



Biochemical Effects of Silver Nanoparticles Prepared by Chemical Reduction Method on Male Rat Kidney Functions and Antioxidant Defense Systems

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ABSTRACT

Background: The present study was conducted at the Animal House Unit, Biotechnology Research Center, Al-Nahrain University, Iraq, Baghdad. This study aimed to synthesize and characterize silver nanoparticles (Ag NPs) and investigate the impact of varying concentrations of Ag NPs on biochemical parameters, antioxidant defense system, lipid peroxidation, Histology and Immunohistochemistry in Male Rat Kidney.

Methods: There were fifty male rats (150-170 g) utilized. Five groups, each with ten rats, were created from the animals. Group II, III, IV and V received oral administration of Silver Nanoparticles (Ag NPs) at doses of 1/150 LD50, 1/100 LD50, 1/50 LD50 and 1/25 LD50 mg/kg BW/day for a duration of three weeks, with the first group serving as the control. At the end of the experiment, blood and kidney samples were collected to look at a number of factors.

Result: Treatment with Silver Nanoparticles (Ag NPs) different concentrations reduced kidney content in comparison to control for glutathione S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR). When rats were treated with varying quantities of silver nanoparticles (Ag NPs), their protein levels were considerably lower than those of the control group. Treatment with various quantities of silver nanoparticles (Ag NPs) raised the urea and creatinine levels significantly, urea and creatinine concentration increases are considered to be significant markers of renal failure.

Key words: CAT, GPx, GR, GST, Silver nanoparticles (Ag NPs), SOD.

INTRODUCTION

Since silver nanoparticles offer a wide range of antibacterial properties, their biological applications are becoming more and more varied. Further research in this area is necessary since the toxicity of nanoparticles is still up for debate (Adeyemi *et al.*, 2014). Particle size, atomic configuration and chemical makeup all affect a nanoparticle's toxicity. Because of its special qualities, namely its antibacterial action, silver is employed extensively in consumer medical items (Sardari *et al.*, 2012). Nanomaterials are the subject of much investigation because of their unique optical, mechanical and electrical capabilities (Olugbodi *et al.*, 2023). Silver nanoparticles are used in many medical applications, including radiosensitizers in radiation therapy, photothermal agents in the treatment of cancer and contrast agents in imaging (Chen *et al.*, 2021; Mustafa, 2023). The unique optical, electrical, magnetic, oxidation resistance and structural qualities of these materials make them extremely relevant in terms of their composition, structure and overall makeup (Al-Mashhadani and Al-Maliki, 2022). Ag NPs are produced by a number of techniques, including chemical reduction, thermal breakdown and electrochemical procedures. Chemical reduction in particular is a widely utilized method due to its simplicity, high yield and ability to produce nanoparticles with exact sizes and shapes (Singh *et al.*, 2020). To ensure consistency and prevent the agglomeration

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of nanoparticles, this procedure often calls for the reduction of silver salts in the presence of substances that stabilize (Huang *et al.*, 2021). Ag NP synthesis remains challenging

despite these advancements, particularly in achieving a uniform size distribution and preventing aggregation. Ongoing research aims to address these issues by examining the use of various capping agents and surfactants to stabilize the nanoparticles (Patra *et al.*, 2022).

Silver nanoparticles (Ag NPs) are becoming more and more appealing as therapeutic agents for the treatment of autoimmune illnesses because of their unique physical and chemical properties. They possess the ability to control immunological reactions, provide specific treatments and have anti-inflammatory qualities (Ghosh *et al.*, 2020). Autoimmune diseases lead to tissue damage and chronic inflammation due to immune system dysfunction. According to recent studies, Ag NPs can help regulate these illnesses in a variety of ways. Firstly, studies have demonstrated the potent anti-inflammatory properties of Ag NPs. They possess the capacity to inhibit the production of pro-inflammatory cytokines and reactive oxygen species, both of which are crucial for the genesis of autoimmune diseases (Singh *et al.*, 2021). Ag NPs have the ability to alter the immune system by interacting with immune cells. They have been shown to have an impact on macrophage and T-cell activity, two crucial factors in the development and progression of autoimmune diseases. By affecting these immune cells, Ag NPs can help restore immunological balance and reduce autoimmunity (Sohrab *et al.*, 2023). The functionalization and synthesis of Ag NPs are necessary for their possible therapeutic applications. Ag NP particle size and shape regulate resonance frequencies (Noshy *et al.*, 2023). Surface modifications, such as coating with certain ligands or polymers, can increase their stability and targeting abilities (Jain *et al.*, 2020).

