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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

kept at −80 °C to follow up the transcriptional level of glutamatergic NMDA receptors (NR1, NMDAR2A, NMDAR2B), MAO-A, MAO-B and metallothionein – III(MT-III).

2.4. Histopathological examination

Rats were sacrificed and brain tissues were harvested from different groups after eight weeks. The samples were fixed in 10% buffered neutral formalin, dehydrated with ethanol, infiltrated and embedded in paraffin. The thickness of paraffin section is five μm that routinely examined under a light microscope after stained with hematoxylin and eosin dye [33].

2.5. Real-time PCR

Total RNA of brain samples was extracted using RNeasy mini kit (Qiagen) following the guidelines of manufacturer's instructions. The high purity of RNA samples was checked using NanoDrop spectrophotometer (NanoDrop technologies, Wilmington, Delaware, USA). Then, 1 μg of total RNA was used to produce cDNA as previously described [34]. Real-time quantitative PCR was done using SYBR-green detection (Applied Biosystems) where 20 μl PCR reaction mixture was made by using 100 ng cDNA, 1 μm forward and reverse primer with 10 μl SYBR premix EXtaq and complete the volume using RNase-free water. The amplification reaction was carried out using Rotor-Gene Q2 plex (Qiagen Inc., Valencia, CA, USA). The primer sequence and PCR amplification condition are given in Table 1 with initial denaturation temperature at 95 °C for 10 min and final extension temperature at 72 °C for 5 min. The 2-DDct method used for calculates the relative quantitation of each transcript in three replicates with regard to β-actin mRNA level [35].

2.6. Statistical analysis

Data are expressed as mean values± standard error (SE) of nine rats and performed using one-way analysis of variance (ANOVA) through SPSS statistical version 21 following Duncan's test for making comparisons among groups.

3. Results

3.1. The detrimental role of Ag-NPs toxicity on brain amino acids concentration and the modulatory effect of rutin

As shown in Table 2, the content of brain Asp and Glu amino acids were significantly ($p \leq 0.05$) increased in Ag-NPs intoxicated group when compared with control rats. Meanwhile, the concentration of brain Gly, threonine (The), serine (Ser), proline (Pro), methionine (Met), tyrosine (Tyr), histidine (His) and phenylalanine (Phe) were noticeably ($p \leq 0.05$) decreased in Ag-NPs intoxicated group when compared with control groups. It is worthy noted that administration of rutin with Ag-NPs showed a significant ($p \leq 0.05$) improvement in all the above-examined amino acids in comparison with animals administered Ag-NPs. Additionally, the concentration of branched chain amino acids [valine(Val); leucine (Leu) and isoleucine(Ile)], alanine (Ala), lysine (Lys) and arginine (Arg) showed no significant changes among experimental groups.

3.2. The antioxidant status in brain homogenate after Ag-NPs toxicity and the role of rutin

There is paradigm imbalance between oxidative and antioxidant parameters in brain homogenate after Ag-NPs toxicity indicated by a statistically significant ($p \leq 0.05$) increase in brain MDA concentration with a significant decrease in GSH level of rats intoxicated with Ag-NPs when compared with control rats (Table 3). Also, the brain activities of CAT, SOD, and GPX showed a significant ($p \leq 0.05$) decrease in Ag-NPs treated rats. Interestingly, the Ag-NPs intoxicated rats treated with rutin significantly returns these changes to control values confirming the antioxidant role of rutin.

3.3. Brain neurotransmitters concentration and Ag-NPs toxicity with special emphasis on the effect of rutin

Table 4 recorded a significant ($p \leq 0.05$) decrease in mono amino neurotransmitters (dopamine, serotonin, and nor-epinephrine) in Ag-NPs intoxicated group when compared with control groups. Moreover, the concentration of GABA showed a significant ($p \leq 0.05$) decrease in Ag-NPs intoxicated rats when compared with control rats. However, the ameliorative role of rutin against Ag-NPs neurotoxicity indicated by a significant ($p \leq 0.05$) enhancement of these values to control levels.

3.4. The effects of Ag-NPs toxicity and rutin on the transcriptional levels of glutamatergic NMDA receptors, MAO-A, MAO-B and MT-III

The brain mRNA levels of NR1, NMDAR2A and NMDA R2 B revealed a statistically significant increase ($p \leq 0.05$) in Ag-NPs intoxicated group when compared with control rats. Meanwhile, the co-administration of rutin with Ag-NPs returned these levels control values (Fig. 1A–C). Also, the transcriptional levels of MAO-

Table 1
Specific primer and real-time PCR conditions.

