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RESEARCH PAPER

Protective and modulatory effects of royal jelly used against the induced changes in silver nanoparticles on the hippocampus of male rats

Mahsa Dalfardi [1], Mohammad Mohsen Taghavi [2], Mahdi Shariati Kohbanani [3], Zahra Taghipour [3], Reza Nosratabadi [4,5], Cyrus Jalili [6], Mohammad Reza Salahshoor [7], Ayat Kaeidi [8], Ahmad Shabanizadeh [9*]

[1]Department of Anatomy, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

[2]Department of Anatomical Sciences, School of Medicine, Social Determinants of Health Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

[3]Department of Anatomical Sciences, School of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan

[4]Department of Immunology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran

[5]Department of Immunology, Immunology of Infectious Disease Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

[6]Department of Anatomical Sciences, School of Medicine, Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

[7]Department of Anatomical Sciences, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran

[8]Department of Physiology and Pharmacology, School of Medicine, Physiology-Pharmacology Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

[9]Department of Anatomical Sciences, School of Medicine, Immunology of Infectious Diseases Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

ABSTRACT

Objective (s): Silver nanoparticles (NPs) have attracted considerable attention owing to their important properties, including antimicrobial and anti-oxidative stress effects. However, high concentrations of silver NPs have been reported to have toxic effects. The present study aimed to evaluate the modulatory and protective effects of royal jelly (RJ) against the harmful effects of silver NPs on hippocampal functions, such as learning and memory.

Materials and Methods: This experimental study was conducted on 40 male Wistar rats. The animals were divided into four groups of 10, including the control group (no silver NPs and RJ), RJ group, silver NPs plus RJ, and silver NPs. Some functions of the hippocampus (e.g., learning and memory) were evaluated using Morris memory function tests for four consecutive days. In addition, the relative expression of TRPV1 was assessed using real-time polymerase chain reaction (RT-PCR). At the final stage, hippocampal tissues were collected for histological studies.

Results: Levels of learning and memory, relative gene expression ratio of TRPV1, and the histological changes in the hippocampus were significantly different in the groups receiving silver NPs compared to the groups administered with RJ.

Conclusion: According to the results, RJ may be the effective in the protection against the adverse effects of silver NPs and improve the function of the hippocampus.

Keywords: *Hippocampus, Memory, Royal Jelly, Silver-nanoparticles, TRPV1*

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* *Corresponding Author Email: shabani54@yahoo.com*

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INTRODUCTION

Nanoparticles are fine, solid particles with dimensions of 1-100 nanometers. Silver nanoparticles (NPs) are widely used for their numerous properties. These particles are used in various sizes and shapes. In large dimensions, silver has low reactivity, while it has various properties when converted into a nanoparticle [1]. Today, colloidal silver NPs are considered to be the most common form of this metal, which are applied in nanotechnology due to their beneficial effects (antiviral, antifungal, antitumor, and antimicrobial effects). Furthermore, silver NPs are widely employed in medical, textile, food, and cosmetics sections [2]. However, the organic solvents used in the production of silver NPs are toxic, which leads to significant toxicity in these particles, thereby inducing oxidative stress and planned apoptosis [3].

Silver NPs are able to cross the blood-brain barrier and accumulate in some tissues, such as the liver, kidneys, lungs, and spleen. On the other hand, they are associated with toxicity, inflammatory responses, alterations of cellular oxidation, abnormal function, apoptosis, and tissue necrosis [4]. According to the literature, the biological activity of nanoparticles increases with the reduced size of particles [5]. Silver NPs may also alter the expression of various genes by increasing the production of active oxygen species and oxidative stress [6].

Hippocampus is one of the most important parts of the central nervous system, which is involved in the functions such as memory and learning. Hippocampal damage could lead to amnesia and weakened short-term and spatial memory [7]. The *TRPV1* receptor is a molecule that plays a key role in memory and learning processes, and any damage to this receptor in the hippocampus could lead to memory impairment. Moreover, such damage has been described in several other tissues, including the peripheral nervous system [8].

