculty in Shahid Chamran University of Ahvaz.

Nanoparticle:

Ag-NPs (size: 10 nm, shape: spherical, purity: 99.9%, density: 1000 ppm) were purchased from Neutrino Corporation (Neutrino Nanovation, Iran). They were prepared by sol-gel method in which silver ions were reduced by sodium borohydride in the presence of citrate (Fig 1).

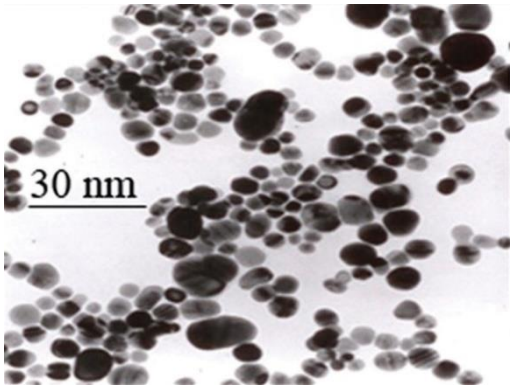

Fig 1: Scanning electron microscope photographs of silver nanoparticles

Animals grouping and Ag-NPs administration:

Harlan Laboratories provides the NMRI outbred mice as a research animal model for fundamental studies. Thirty virgin female and ten male NMRI (US Naval Medical Research Institute) mice, aged five to six weeks, were acquired from the Laboratory Animal Facility of Jondi Shapoor University in Ahvaz, Iran. Males and females were kept apart in ordinary plexiglass cages at a temperature of 23±2º. The 12:12-hour light/dark cycle was used to automatically arrange the lightness program. The animals were given tap water and a standard pellet meal (Pars Animal Feed, Iran) on an as-needed basis. The female mice were split into three groups (n = 10 for each group) after a week of acclimatization: the control, NP 0.2, and NP 2 groups. They received subcutaneous (SC) injections of an identical amount of 0.2 ml of solution containing zero, 0.2, and 2 mg/kg of Ag-NP, respectively. We take into account the lowest dosage to ensure that Ag-NPs were delivered to the majority of the mother's and the embryo's organs and extra-embryonic tissues without creating any anatomical abnormalities since it has been previously documented that an excess of Ag-NPs may result in embryo-malformation [21]. As a teratogen, Ag-NPs may thus be the cause of postpartum neurobehavioral problems, which are unrelated to physical dysmorphology [22]. Therefore, the potential morphological anomalies caused by our selected dosage of Ag-NPs were initially examined in a preliminary experiment. Only female mice were then administered Ag-NPs just after mating, and this procedure was repeated once every three days until delivery. The males and females were separated fourteen days later. In order to guarantee effective mating, this period was chosen. The females were moved to separate cages (26 x 47 x 20 cm3) 17 days after mating in order to provide them adequate room for a comfortable parturition. Ten males and ten females were selected at random from several colonies of 45-day-old mice for behavioral testing.

Behavioral tests:

Every trial was carried out between 13:00 and 17:00, and behavior characteristics were noted as duration (s). Each FST and TST test's depressive behaviors were assessed independently using the conventional behavioral mice model. Every exam was videotaped, and the videotapes were graded by a blind observer.

Forced swim test:

To put it briefly, each mouse was made to swim for six minutes on its own. Fresh water (25±1º) was employed in an open glass box (25×15×25 cm) up to a height of 15 cm. Mice were removed from the water and given time to dry at the conclusion of each session (after every six minutes). Water was replaced after every session since it has been shown in the past that used water may modify an animal's behavior. Every mouse underwent this test twice. The mice were very

active during the first FST session, attempting to climb the chamber wall or plunge to the bottom while swimming vigorously in a circle. They were made to swim in the same setting for an additional six minutes after a 24-hour period. The mice floated in the water once they stopped trying. This was seen as immobility. The immobility time was measured during the last four minutes of the six-minute period [23, 24].