Gene name	Accession number	Primer sequences 5′ → 3′	Reaction conditions
NR1	L08228	F-CTCATCTCTAGCCAGGTCTA R-TCGCATCATCTCAAACCAGAC	94 °C–30s/55 °C–1 min/72 °C–1 min (33cycle)
NMDAR2A	M91561	F-ACTCCACACTGCCCATGAAC R-TTGTTCCCCAAGAGTTTGCTT	94 °C–30s/55 °C–1 min/72 °C–1 min (33cycle)
NMDAR2B	M91562	F-GTTTGATGAAATCGAGCTGGC R-TCCAGTTCCTGCAGGGAGTT	94 °C–30s/55 °C–1 min/72 °C–1 min (33cycle)
MAO-A	NM_033653.1	F- GCCAGGAACGGAAATTTGTA R-TCTCAGGTGGAAGCTCTGGT	95 °C–15s/60 °C–30s/72 °C–60 s (40 cycle)
MAO-B	NM_013198.1	F-TGGGAAGATTCCAGAGGATG R-GCTGACAAGATGGTGGTCAA	95 °C–15s/60 °C–30s/72 °C–60 s (40 cycle)
MT-III	NM_053968.3	F-ATGGACCCTGAGACCTGCCCCTGT R-TCACTGGCAGCAGCTGCATTTCTCG	94 °C–60s/60 °C–1 min/72 °C–1 min (25cycle)
β-actin	BC063166.1	F-ACCACAGCTGAGAGGGAAATCG R- AGAGGTCTTTACGGATGTCAACG	94 °C–45s/59 °C–44s/72 °C–60 s (30 cycle)

F indicates a forward primer; R indicates a reverse primer.

Table 2
Effects of silver nanoparticles (Ag-NPs) toxicity and/or rutin administration on brain amino acids concentration in experimental rats.

Amino acids (mg/g tissue)	Control group	Rutin-treated group	Ag-NPs intoxicated group	Rutin + Ag-NPs treated group
Asp	7.61 ± 0.08^{c}	7.31 ± 0.09^{b}	8.07 ± 0.06^{a}	7.42 ± 0.07^{b}
The	4.51 ± 0.02^{b}	4.71 ± 0.01^{a}	4.24 ± 0.02^{c}	4.43 ± 0.03^{b}
Ser	6.12 ± 0.01^{a}	5.96 ± 0.02^{a}	5.45 ± 0.02^{b}	6.08 ± 0.01^{a}
Glu	8.92 ± 0.08^{b}	7.99 ± 0.06^{c}	9.12 ± 0.08^{a}	8.85 ± 0.05^{b}
Pro	0.53 ± 0.01^{b}	0.61 ± 0.01^{a}	0.56 ± 0.01^{b}	0.52 ± 0.01^{b}
Gly	3.11 ± 0.06^{b}	3.51 ± 0.02^{a}	2.01 ± 0.03^{b}	3.32 ± 0.01^{ab}
Ala	10.51 ± 0.07^{a}	10.53 ± 0.06^{a}	10.52 ± 0.07^{a}	10.54 ± 0.08^{a}
Val	5.12 ± 0.01^{a}	5.13 ± 0.01^{a}	5.14 ± 0.02^{a}	5.11 ± 0.02^{a}
Met	1.62 ± 0.008^{b}	1.83 ± 0.01^{a}	1.27 ± 0.03^{c}	1.58 ± 0.01^{b}
Ile	4.78 ± 0.05^{a}	4.75 ± 0.03^{a}	4.77 ± 0.05^{a}	4.75 ± 0.05^{a}
Leu	8.68 ± 0.06^{a}	8.65 ± 0.02^{a}	8.63 ± 0.03^{a}	8.87 ± 0.04^{a}
Tyr	3.31 ± 0.02^{b}	3.59 ± 0.02^{a}	2.73 ± 0.09^{c}	3.38 ± 0.02^{ab}
Phe	3.62 ± 0.01^{b}	3.99 ± 0.02^{a}	2.91 ± 0.03^{c}	3.79 ± 0.02^{b}
His	3.57 ± 0.01^{b}	3.68 ± 0.02^{a}	3.09 ± 0.01^{c}	3.64 ± 0.04^{ab}
Lys	8.61 ± 0.06^{ab}	8.72 ± 0.05^{a}	8.56 ± 0.07^{b}	8.68 ± 0.05^{ab}
Agr	7.34 ± 0.03^{a}	7.33 ± 0.04^{a}	7.35 ± 0.02^{a}	7.32 ± 0.03^{a}

Values are the mean ± SE in each group.
[a,b,c] The means within the same column and bearing different superscripts are significantly different at $p < 0.05$.