Use of natural substances containing antioxidants (e.g., herbal medicines and non-chemical products) has been shown to reduce the side-effects of drugs and their destructive effects. Such example is royal jelly (RJ), which is used for the treatment or alleviation of the complications caused by diseases and medications. RJ is a milk-white food with a fruity aroma, which is used by young bee and the queen larvae. This substance is secreted from the submandibular glands of young laborer bees [9]. RJ has remarkable nutritional value and contains compounds such as amino acids, sugars, fatty acids, and small amounts of minerals and vitamins [10]. As a beneficial nutritious ingredient, RJ has several pharmacological properties, including antimicrobial, anti-inflammatory, and antitumor effects, vasodilatation, blood pressure suppressant properties, and anti-hypercholesterolemic stimulants [11].

Several studies have indicated that RJ contains a substance known as acetylcholine, which is the main ingredient involved in the transmission of neurological messages and prevention of Alzheimer's disease and amnesia [12]. Furthermore, RJ plays a pivotal role in the differentiation of brain cells from neuronal stem cells [13]. This milk-white food and its compounds facilitate the neurogenesis processes in the hippocampus and improve the memory [14]. Due to the widespread use of silver NPs in the food and medical industries, it appears that complementary components are required to reduce the side-effects of silver NPs.

The present study aimed to explore the effects of RJ on diminishing the side-effects of silver NPs *in-vivo*.

MATERIALS AND METHODS

Preparation and characterization of particle suspensions

In accordance with the approval of the manufacturer of USA Nano, we purchased the silver NP solution with the concentration of 8,000 ppm, size of less than about 100 nanometers, and 99.98% purity from Nanophishgaman Company in Mashhad, Iran. In addition, 20 grams of the RJ vial was purchased from Pars Asal Company in Shiraz, Iran and prepared in one liter of sterilized double-distilled water in accordance with the instructions of the manufacturer [10].

Table 1. Primer Sequences Used in Study

	Primers and Their Sequences	Annealing	Size (bp)
TRPV1	F:5′-ACTCCTGACGGCAAGGATGAC-3′ R:5′-ACCCACATTGGTGTTCCAGGTAG-3′	60	81
β-actin	F:5′-ATGGTGGGTATGGGTCAGAAGG-3′ R: 5′-TGGCTGGGGTGTTGAAGGTC -3′	60	265

Experimental animals

The present study was conducted in accordance with the research ethics in animal experiments,

monitoring of the nutritional status, and principles for animal use. In total, 40 male Wistar rats weighing 200-250 grams were obtained and kept in standard light, temperature, and humidity. The animals were provided with adequate water and food for several days before the experiments.

The rats were randomly divided into four groups of 10, including the control group (no administration), RJ group (100 mg/kg of RJ), RJ and silver NPs group (30 mg/kg of silver NPs and 100 mg/kg of RJ), and silver NPs group (30 mg/kg of silver NPs).

The substances were daily prepared and administered to the animals via gavage for 28 days [15].

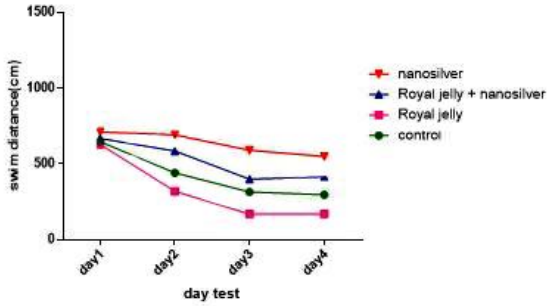

Fig 1. Mean Distance to Reach Target Platform to Evaluate Memory and Learning Processes on Days 1-4 in Experimental Groups (distance parameter to reach platform decreased significantly in RJ group compared to silver NPs groups; P <0.05)

Behavioral tests

Behavioral tests were performed in the Morris water maze in order to assess learning and memory using a dark, circular water tank with the diameter and height of 160 and 60 centimeters, respectively.

The tank was geographically divided into four quadrants. On days 1-4, the traveled distance to reach the target quarter had a resting platform and was recorded for access to the learning and memory functions of the animals [16].

Histological evaluation

After the behavioral tests, the brain tissues of the animals were fixed in 10% formalin for two days. Afterwards, five-micron coronal sections of the brain tissue were prepared for toluidine blue staining. In addition, a dark cell with a nucleus shrinkage parameter was analyzed to detect unhealthy neurons [17].

