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Original article

Neurotoxic effects of silver nanoparticles and the protective role of rutin

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ABSTRACT

The toxicological studies on silver nanoparticles (Ag-NPs) have become a hot topic over the past few decades due to their unique properties on the nanoscale and widespread in many commercial products that launched into the market recently. This study was undertaken to shed light on Ag-NPs toxicity on neurotransmitters with special emphasis on the impact of concurrent administration of rutin with Ag-NPs in the experimental rats. The oral administration of Ag-NPs in rats induced brain oxidative stress, significant alterations in neurotransmitters and amino acids. Furthermore, transcriptional levels of glutamatergic N-methyl-D-aspartate (NMDA) receptors, monoamino oxidases (MAO-A, MAO-B) and metallothionein-III (MT-III) showed a significant elevation in Ag-NPs intoxicated rats. Moreover, histological examinations revealed astrogliosis and demyelination of neurons concomitant with neuronal degeneration and vacuolation. Strikingly, oral administration of rutin counterbalanced the toxic effects triggered by Ag-NPs. Taken together, our findings suggested that oral administration of Ag-NPs induced neurotoxicity in rats and rutin mitigates these effects.

1. Introduction

Undoubtedly, human became in a vicious circle of risks induced by exposure to nanoparticles (NPs; diameter < 100 nm) either from ambient air or therapeutic uses as drug delivery. There are two main types of NPs; combustion-derived NPs (e.g., particulate matters, diesel exhaust particles, and welding fumes) and manufactured or engineered NPs (e.g., titanium dioxide, carbon black, carbon nanotubes, silver, zinc oxide and copper oxide) [1].

Silver nanoparticles (Ag-NPs) are one of the fastest-growing product categories due to their excellent antimicrobial properties and commonly used in a myriad of applications including water disinfection, the coating of refrigerators, cosmetics, wound dressing, baby bottles, food containers and household goods [2]. Although various organs can get rid of Ag-NPs, these nanoparticles tend to reside for a considerable time and exhibit a longer half-life within the CNS rather than other organs, thereby causing neural damages following prolonged exposure [3].

The brain considered one of the most susceptible organs to oxidative stress-induced damage because of its high oxygen consumption, relatively high concentration of iron and ascorbates that carry out the radical generating fenton reaction. With respect to blood-brain barrier (BBB) function in safeguarding the brain from harmful endogenous and exogenous substances circulating in the blood and restricts the entry of most therapeutic agents [4], Ag-NPs have been shown to enter the brain by crossing the BBB inducing BBB dysfunction, astrocyte swelling and neural degeneration [5].

The neurotransmitters are endogenous chemical messengers-stored in synaptic vesicle- that conduct information throughout the body and mediating signal states between nerve cells and other cells through tuning the signals [6]. They are released from presynaptic nerve terminals by exocytosis through the fusion of synaptic vesicles with the plasma membrane of nerve terminal [7].

Amino acids play a vital role in information transmission between neurons like the role of neurotransmitters in the central nervous system where there are two types of neurotransmitters, excitatory such as glutamate and aspartate (Asp) while inhibitory ones including γ-aminobutyric acid (GABA) and glycine (Gly). However, the imbalance between these two types caused deregulation of intracellular calcium pathway, intracellular calcium overload, which eventually leads to mitochondrial dysfunction, ROS release and neuronal death [8].

Glutamate (Glu) is the key excitatory neurotransmitter implicated in many neural functions such as learning and memorizing. Under normal status, the level of Glu and GABA in

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synaptic cleft are maintained at a low level to prevent sustained activation of their receptors which mostly related to neurotoxicity [7]. The Na^+-coupled neurotransmitter transporters are paramount players in the synaptic neurotransmission termination where they allow the amino acid neurotransmitters uptake by cytosol through the Na^+/K^+ electrochemical gradients across plasma membrane which represents the driving force for neurotransmitters release [9].

Dopamine is a major neurotransmitter in brain′s neural circuits as it involved in learning, reward, emotion and motor control where its depletion results in movement disorders. Moreover, nor-epinephrine exerts its neuromodulatory effects on synaptic transmission [10].

The characteristic features of synaptic currents are maintained through specific ionotropic glutamatergic N-methyl-D-aspartate (NMDA) receptors which orchestrate synaptic information competence and cell excitability, and changes in these currents will impact upon neuronal activity in both physiological and pathological status [11].

Monoamino oxidases (MAOs) are flavoenzymes located on the outer membrane of mitochondria; they catalyze the oxidative deamination of biogenic amines such as dopamine, serotonin, and nor-epinephrine. They are classified into two subtypes; MAO-A and MAO-B where the regulation of MAOs activities was important for the treatment of neurodegenerative disease [12].

The exposure to Ag-NPs induced hepatotoxicity and changes in blood chemistry beside neurotoxic effect as a result of generating oxidative stress [13] where the antioxidant status become exhausted [14]. Also, the deposition of Ag-NPs in primary mixed neural cells acts as a catalyst and produce reactive oxygen species (ROS) that induced neuronal oxidative damage [15].