Table 3
Effects of silver nanoparticles (Ag-NPs) toxicity on brain antioxidant status and the role of rutin in experimental groups.

Treatments	Control group	Rutin-treated group	Ag-NPs intoxicated group	Rutin + Ag-NPs treated group
MDA (μmol/g tissue)	33.39 ± 1.21^{c}	28.67 ± 1.13^{b}	48.56 ± 2.23^{a}	32.98 ± 1.23^{c}
GSH (mg/g tissue)	19.61 ± 1.76^{b}	27.36 ± 1.95^{a}	12.98 ± 1.21^{c}	19.56 ± 2.02^{b}
CAT (μmol H_2O_2 decomposed/g tissue)	51.67 ± 1.88^{b}	62.14 ± 2.35^{a}	36.87 ± 2.61^{c}	53.45 ± 1.45^{b}
SOD (U/g tissue)	21.34 ± 0.89^{b}	28.07 ± 0.58^{a}	12.35 ± 1.54^{c}	20.63 ± 1.77^{b}
GPX (μmol NADPH/g tissue)	11.33 ± 0.81^{ab}	15.64 ± 1.21^{a}	4.32 ± 0.76^{c}	10.08 ± 1.15^{b}

Values are the mean ± SE in each group.
[a,b,c] The means within the same column and bearing different superscripts are significantly different at $p < 0.05$.

Table 4
Effects of silver nanoparticles (Ag-NPs) toxicity and/or rutin supplementation on brain mono amino neurotransmitters and gamma aminobutyric acid (GABA) levels in experimental rats.

Treatments	Control group	Rutin-treated group	Ag-NPs intoxicated group	Rutin + Ag-NPs treated group
Dopamine (μg/g tissue)	1.75 ± 1.09^{b}	2.86 ± 0.98^{a}	0.53 ± 0.06^{c}	1.77 ± 1.11^{b}
Serotonin (μg/g tissue)	7.72 ± 1.31^{b}	8.88 ± 1.23^{a}	5.86 ± 1.12^{c}	7.68 ± 1.28^{b}
Nor-epinephrine (μg/g tissue)	1.28 ± 0.09^{b}	2.17 ± 0.75^{a}	0.79 ± 0.03^{c}	1.26 ± 0.13^{b}
GABA (μg/g tissue)	3.39 ± 0.18^{b}	4.72 ± 0.33^{a}	2.19 ± 0.17^{c}	3.42 ± 0.19^{b}

Values are the mean ± SE in each group.
[a,b,c] The means within the same column and bearing different superscripts are significantly different at $p < 0.05$.

A, MAO-B, and MT-III exhibit a significant increase ($p \leq 0.05$) in Ag-NPs intoxicated rats when compared with control group and the co-treated of rutin with Ag-NPs succeeded to return these values to control one (Fig. 2A–C).

3.5. Histopathological findings in brain after Ag-NPs toxicity and rutin treatment

The brain sections from control and rutin administered group revealed normal histological architecture and structure of the neurons and neuropil (Fig. 3a).Brain section of Ag-NPs intoxicated rats (Fig. 3b) portrayed severe congestion, perivascular edema (arrow) and hemorrhage of blood vessels (arrowhead) with neuronal degeneration (zigzag) and vacuolation (V). Moreover, astrogliosis (arrow) and demyelination of neurons (D) were recorded in the same previous group (Fig. 3c).On the other hand, co-administration of rutin with Ag-NPs improved the histopathological changes indicated by very mild demyelination (arrow), few extravasated erythrocytes (zigzag) (Fig. 3d), mild vacuolation (arrow, arrowhead) (Fig. 3e).

4. Discussion

The exacerbated risks arising from long-term retention of Ag-NPs in the brain despite its clearance from the most organs such as liver and the broad range of their applications have drawn a lot of our attention to the importance of resorting to natural products that lessen their neurotoxic effects.