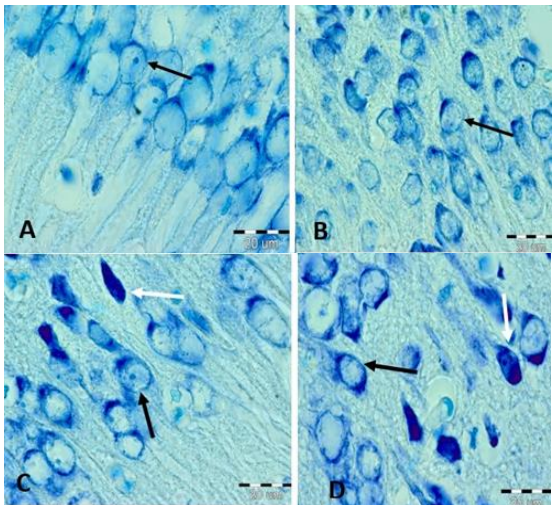

Fig 2. Coronal Sections of Hippocampal Tissues in a) Control Group; b) RJ Group, c) RJ-Silver NPs Group and d) Silver NPs Group (100X Magnification, Toluidine Blue Proprietary Staining, Black Arrow: Healthy Neurons, White Arrow: Dark Neurons)

Real-time reaction

The effects of RJ and silver NPs on the mRNA expression of *TRPV1* were evaluated using RT-PCR. Initially, 40 milligrams of hippocampal tissue was removed from the brains of the animals via surgery, and total mRNA extraction was performed in accordance with the instructions of the kit (Sinaclon Co., Tehran, Iran). The nano-drop device was also used to evaluate the mRNA quantity. Moreover, the quality of the total mRNA extracted via electrophoresis was determined using 1.5% agarose gel.

Following that, RNA was converted into cDNA in accordance with the protocol of the cDNA synthesis kit (Parstous Co., Tehran, Iran).

Real-rime reactions were performed using the ABI RT-PCR device. In addition, appropriate primers were designed for $TRPV_1$ (gene of interest) and *β-actin* (reference gene) (Table 1). The reaction was carried out in the final volume of 20 microns using a SYBR green mix master (Pars Tous Company, Tehran, Iran) [18].

Statistical analysis

Data analysis was performed in SPSS version 18, and the quantitative data were compared using one-way analysis of variance (ANOVA) and LSD test. In all the statistical analyses, P≤0.05 was considered significant.

RESULTS

Behavioral outcomes

According to the obtained results, RJ significantly improved the Morris water maze and behavioral outcomes compared to the animals administered with silver NPs ($P< 0.05$).

Fig 1 illustrates the results of the Morris water maze and behavioral tests in the study groups.

Toluidine blue staining

The neurons of the animals in the control group were characterized by a round, dominant nucleus. In the RJ group, the round and dominant nucleus of the neurons was identical to the control group, while it had higher density. In the RJ-silver NPs group, the density of the healthy neurons was higher compared to group four, while it lower than the first and second experimental group, in which the dark neurons with a dense nucleus indicated a significantly lower coloration compared to the fourth experimental group, in which the neurons were similar to the control group. On other hand, the density of the healthy neurons in the animals administered with silver NPs was lower compared to the third experimental group (Fig 2).

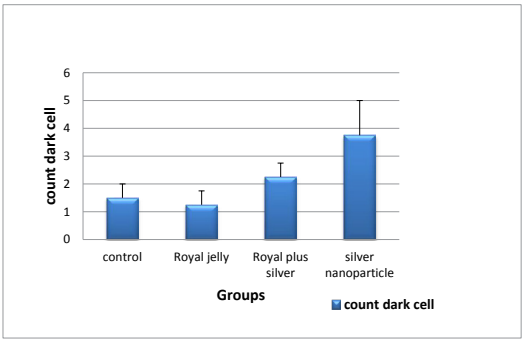

Fig 3. Comparison of Mean Number of Pyknotic and Dark Neurons (Results of neuron counting indicated that the number of the healthy neurons in the RJ group was relatively higher than the other groups. Also, the number of pyknotic and dark neurons in the groups administered with silver NPs [P=0.001] and RJ plus silver NPs [P=0.05] had a significant difference with the RJ group)

Results of neuron counting

According to the findings, silver NPs significantly increased the number of the dark cells when used alone and in combination with RJ (Fig 3).

Although RJ decreased the cytotoxicity of silver NPs, the difference was not considered significant ($P>0.05$).