Applications for silver nanoparticles (AgNPs) in biomedicine are becoming more and more common. This is dependent on the fact that AgNPs have a broad range of antibacterial activity reported. Because of its unique qualities, including strong conductivity, chemical stability, catalytic activity, antibacterial activity, antifungal, antiviral and anti-inflammatory qualities, AgNP, an ancient product of nanotechnology, has drawn attention (Sulaiman *et al.*, 2015). Silver nanoparticles, or AgNPs, have garnered a lot of interest in the medical and environmental sectors among other uses because of their potent antibacterial capabilities.

AgNPs have benefits, but there are certain risks to health as well. The potential impact they may have on biological systems is especially concerning. An essential part of their safety profile is understanding how they impact GI immune system and renal function (Li Wang, 2023). The gastrointestinal tract is immune response central, acting as a site of high immune activity and a barrier against pathogens. AgNPs have the ability to interact with the gastrointestinal tract mucosa and impact the immune system when ingested or absorbed systemically. These nanoparticles have the potential to alter gut flora and immune cell function, which might lead to inflammation and immune system interference (Zhao and Zhang, 2022). Because the kidneys are essential for maintaining fluid and electrolyte balance as well as filtering metabolic waste, they are also susceptible to exposure to nanoparticles. Recent studies have shown that AgNPs can accumulate in renal tissues and impair renal function by triggering inflammatory responses and oxidative stress. The specific effect of AgNPs on renal health has to be further studied because their accumulation in the kidneys may cause both structural damage and functional issues (Nguyen and Park, 2024).

The objective of this study is to clarify how silver nanoparticles affect the immune system in the gastrointestinal tract and how that affects kidney function in mice. Through examining these interplays, the research aims to offer thorough understandings of the possible health hazards linked to AgNPs, guiding treatment plans and safety protocols.

MATERIALS AND METHODS

liquid sample

Trisodium citrate ($C_6H_5O_7Na_3$) (purity 99%) and silver nitrate ($AgNO_3$) (purity >98%) were used as starting materials without additional purification (Ismaeil and Al-Awadi, 2023). In a typical experiment, 62.5 mL of $AgNO_3$ solution was heated to boiling. 2.5 mL of 5% trisodium citrate were added to this solution drop by drop. Throughout the procedure, the fluid was violently churned. The solution was heated to produce a pale yellow color shift (Fig 1). After that, the heating element was removed and it was swirled until it attained room temperature. The following is one way to express the mechanism of reaction (Cannas *et al.*, 2001).

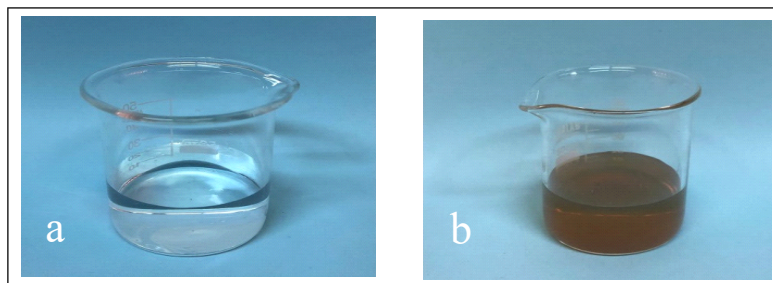
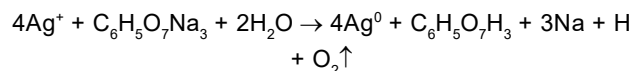


Fig 1: (a) The $AgNO_3$ solution (b) The Ag NPs solution.



Silver nanoparticles were applied using the drop casting process on a glass microscope slide to get a uniform coating that may be used in metal enhance fluorescence technique and to get a structure testing to silver nanoparticles such as FE-SEM and X-Ray diffraction (Fig 1).

Characterization of the synthesized sample

Using a Philips X-ray diffractometer (model 6000), provided by Shimadzu, X-ray diffraction (XRD) was used to assess the obtained materials structure. Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) was used to operate it at 40 kV and 30 mA in the 2θ range from 5° to 80° . To record the UV-Vis-NIR optical absorption spectra of the materials developed, the Centra-5 UV-VIS Spectrometer was powered by a dual beam tungsten lamp and a deuterium lamp. An FE-SEM, or field emission scanning electron microscope, uses an Inspect F 50 to create an image of a sample. The samples were produced under ideal circumstances and the production outcomes were analyzed to confirm the formation of nanoparticles.

Animals

Forty male Wistar rats, a weight of approximately 150 g, were used for the experiments and obtained from the zoology department farm. The male rats were housed in the laboratory room for seven days before the start of the research and provided with a standardized diet ad libitum.

Experimental groups

The following five groups comprised male rats in a similar manner.

G1 (Control): For a duration of three weeks, distilled water was given orally to control rats with a ball-tipped, curved intubation needle, every day.

G2: For a duration of three weeks, rats were given an oral dosage of 1/150 LD50 mg/kg BW/day of Gp2: (Ag NPs).