Ag-NPs evoked their neurotoxicity through three main pathways, oxidative stress, mechanical damage and increase cytoplasmic Ca^{+2} [36].The oxidative stress induced toxicity of Ag-NPs relies on different mechanisms, the first depends on the liberation of Ag ions which interact with molecular oxygen and oxidized to silver oxide [37] that interacted with sulfur containing proteins as consequence to its high affinity to thiol group [38] resulting in formation of protein corona and alter protein function relationship of mitochondrial inner membrane [39]. Meanwhile, the second mechanism involved decrease in the activity of complex I, II, III and IV of mitochondrial respiratory chain leading to drop of ATP levels and increased rates of ROS [40]. Hence, diminishing the level of

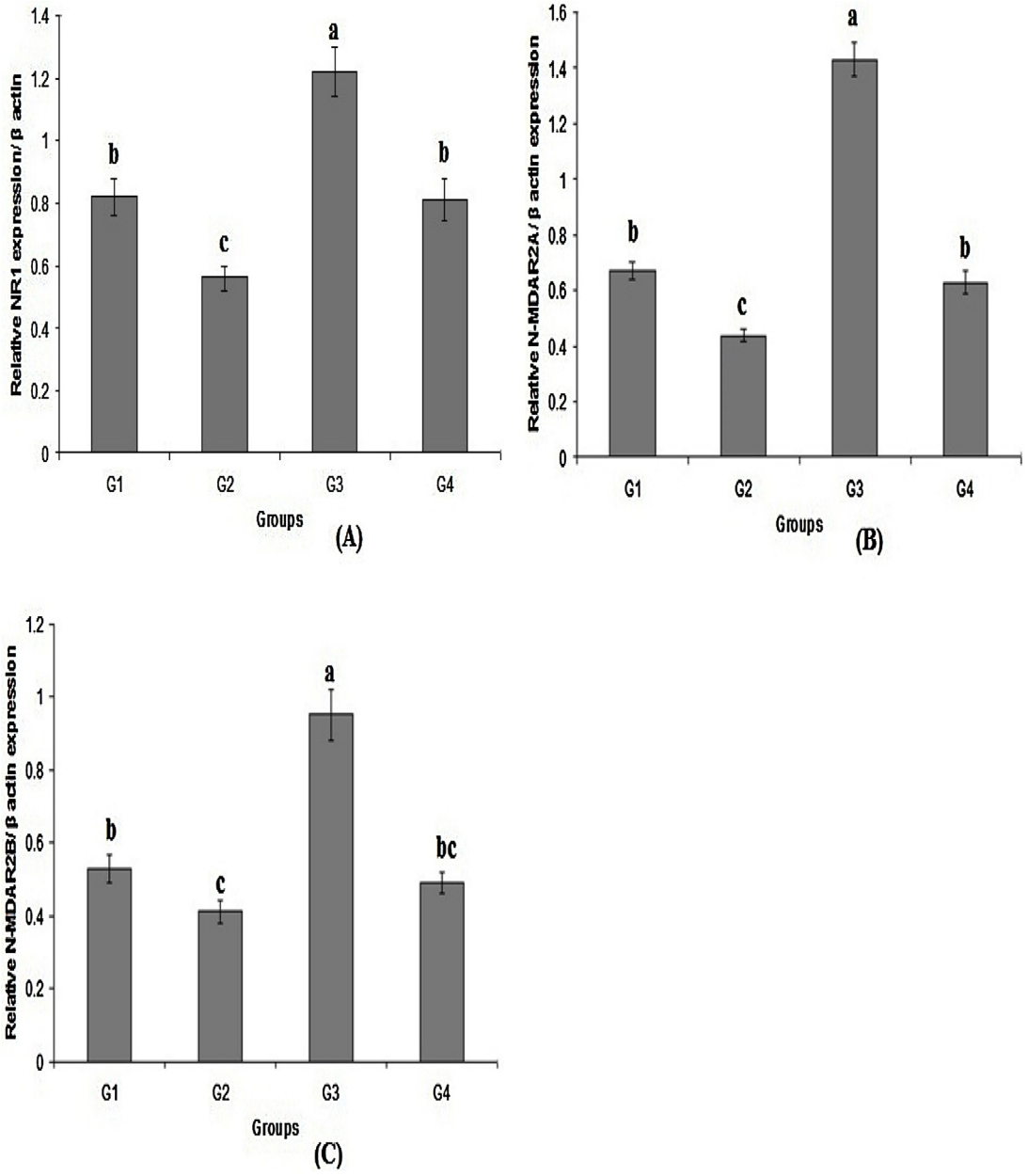

Fig. 1. Quantitative real time-PCR analysis of glutamatergic N-methyl-D-aspartate receptors [NR1(A), NMDAR2A(B) and NMDAR2B(C)] expression in the brain of rats of control group (G1), rutin treated group (G2), Ag-NPs intoxicated group (G3) and co-administered rutin with Ag-NPs treated group (G4) for eight weeks as described in the Material and Methods section. Total RNA was extracted and reverse transcribed (1 μg) to form cDNA followed by PCR amplification. Data analysis was carried out on three different rats. Values are statistically significant at $p < 0.05$.

ROS considered a possible candidate for elucidation of Ag-NPs toxicity and therapeutic agents.