Tail suspension test:

This test is often used to identify depressant-like activity in mice using a behavioral paradigm. A 25 x 25 x 30 cm box was supplied, and the testing were conducted in a quiet, dark environment to prevent any disruptions. Mice were hung by a clamp at a distance of 1 cm from the end of their tails, resulting in a head-to-bottom distance of 5 cm. The animals were separated from one another both visually and aurally. The length of immobility was assessed during the last 4 minutes of the test after the mice were suspended for a total of 6 minutes. Only when the mouse was totally still was the time of immobility taken into account [25].

Statistical analysis:

Using SPSS16 (Chicago, USA), statistical analyses were carried out by contrasting the treatment groups with the control group. The mean±SEM was used to represent all values. One Way Analysis of Variance (ANOVA) was used to conduct statistical comparisons. The Tukey test was used to do post-hoc comparisons between individual groups. A P value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Table 1 displays all of the findings. Prenatal exposure to both doses of 0.2 and 2 mg/kg decreased mobility in male offspring (control: 90.6±19.5 s, NP 0.2 mg/kg: 56.8±11.67 s, NP 2 mg/kg: 64.7±14.5 s, P<0.05) and increased immobility (control: 149.4±19.5 s, NP 0.2 mg/kg: 183.2±11.67 s, NP2 mg/kg: 175.3±14.5 s, P<0.05, fig. 2). However, there was no change in the FST test mobility or immobility times in females (P>0.05). In the tail swimming test, the mobility and immobility periods of male offspring were unaffected by the doses of 0.2 and 2 mg/kg of Ag-NPs. However, in females, the NP 2 dose caused a decrease in mobility (NP 0.2 mg/kg: 210.5±6 s, NP 2 mg/kg: 159±11.4 s, P<0.05) and an increase in immobility periods (NP 0.2 mg/kg: 29.5±6 s, NP 2 mg/kg: 71.1±12 s, P<0.05, fig. 3).In our investigation, FST increased immobility and decreased mobility in males, whereas TST had the similar effects in females. Gender-specific behaviors resembling depression were probably seen. The dose-dependent rise in immobility and fall in mobility in FST in the male progeny was an intriguing result of the current research that may be connected to behaviors similar to depression. Physiological, neurological, and behavioral factors combine to cause depression, an emotional illness [26]. According to FST, the sad mice stop swimming and stay motionless after floating in the water. The duration of immobility is seen as a measure of depression [27]. The immobility period is regarded as a depressive state in TST as well [28]. This time, the antidepressant ingredients decreased since they raise the

Table 1: Summary of Results

Genders	FST (0.2 and 2 mg/kg)		TST (0.2 and 2 mg/kg)	
	Mobility	Immobility	Mobility	Immobility
Male	Decrease	Increase	-	-
Female	-	-	Decrease	Increase