G3: For a duration of three weeks, rats were given oral treatment with Ag NPs at a dosage of 1/100 LD50 mg/kg BW/day.

G4: For a duration of three weeks, rats were given an oral dosage of 1/50 LD50 mg/kg BW/day of Ag NPs (Gp4).

G5 (Ag NPs): For a duration of of three weeks, rats received an oral dosage of 1/25 LD50 mg/kg BW of Ag NPs.

This study began in Unit Animal House, Biotechnology Research Center/ Al-Nahrain University in July 2020 and the practical part was completed in April 2022. The theoretical part and writing of the research began and the research was finally completed in January 2024.

Blood and serum samples

Rats after starving each group for ten to twelve hours, the rats were put to sleep with sodium pentobarbital so that they could be thoroughly dissected. The serum from the inferior vena cava was extracted and separated using centrifugation for 15 minutes at 3000 rpm. The obtained serum was maintained at -18°C to facilitate analysis and estimation of blood parameters.

Oxidative and antioxidants parameters Estimations

Oxidative and antioxidant factors were estimated in the homogenate of testis according to the methods described by Alankooshi *et al.* (2023) and Cannas *et al.* (2001).

Statistical analysis

The results were provided as mean values \pm standard error (SE) and statistical analysis was performed using an unpaired t-test to assess significant differences across experimental groups. For biochemical data, a significance level of 0.05 was used as the cutoff point, signifying statistically significant outcomes. The SPSS statistical version 21 software program (SPSS® Inc., USA) was used for all statistical analyses

RESULTS AND DISCUSSION

Chemical reduction method

The XRD pattern in Fig (2), which was obtained at diffraction angles of 32° (111), 29° (200) and Bragg's reflections planes representing the (111) and (200) planes of the FCC crystal structure of metal silver, shows two peaks with their

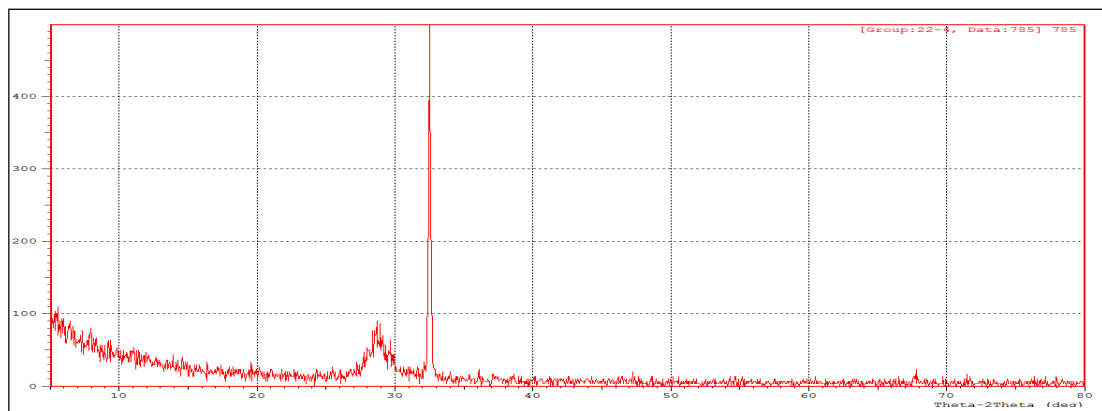


Fig 2: The X-ray pattern of Ag NPs synthesized at 9 min reduction period and $5.0 \times 10^{-3} \text{ mol/L}$ concentration.

corresponding planes, which can be used to look at the produced Ag nanoparticles' phase structure (NPs). There are no additional or impurity peaks in the XRD pattern, indicating that the processed samples are free of impurities. Ag nanocrystals' prominent, pointed peaks show how well orientated they are. The patterns show that all of the diffraction peaks are correlated with the metal silver's characterization and are comparable to the pure silver samples that were created according to (Al-Mashhadni and Kadhim, 2023).

The silver nanoparticles produced in this work were imaged using FE-SEM. Upon initial observation, a consistent dispersion of nanosphere-shaped silver nanoparticles, with particle sizes falling within the nanoscale, is discernible. Upon closely examining this image, it can be observed that at concentrations of 5×10^{-3} mol/L, the nanoparticle

distribution is regular, with particle sizes falling between 14.86 and 65.94 nm (Fig 3). The light yellow in this result indicates a lower particle size, whereas the dark yellow indicates a bigger particle size. This suggests that the particle size may be inferred from the color of the colloidal silver sample. The color changing to brown could be a sign of an increased chance of aggregation through the colloidal solution

The surface plasmon resonance was studied and measured using UV-visible spectroscopy. With a concentration of 5.0×10^{-3} M and a reduction period of 5 minutes after the boiling point, the absorption spectra of Ag NPs produced by the chemical reduction technique are displayed in Fig 4 (Putra *et al.*, 2019). At 423 nm, the surface plasmon resonance absorbance band's peak was discovered (Fig 5).