The oxidative stress of Ag-NPs represented in depletion of GSH level, increase MDA level in rat's liver [38] and reduction of level of glutathione peroxidase gene expression in PC12 cells which clearly demonstrated their effect on glutathione system [13].

Initially, the brain is the most vulnerable to damage and oxidative stress by Ag-NPs exposure due to its high energy demand with high lipid and protein content [15].

In our study, we found that the oral administration of Ag-NPs evoked neurotoxicity indicated by increasing excitatory amino acids neurotransmitters (Glu and Asp) and decreasing the inhibitory ones (GABA and Gly).

The brain amino acids neurotransmitter imbalance was induced after chronic intoxication of rats with silver, copper and aluminum nanoparticles indicated by a significant increase in excitatory amino acids neurotransmitter (Glu and Asp) with a sharp decrease in inhibitory ones (Gly and GABA) and this confirmed the brain damage and edema triggered by these engineered nanoparticles [41].

The downstream level of brain mono amino neurotransmitters (dopamine, serotonin, and nor-epinephrine) after Ag-NPs toxicity was confirmed by Hadrup et al. [42] who investigated the alteration in dopamine, serotonin and nor-epinephrine in the brain homogenate of rats orally given Ag-NPs for 14 days with similar effect of ionic silver.

The active transport of acetylcholine, Glu, monoamines, GABA/glycine across synaptic vesicles, which are the acidic component of nerve terminal, are depended mainly on the proton gradient generated by V-ATPase that pump protons inside vesicle and this