RT-PCR

As is depicted in Fig 4, the relative expression of *TRPV1* significantly decreased in the animals administered with silver NPs and RJ plus silver NPs compared to the RJ and control groups.

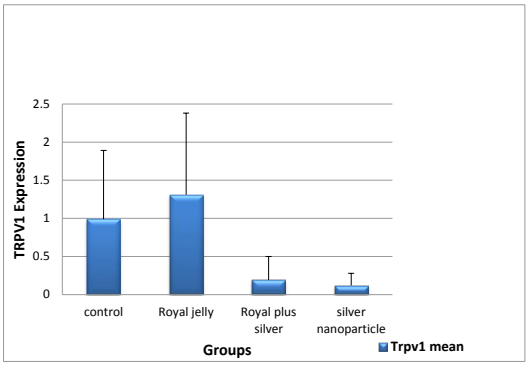

Fig 4. Comparison of TRPV1 Gene Expression in Study Groups (significant reduction in expression of TRPV1 in RJ-silver NPs group [P=0.02] and silver NPs group [P= 0.03] compared to RJ group)

DISCUSSION

The results of a previous in this regard denoted that 30 mg/kg of silver NPs could cause inflammation and necrotic effects on the hippocampus of mice. Furthermore, it was reported to disrupt DNA replication and ATP synthesis. This is the minimum effective and non-lethal dose of silver NPs [15]. In the present study (Fig 1), the distance parameter to reach the target platform decreased significantly in the RJ-silver NPs and silver NPs groups compared to the RJ group. Therefore, it could be inferred that RJ could facilitate the function of the hippocampus neurons. In contrast, the silver NPs were observed to neutralize the effects of RJ in the current research, and the neurons were damaged in the animals receiving silver NPs and those administered with RJ in combination with silver NPs. On the other hand, RJ could not reduce the effects of silver NPs, which could be due to the high penetration power of silver NPs or the low concentration of RJ.

According to the results of the present study, the number of the dark and pyknotic neurons increased significantly in the silver NPs group compared to the RJ group. Therefore, it could be concluded that silver NPs could penetrate into the hippocampus and cause cell necrosis and apoptosis. In addition, a significant difference was observed in this regard in the animals administered with RJ and silver NPs compared to the RJ group,

which indicated that the dosage of RJ in could not inhibit the harmful effects of silver NPs on the hippocampus neurons. Higher levels of RJ may protect the neurons from the toxicity induced by silver NPs.

In the current research, the expression of *TRPV1* showed that RJ could not inhibit the down-regulation of the molecules in the animals receiving silver NPs. However, the gene expression was higher in the RJ group although not significant. Therefore, it seems that RJ led to the higher expression of *TRPV1* in the hippocampus neurons, thereby enhancing the memory and learning functions, while in the animals receiving silver NPs and RJ plus silver NPs, the silver NPs decreased *TRPV1* expression, and the used concentration of RJ could not neutralize the harmful effects of silver NPs.

Another research in this regard aimed to evaluate the apoptotic effects of silver NPs at the concentrations of 100, 200, and 400 ppm. According to the findings, the effects of silver NPs on the body were dose-dependent, leading to increased apoptosis through the destruction of the blood-brain barrier and producing free radical by breaking the balance between the production of free radicals and oxidative system [19, 20].

One of the most important compounds found in RJ is 10-hydroxytrans-2-decanoic acid, which is an unsaturated acid that could easily pass through the blood-brain barrier and has similar effects to the neurotrophic factor. Moreover, the protein content of RJ has high antioxidant activity, and these proteins are able to inhibit the activity of free radicals and improve learning and memory through neurogenesis, prevention of neuronal loss, and altering *TRPV1* gene expression [21-25].

According to the findings of the current research, RJ could effectively decrease inflammatory and necrotic cells in the hippocampus through relative inhibition and interaction with silver NPs. Furthermore, it seems that silver NPs disrupt the learning process by reducing *TRPV1* expression or the function of *TRPV1* gene receptors.

CONCLUSION

According to the results, RJ could effectively protect the cells against the adverse effects of silver NPs, while higher concentrations of this compound may be required to protect the hippocampus neurons. In addition, the nano jelly shape of RJ may be associated with remarkable effects against silver NPs.

Royal nano gel could be used in combination with silver NPs in food, healthcare, and medical industries in order to diminish the adverse effects of the nanoparticles.

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