RESEARCH PAPER

Association Between Silver Nanoparticle Dose and Brain or Renal NF-κB Gene Expression

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ARTICLE INFO

Article History:
Received 28 August 2024
Accepted 20 December 2024
Published 01 January 2025

Keywords:
AgNP
CNS
NF-κB Gene
Renal abnormalities

ABSTRACT

Several studies have claimed that AgNPs' safety is not completely verified. The current work studied the CNS and renal genetic abnormalities (as manifested by modifications in NF-κB transcription) in mice treated with diverse AgNP concentrations. The findings revealed that as the amount of AgNPs was elevated, NF-κB production increased considerably.

How to cite this article
Nasir G., Najm M., Mahmood H. Association Between Silver Nanoparticle Dose and Brain or Renal NF-κB Gene Expression. J Nanostruct, 2025; 15(1):249-254. DOI: 10.22052/JNS.2025.01.024

INTRODUCTION

Nanoparticle synthesis is on the rise these days for utilization in several essential applications. [1-3]. Because of their unique features, AgNPs are broadly applied in different daily goods [4-12], but the probability of Ag+ ions leaking may adversely impact their safety [13-17]. As per numerous studies, the toxic effects of AgNPs and Ag+ release have no connection [18, 19]; nevertheless, some analysts have said that expected toxicities coming from silver nanoparticle uses may be higher than the cytotoxic effects of silver ions singly [20, 21]. NF-κB is a negative and positive regulator of gene expression [22], its activity modifies immunity, inflammatory response, and nervous system function & enhances cancer development [23, 24]. This study is carried out to see how AgNPs affected CNS and renal NF-κB expression.

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MATERIAL AND METHODS

Synthesis of AgNPs

The prepared AgNPs were (40±5) nm in size and spherical form. D-sorbitol was used as a capping agent and olive leaf plant extract as a reducing agent [25, 26].

Experimental Animals

Male mus musculus (Balb/C) mice, (Fifty-six) weighting (23–35) g and aged eight weeks, were used. They came from the Al-Nahrain Centre of

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Biotechnology's animal house. They were held with a 12-hour light/dark cycle [27], at (23-25) °C, and a 7-day acclimatization period before the experiment began [28], and were randomly distributed into 7 groups, each with eighth mice in isolated cages, and provided a standard pellets meal and adequate water every day as follows:

-The first and fourth groups were given an intraperitoneal quantity of 0.25 mg/kg of AgNPs (50 µl) for one and two weeks.

-Second, and fifth groups were given 0.5 mg/kg of silver nanoparticle solution (50 µl). Intraperitoneal for one and two weeks, respectively.

-The third and sixth groups received a dose of 1 mg/kg body weight of AgNPs.

- The seventh group received an intraperitoneal dosage of distilled water (50 µl) instead of an AgNP solution.

Tissue Sampling

The fourth, fifth, and sixth groups were sacrificed the day after the treatment period ended (after 14 days), while the first, second, and third groups were sacrificed via cervical dislocation (Euthanasia) the day after the dosing period ended (after 7 days).

Tissue preparation for RNA extraction

Tissues were processed for histological evaluation using Junqueira and Carneiro's (2003) procedure, with paraffin slices used for livers, spleens, kidneys, and brains [29]. 5-8 semi-thin slices (0.5-10µm) were prepared from tissue paraffin blocks using an electrical ultra-microtome. The sections were used for RNA extraction.

Pre-Extraction Preparations

1- The lyophilized enzyme was dissolved in 550 µl of RNase-free water to make the DNase I Stock Solution.

2- In the preparation of RPE buffer, absolute ethanol, 44 µl (96-100%) was added to a container

Table 1. PCR Condition Program.

Temperature	Heated Lid	Step 1	Step 2	Step 3	Step 4	Step 5
	111 °C	25 °C	37 °C	42 °C	75 °C	4 °C
	--	10	10	60	5	∞

Table 2. Control gene (B-ACTIN) & the target gene (NF-κB) primers.

Primers	(5 →3)	Melting Temperature
Beta-ACTIN - F	CCTGAACCCTAAGGCCAAC	60 °C
Beta-ACTIN - R	ACGTACATGGCTGGGGTGT	62 °C
NF-κB - F	GTCAACAGATGGCCCATACC	62.0 °C
NF-κB - R	GATGGGCCTTCACACACATA	60.0 °C

Table 3. NF-κB measured concentrations.