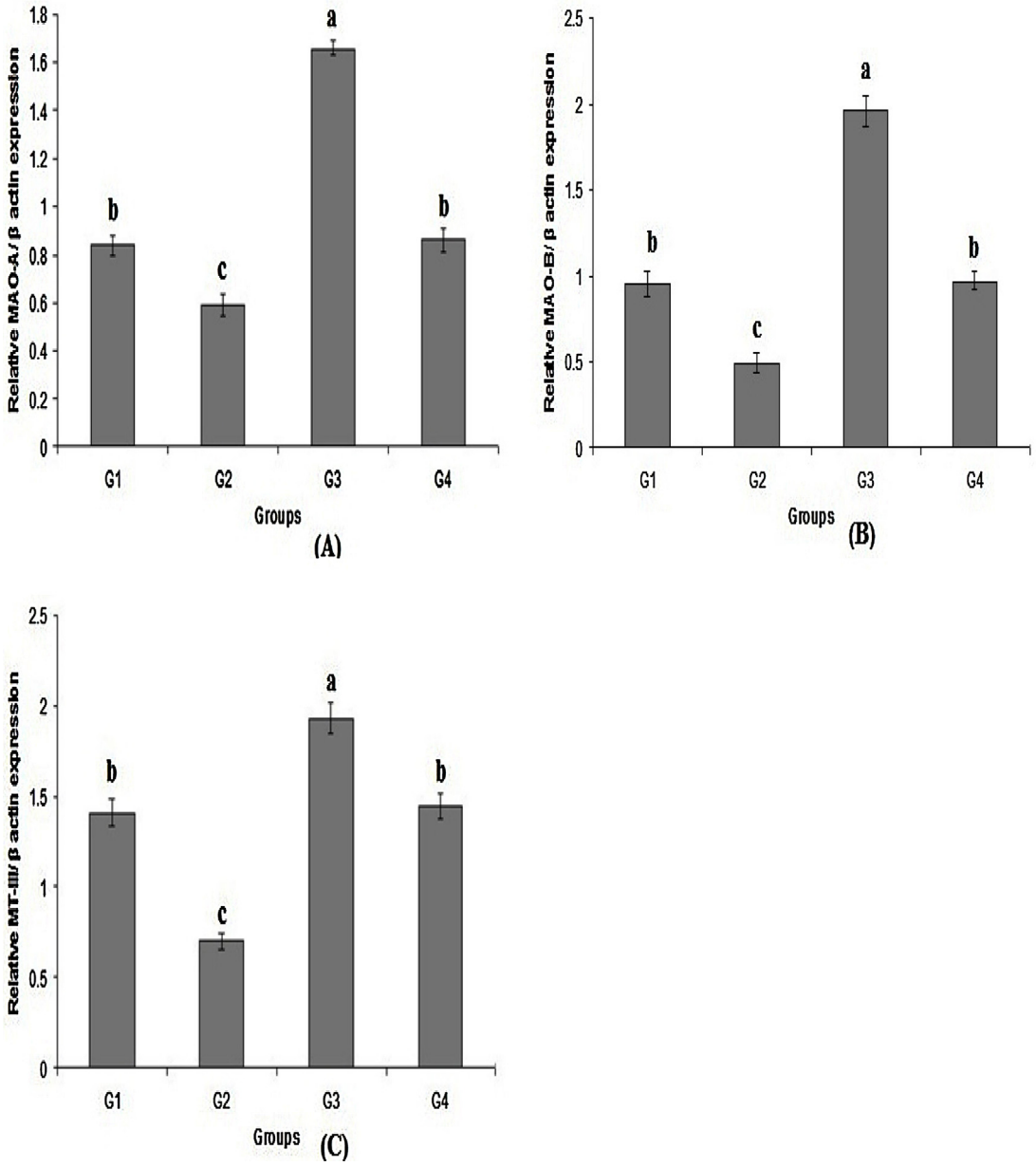

Fig. 2. Quantitative real time-PCR analysis of monoamino oxidases [MAO-A(A), MAO-B(B)] and metallothionein-III [MT-III(C)] expression in the brain of rats of control group (G1), rutin treated group (G2), Ag-NPs intoxicated group (G3) and co-administered rutin with Ag-NPs treated group (G4) for eight weeks as described in the Material and Methods section. Total RNA was extracted and reverse transcribed (1 μg) to form cDNA followed by PCR amplification. Data analysis was carried out on three different rats. Values are statistically significant at $p < 0.05$.

mechanism confirm that the lower the proton gradient in vesicles, the easier release of neurotransmitters through exocytosis. The reduction in acidification of synaptic vesicles and low activity of Na^+ coupled neurotransmitter transporters of Glu are the triggers for extracellular glutamate augmentation without effect on action potential of nerve terminal membrane [7]. Moreover, transportation of mono amino neurotransmitters after Ag-NPs toxicity depends mainly on voltage-gated sodium currents which have been reduced as a result of decreasing Na^+ influx [11].

Neural synaptic transmission requires normal neural cell membrane to allow passage of Ca^{+2}, Na^+, K^+ and CL^- but the smaller sized Ag-NPs and/or released ions disrupted the nerve cell permeability and increase intracellular calcium concentration [43]. The reduction of ATP within neurons disrupts maintenance of pumps regulating voltage and ionic gradient across cell membranes especially Na^+/K^+ ATPase which leads to partial depolarization that allows normally inert Glu to over stimulate glutamatergic NMDA receptors, increase Ca^{+2} influxes, up-regulate nitric oxide synthase activity and ROS production [44].

Ag-NPs markedly increase excitatory synaptic transmission in a dose dependent manner through presynaptic Ca^{+2} influxes which trigger neurotransmitters release in rat CA1 pyramidal neurons [11].

The higher level of extracellular glutamate in the brain induced neuronal death mainly through over-activation of glutamatergic NMDA receptors [45]. On the same ground, Zieminska and Struzynska [46] recorded that Ag-NPs induced over-activation of these receptors in CGCs in response to elevated level of extracellular glutamate. These results may be explained by the ability of Ag-NPs to increase intracellular zinc level where it released into the synaptic space and activate units of glutamatergic NMDA receptors [43]. Also, Ag-NPs can directly interact with