FST: Forced swimming test, TST: tail suspension test

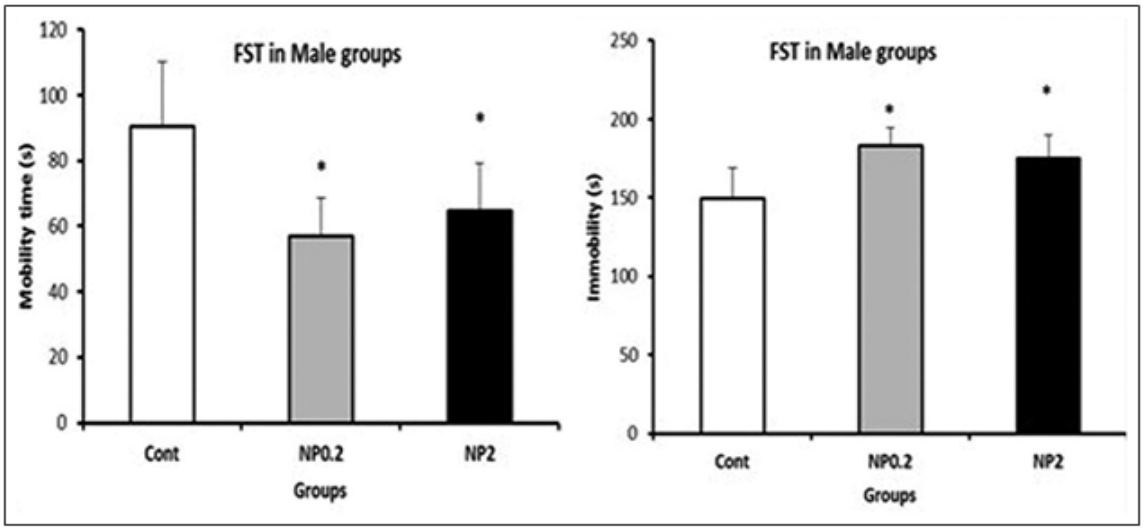

Fig 2: Effect of different dose of Ag-NPs on male offspring mouse in FST

Effect of different dose of Ag-NPs (0.2, 2 mg/kg, sc) on mobility and immobility period in male offspring mouse FST. The values are expressed as mean±SEM (*n*=10). Data were analyzed by ANOVA followed by Tuky test.

Cont=control group, NP0.2= group, which received dose 0.2 mg/kg of Ag-NPs, NP2= group, which received dose 2 mg/kg of Ag-NPs. *Indicate significant difference *vs.* control group (*P*<0.05).

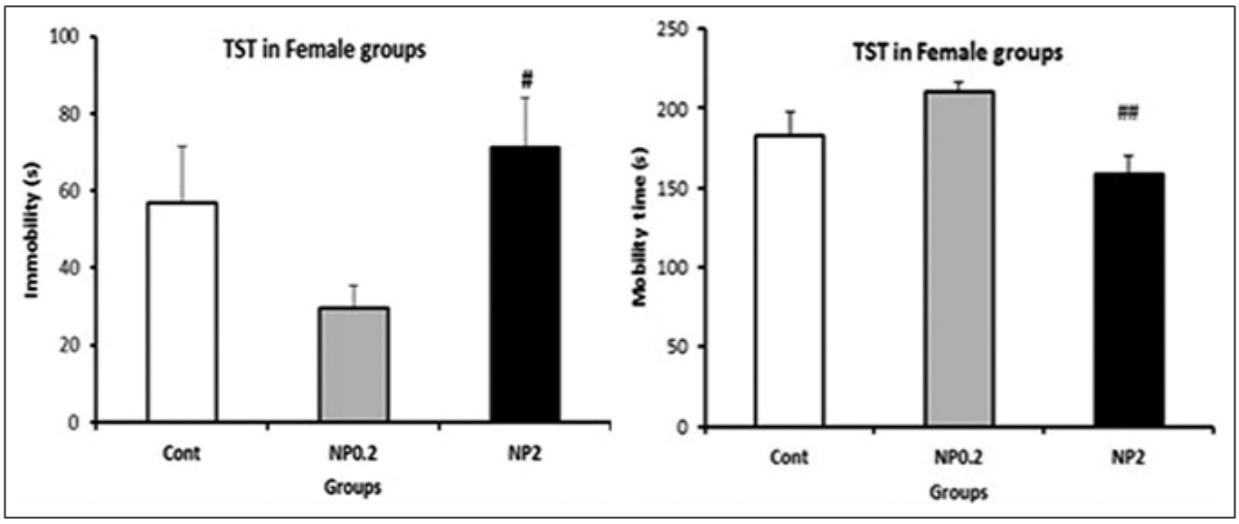

Fig 3: Effect of different dose of Ag-NPs on female offspring mouse in TST

Effect of different dose of Ag-NPs (0.2, 2 mg/kg, s.c) on the mean mobility and immobility period in female offspring mouse TST. The values are expressed as mean±SE (*n*=10). Data were analyzed by ANOVA followed by Tukey test. Cont = control group, NP0.2 = group, which received dose 0.2 mg/kg of Ag-NPs, NP2 = group, which received dose 2 mg/kg of Ag-NPs. # indicate significant difference *vs*. NP0.2 group (*P*<0.05).