Period	AgNPs (mg)	Mean ± SE	
		CNS levels	Renal levels
Week	0.025 mg	0.269 ± 0.043 bc	0.081 ± 0.014 a
	0.5 mg	0.217 ± 0.066 bcd	0.069 ± 0.014 ab
	1 mg	0.296 ± 0.081 b	0.078 ± 0.001 a
Two weeks	0.025 mg	0.6128 ±0.058 a	0.0347 ±0.012 cd
	0.5 mg	0.1117 ±0.017 cd	0.0164 ±0.004 d
	1 mg	0.2982 ±0.056 b	0.0450 ±0.004 bc
	Control	0.0678 ±0.064 d	0.0154 ±0.002 d
	LSD value	0.169 **	0.027 **

** (P<0.01).

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with 11 µl of buffer RPE concentration.

3-The Qiagen Kit was used to purify total RNA from Formalin Fixed Paraffin Embedded (FFPE) tissue slices and extract RNA, which were then stored at -20°C.

cDNA Synthesis

Using an Applied Biosystem RNA-to-cDNA kit (Part No. 4387406), RNA was reverse-transcribed to complementary DNA (cDNA). For use in real-time PCR, cDNAs were stored at -20°C. RT-PCR.

Each sample was quantified in duplicate using the SYBR Green master mix. In addition, each run contained two non-template controls. Control gene mRNA quantities were employed to normalize NF-κB mRNA levels, ensure proper cDNA synthesis, and describe computations. Primer 3 Plus was used to generate primers for the NF-κB gene, with B-ACTIN as a reference gene.

The change in the target's expression in a sample as compared to the same sample at time zero [30] was determined using the relative quantitation (RQ) technique, which relies on cycle threshold (CT).

A 20µl reaction was applied for the SYBR Green assay. A 96-well plate was given to each gene. The qRT-PCR procedure was as follows.:

Stage 1 was 50 °C / two minutes, Stage 2 was 95 °C / 10 minutes, and Stage 3 had a three-step cycle process (denaturation 95 °C for ten seconds, annealing 62 °C /60 sec., and extension 72 °C for forty-five seconds) that was performed fifty times

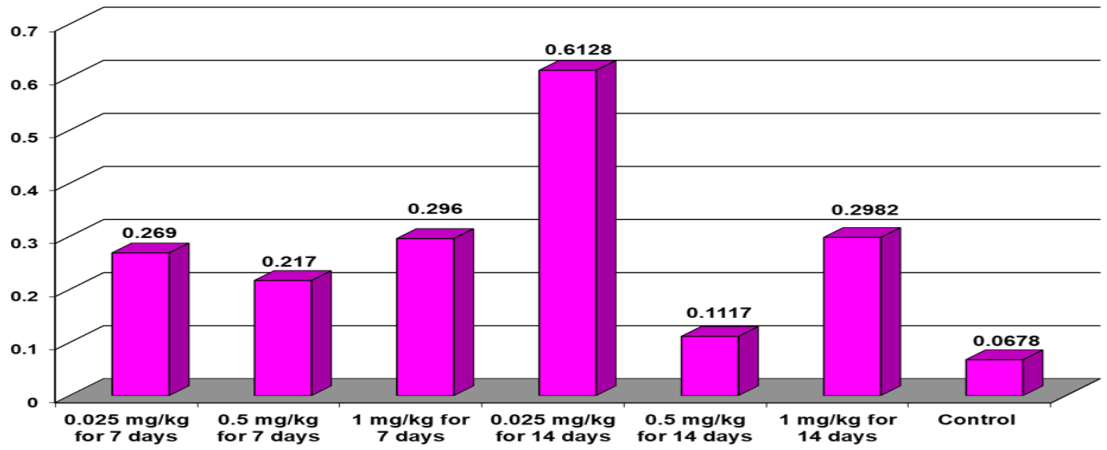

Fig. 1. Effect of study groups in Brain/NFKB gene.