In the recent years alleviating the harmful effects of most therapeutic agents through herbal compounds has captured a lot of attention. Rutin (quercetin-3-O-rutinoside) is – a member of bioflavonoids and also called vitamin P- considered a common dietary flavonol glycoside, composed of quercetin and disaccharide rutinose with some established pharmacological effects thanks to its antioxidant, anti-inflammatory, anti-carcinogenic and antiviral properties [16]. It attaches to the iron ion, preventing it from binding to hydrogen peroxide which would otherwise create a highly reactive free radical that may damage cells [17].

There are many food sources provide human with rutin including, tartary buckwheat seeds, asparagus, red pepper, apples, cherries, aronia berries, citrus fruits and leaves of many herbs such as rue, rosemary and green tea [18]. It has an effective role in retarding memory dysfunction resulting from hippocampal pyramidal neuron loss due to its ability to cross the BBB in trimethyltin toxicity [19]. Also, it has antidepressant activity mediated by its interaction with α 2-adrenoreceptors [20].

It is imperative to give special attention to the neurotoxic effect of Ag-NPs especially with the vast daily use in consumer products and the dearth of data which focused on their effect on neurotransmitters, our study aimed to evaluate to what extent the rutin could combat the neurotoxic effect arising from Ag-NPs intoxication in rats.

2. Material and methods

2.1. Chemicals

Silver nanoparticles nano powder (Ag-NPs), CAS registry number 576832, purity 99.5%, surface area 5.0 m^2/g with diameter < 100 nm, stabilized and coated with Poly Vinyl Pyrrolidone as manufacturer's suggestion to prevent their sedimentation and agglomeration, thereby maintaining their dispersed form, rutin hydrate [($C_{27}H_{30}O_{16}$. xH_2O), CAS registry number 207671-50-9, purity ≥ 94%], NADPH, epinephrine, reduced glutathione and DTNB (5,5 dithiobis 2-nitrobenzoic acid) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethylenediaminetetraacetic acid (EDTA), glacial acetic acid, potassium dichromate, diethyl ether, hydrochloric acid and hydrogen peroxide were purchased from El-Nasr Company (Cairo, Egypt).

2.2. Animals and experimental design

Male Wistar rats from the Animal House in Faculty of Veterinary Medicine, Zagazig University, Sharkia, Egypt were used throughout the study. They were left for two weeks in standard cages with12 h/12 h dark light cycle and free access to water and food for adaption. All the experimental protocols were approved by the Ethics Committee of Faculty of Veterinary Medicine, Zagazig University, in accordance with the guiding principles of The European Community Directive (86/609/EEC) on animal care. Thirty-six adult male rats (140–150 g) were randomly assigned into four groups (9 rats per group).Group I (control group) rats received normal saline. Group II (rutin-treated group) rats received orally 50 mg/kg body weight rutin once daily and this protective dose improved rat's episodic memory deficits [21]. Also, this dose was protective against haloperidol- induced orofacial dyskinesia and associated neurochemical changes [22]. Group III (Ag-NPs – intoxicated group) rats received orally dose of 30 mg/kg body weight Ag-NPs dissolved in distilled water once daily [23] and the dosing volume was 2 ml/kg body weight. This toxic dose has a serious impact on synaptic plasticity of the hippocampus and spatial cognition in rats [24]. The oral route of Ag-NPs administration was applied due to extensive use of Ag-NPs in food packaging materials[25]. Group IV (Rutin + Ag-NPs treated group) rats received rutin and Ag-NPs concomitantly at the same dose and route of group2 and 3.All treatments were given orally for eight weeks. Every day, the suspensions were prepared freshly before the administration.

2.3. Sampling and biochemical analysis

Rats from different groups were killed by decapitation after the end of experimental period. The brains were dissected, rinsed with sterile physiological saline (0.9%). Amino acids concentrations in brain tissues were determined in triplicate/group using Sykam amino acid analyzer (Sykam S334D, Sykam GmbH, Germany) after acid hydrolysis using 6N HCL for 24 h at 100 °C [26] in sealed glass tubes following the manufacturer's instructions. For antioxidant status, brain samples from different groups were homogenized as previously described [27]. In brief, one gram of brain tissue was weighed and homogenized in 0.1 M chilled potassium phosphate buffer (PH 7.4) using Potter-Elvehjem tissue homogenizer for 5 min. The homogenate was centrifuged at 14000 rpm at 4 °C for 15 min to obtain supernatant that used for evaluate the concentration of malondialdehyde-lipid peroxidation marker- (MDA) [28], reduced glutathione (GSH) [29] with catalase (CAT; EC 1.11.1.6) [30], superoxide dismutase (SOD; EC 1.15.1.1) [31] and glutathione peroxidase (GPX; EC 1.11.1.9) [32] activities at Shimadzu spectrophotometer (UV120-02).The concentration of mono amino neurotransmitters (dopamine, serotonin, and nor-epinephrine) and GABA were measured in brain homogenates. Quantitative measurement of dopamine was done using ELISA immunoassay kit, a product of Cusabio (China) following the manufacturer's instructions. The level of serotonin and nor-epinephrine were measured using a specific rat sandwich enzyme immunoassay technique (MyBioSource, San Diego, CA, USA). Rat specific ELISA kit provided from EIAab (China) was used for GABA quantitation in brain homogenate. A small portion of brain samples was harvested, snap frozen by immersion in liquid nitrogen and