CNS monoaminergic transmitter level [29]. The main causes of mental depression are deficiencies in certain monoaminergic transmitters, such as noradrenalin, serotonin (5-HT), and dopamine (DA), or dysregulation of their receptors in the brain [30]. Ag-NPs (15 nm) have been shown to decrease DA and its metabolite (dihydroxyphenylacetic acid). They affect the ability of cells to produce DA because they hinder neuronal cell differentiation [31]. Additionally, they lower the amount of gene expression associated with the dopaminergic system. Ag-NPs of small sizes go across the blood-brain barrier. Ag-NPs that are 50–100 nm in size may alter the action potential of hippocampal CA1 neurons [21], whereas those that are 15 nm in size decrease dihydroxyphenylacetic acid and DA. 20 nm-sized silver nanoparticles were shown to be more hazardous than bigger NPs. Additionally, Ag-NPs are more hazardous than Ag ions. The toxicity of Ag-NPs depends on the size of the NPs, the type of cell, and their capacity to permeate the cell membrane [35]. Ag-NPs with a size of 10 nm were employed in this investigation. Because of their size, NPs are able to pass through biological barriers. In an in vitro BBB model made of rat brain vascular endothelial cells, Ag-NPs may build up. Since it has been previously documented that SC injection of Ag-NPs may pass through the BBB and produce pathological alterations, necrosis, and neuronal degeneration, we delivered Ag-NPs subcutaneously. Additionally, oral treatment caused buildup in the brains of dams [13]. Ag-NPs have sufficient opportunities to affect the brain due to their passage via the blood-brain barrier, entering fetal circulation, accumulation in the brain, and prolonged maintenance. Ag-NPs have been linked to brain injury via a number of processes, including activating the ROS system, affecting the immune system, causing ion channel malfunction, impairing neurotransmission in the brain, and altering the function of glutamate, the primary excitatory neurotransmitter in the brain. Numerous cellular pathogenic processes, such as DNA damage, cell stress, tissue inflammation, and apoptosis, may be caused by oxidative stress and the ROS system. Additionally, oxidative stress caused by ROS is directly linked to depressive illness [14]. Ag-NPs may reach the fetus's bloodstream, activate the ROS system, and cause fetal cell dysfunction because they cause ROS formation in the mother's body and can cross the blood-brain barrier. Because inflammatory variables and depression are related, persons with depression have higher levels of IL-6 and IL-1. As a consequence, their serum level may alter the FST and TST outcomes. By using TST and FST examination, it was previously shown that nanoparticles produce depressive disorders in both genders. Additionally, multiwall carbon nanotubes may cause depressive disorders by triggering the inflammatory cascade, according to Ivani *et al*.,'s FST evaluation. These are the two primary pathways linked to Ag-NPs toxicity because, in addition to oxidative damage, Ag-NPs affect the immune system and cause the generation of certain inflammatory agents like cytokines and interleukins. Ion channels are essential for coordinating cell survival and function in the central nervous system. In addition to producing action potentials, sodium and potassium currents are crucial for the release of neurotransmitters. Nano-Ag (50–100 nm) has been shown to alter the action

potential of hippocampal neurons by lowering the intracellular Na+ concentration and disrupting the voltage current.

The primary excitatory neurotransmitter in the brain, glutamate, is responsible for another process. Ag-NPs have been shown to enhance NMDA receptors. Impaired cell excitation and glutamatergic neuron degeneration are caused by these receptors. This mechanism contributes to the pathophysiology of depression. Certain consequences of prenatal exposure to Ag-NPs were more severe in females in our investigation. Therefore, sex-dependent alterations in the brain are likely linked to sex variations in brain neurotransmitters and neurochemistry. Additionally, progesterone and estrogen have neuroprotective and neuroregenerative properties and strengthen antioxidant systems. It may be one of the causes of the variations in Ag-NP toxicity across genders. All things considered, their impact may depend on the time and length of exposure, the kind of maternal administration, the amount utilized, the gender, and the age of the fetus. Ag-NP exposure therefore led to a rise in depression-like behaviors in the offspring, particularly in males. Additionally, oxidative damage, an increase in inflammatory markers, and a lack of monoaminergic transmitters are likely the primary mechanisms by which Ag-NPs induce depression. As a result, the safety of using nano silver in psychopharmacology during pregnancy is still up for debate. These results are crucial for alerting women who are already pregnant or want to get pregnant.

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