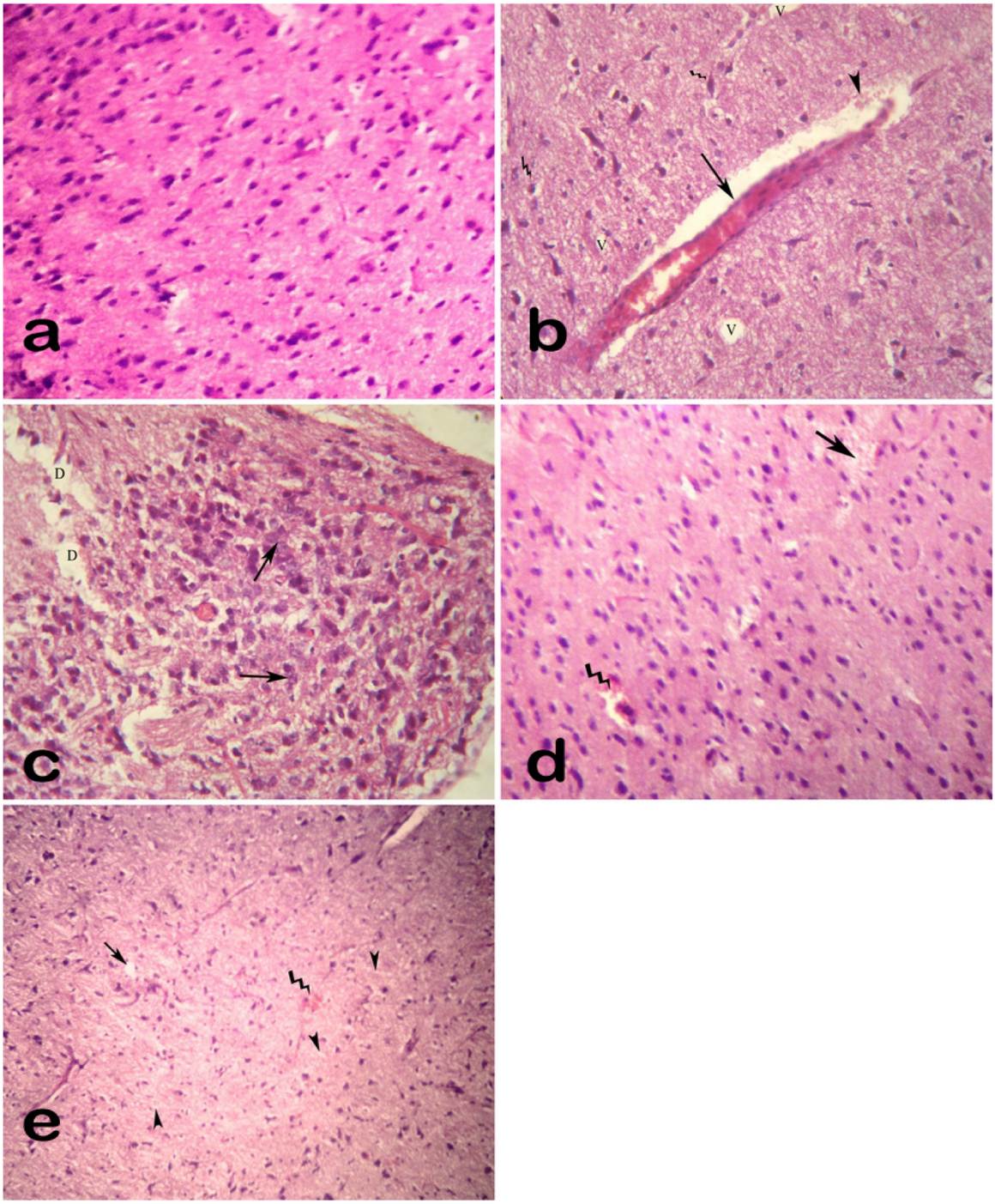

Fig. 3. Photomicrograph of rat's brain section stained with hematoxylin and eosin of control and rutin treated groups show normal neuron architecture and neuropil (a).The brain of silver nanoparticles (Ag-NPs) intoxicated group (b) shows perivascular edema (arrow) with neuronal degeneration (zigzag) and vaculation (V). Neuronal demyelination (D) and astrogliosis are record in Ag-NPs intoxicated group (c) while, the brain's section co-administered rutin with Ag-NPs (d) reveals mild demyelination (arrow) and few extravasated erythrocytes (zigzag), mild vacuolation (arrow, arrowhead) (e).

calcium responses and subsequently disrupt neural synaptic transmission with inhibition of neuronal branches sprouting and elongation [47].