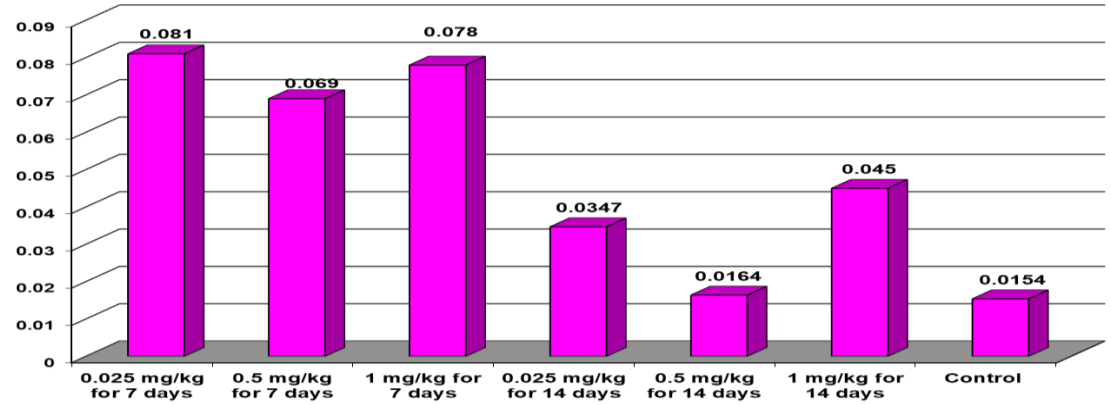

Fig. 2. Effect of study groups in Kidney/NFKB gene.

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before being cooled to 40 °C for ten seconds.

QRT-PCR Data Analysis

The threshold cycle (CT) was used to measure the fold change and gene expression levels. The reference gene (B-ACTIN gene) was used to standardize the CT data.

ΔCT sample = Ct Sample-Ct endogenous control

ΔCT calibrator = Ct control- Ct endogenous control

The relative fold change of gene expression is measured using the normalized ΔCT data and particular calibrators:

ΔΔCT = ΔCT sample - ΔCT calibrator

Relative copy number =Fold change = $2^{-\Delta\Delta CT}$

RESULTS AND DISCUSSION

The study found varying doses and durations of AgNPs administration affected tissue expression levels of the NF-κB gene in many organs, including the brain and kidneys (Table 3).

The lowest value of NF-κB gene expression in the brain was 0.1117, which was obtained from the lowest AgNPs dose (0.025 mg/kg) for two weeks (P<0.01), while the highest value was (0.6128) only after daily administration of the lowest AgNPs dose (0.025 mg/kg of body weight) for two weeks (P<0.01), as shown in Fig. 1.

After one week of treatment with the highest dose of AgNPs (0.025 mg/kg of body weight), kidney NF-κB gene expression was 0.081 (P<0.01), but after two weeks at a dose of 0.5 mg/kg, kidney expression was 0164 (P<0.01). as shown in Fig. 2.

Utilizing products even if they contain a small amount of silver, can have a bad impact on immunity and general health. [31-35]. The recent findings discovered that variation in the given AgNP doses led to different elevated CNS levels of NF-κB, regardless of the duration of mice exposure.

Prolog exposure to the excitatory glutamatergic stimuli was demonstrated to produce a harmful NF-kB activation in the neurons [36-41].

It has been found that the non-regular NF-kB activation or abnormal production of NF-kB can lead to many CNS abnormalities, like modification of the apoptotic response to neurodegenerative cells and, an enhance the inflammation of neurons that may lead to neuronal cell death [42].

The current investigation found that the varied supplied AgNP dosages resulted in varying concentrations of renal NF-κB expression during the two periods of mice exposure.

The NF-kB contribution to the incidence of acute kidney injury or other kidney damage has been recognized over time [43–51]. First of all, NF-kB affects the renal expression of inflammatory genes [52, 53], furthermore, cytokines, genotoxic stress, mechanical stress, mechanical stress, and other mediators are among the triggers that activate NF-kB in kidney injury [54, 55].

The tumor suppressor protein, p53, interrupts the cell cycle and promotes cell death in response to DNA damage and cellular stress [56, 57]. A current study found that the lack of p53 during kidney damage confers a protective effect [58–61]. Increasing evidence suggests that p53 has a kB site, implying that NF-kB can bind to it and control p53 gene production [62, 63]. Which in turn, enhances the inflammatory and apoptotic response which leads to further kidney damage.

CONCLUSION

The results here revealed a substantial relationship between NF-kB expression and AgNP administered dose, suggesting a potential risk associated with utilizing AgNP goods.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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