Impairment of Glu uptake by glutamate transporter leads to abnormal increase in glutamatergic NMDA receptors in hippocampus which act as a compensatory way to the reduction in the number of functional receptors that lead to blemish in spatial learning. The highly increased Glu synthesis, reduced uptake or defect in glutamate receptors on post synaptic membrane may be a marker for neurotransmission changes after Ag-NPs administration [1]. The de novo synthesis of Glu is mainly occurred in astrocytes through using glutamine. Metabolically, Glu and glutamine shuttle between astrocytes and neurons through glutamate-glutamine cycle and the significance of this cycle is twofold. First, Glu is easily recycled from glutamine. Second, the relative inert glutamine rather than the excitatory Glu is returned back to neuron [48].

As healthy cytoskeleton of neurite plays an effective role in the trafficking and localization of synaptic neurotransmitters and receptor proteins. Alteration in hippocampal synapses structure

and their protein after prolonged exposure to oral Ag-NPs may suggest neurotransmission disturbance and predict impairment of cognitive processes [49].

One of the suggested mechanisms of rutin as antioxidant involved its ability to scavenge free radicals potentiates the antioxidant capacity of vitamin E and vitamin C [50] and increased GSH level with endogenous antioxidant enzymes activities [51]. Additionally, dopamine oxidation by MAO leads to release of hydrogen peroxide and superoxide anion which conjugated with ferrous ions through fenton reaction forming hydroxyl free radicals and induced lipid peroxidation [52].

The significant escalation in antioxidant enzyme activities (CAT, SOD and GPX) and GSH concentration with the reduction of MDA level in rutin co-treated with Ag-NPs group were attributed to a glycon quercetin in rutin that improved chelation of free radicals and metal ions especially ferrous ions with decreased the dopamine turnover through MAO inhibition [22].

Exposure of rats to Ag-NPs causing accumulation of Ag in the brain cells resulting in a glitch in expression of genes involved in neural function indicated by a significant up regulation of MAO-A and MAO-B mRNA. Controversially, co-administration of rutin with Ag-NPs in rats exhibits a significant decline in these levels to control values.

Our previously mentioned results deucedly paved to the mechanism of treatment, which relies on increasing antioxidant status and inhibiting MAO activities that may have great therapeutic potential against neurodegenerative diseases [53].

Regarding to MT-III gene expression, our study detected up regulation of its mRNA in Ag-NPs intoxicated group, we owed this result to the higher affinity of Ag-NPs to MTs than zinc ions which liberated from zinc-containing proteins and activated the metal regulatory transcription factor 1 that binds to metal response elements in the MT genes and induced MT synthesis [54]. Another elucidation of MT-III mRNA up regulation is that Ag-NPs induced oxidative stress which stimulates binding of the transcription factor Nrf1- member of the vertebrate cap ′n′ collar transcription factor- to the antioxidative response element within the MT gene [55].

On the level of the histopathological findings, we noticed structural alteration represented by perivascular edema and demyelination of neurons due to generation of ROS after Ag-NPs administration, which caused damage to cellular components such as membranes and induced apoptosis in the neural cells, immediately after crossing the BBB.

The presence of brain edema and neurotoxicity as a result of Ag-NPs exposure significantly inhibit biosynthetic processes in astrocytes and may decrease the secretion of important nutrients and signal factors [56].

There is convincing evidence about the betterment of dopamine biosynthesis in rutin co-treated with Ag-NPs rats through up regulation of anti-apoptotic genes and tyrosine hydroxylase activity with down regulation of apoptotic genes [57,58]. Also, rutin has been reported to induce inhibitory effects on neuronal necrotic pathways and this nominated it to be a potential promising neuroprotective agent [59]. Additionally, it reduced infarct size and neurological defects in rats after middle cerebral artery occlusion as well as protected the activities of brain antioxidants enzymes and dopaminergic neuron by directly suppress oxidative damage and inflammatory response [60].

5. Conclusion

We surmise from our study that, although the boom enormous field of Ag-NPs therapeutic uses, their toxicity has been neglected. Imbalance in brain neurotransmitters, oxidative stress, potential neuronal damage and robust alteration in glutamatergic NMDA receptors gene expression confirmed these neurotoxic hazards. Impressively, the use of rutin overwhelms these toxic effects through improving the brain antioxidant status, correcting the neurotransmitters imbalance. These roles confirmed its therapeutic potential against neurotoxic effect of Ag-NPs.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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