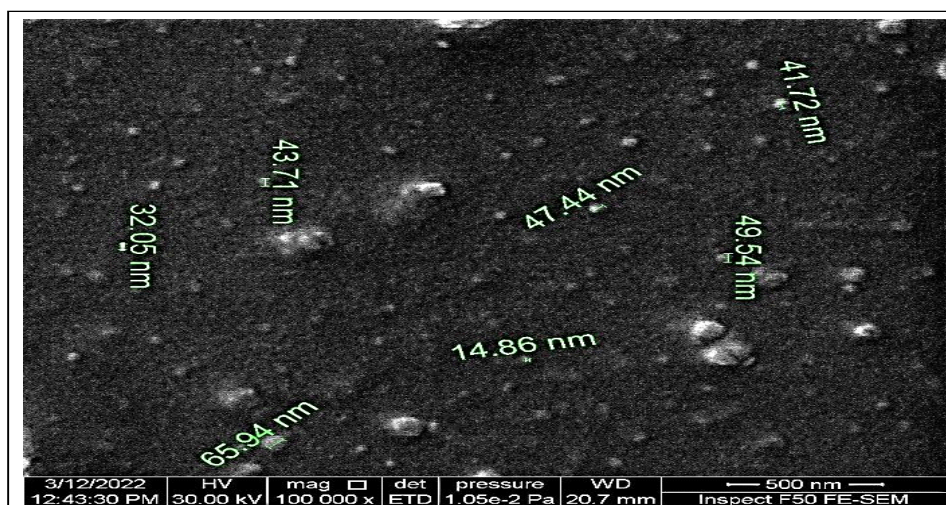


Fig 3: The FE-SEM image of Ag NPs synthesized at concentration 5×10^{-3} mol/L and 9 min reduction period after boiling.

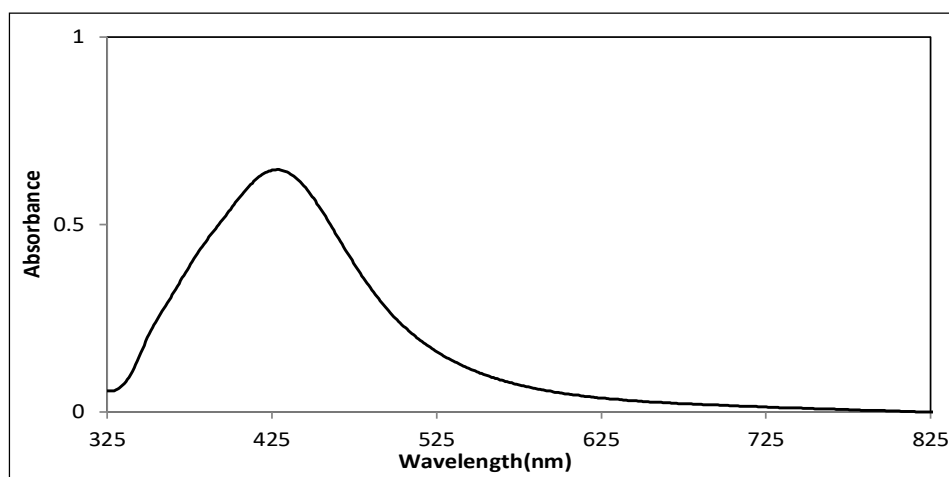


Fig 4: The absorption spectrum of Ag colloidal which is prepared by chemical reduction method at 9 min reduction period and 5.0×10^{-3} mol/L concentration.

Effect of silver nanoparticles (Ag NPs) different concentrations on antioxidant enzymes activity in rat kidney

Table 1 provides information on kidney antioxidant enzyme activity (SOD, CAT, GPx, GR and GST). Several rat groups treated with Ag NPs nanoparticles showed a substantial ($P<0.05$) decrease in the activity of antioxidant enzymes when compared to the control.

Effect of silver nanoparticles (AgNPs) different concentrations on kidney function biomarkers and enzymes activity in rat kidney

As shown in Table 2, serum urea and creatinine were significantly increased while protein content in rat kidney

was significantly decreased in Ag NPs nanoparticles different concentrations treated groups as compared with control.

In this study, oxidative stress caused by SnO_2 nanoparticle exposure included suppression of the enzymatic antioxidant defense system in addition to an increase in lipid peroxidation and GSH depletion. When amounts of ROS are appropriate, antioxidant enzymes offer a remarkable variety of defensive mechanisms that effectively contain them. SOD, CAT, GPx, GR and GST are some of the antioxidant enzymes that counteract oxidative cellular damage by neutralizing free radicals and working in concert with non-enzymatic antioxidants like GSH.

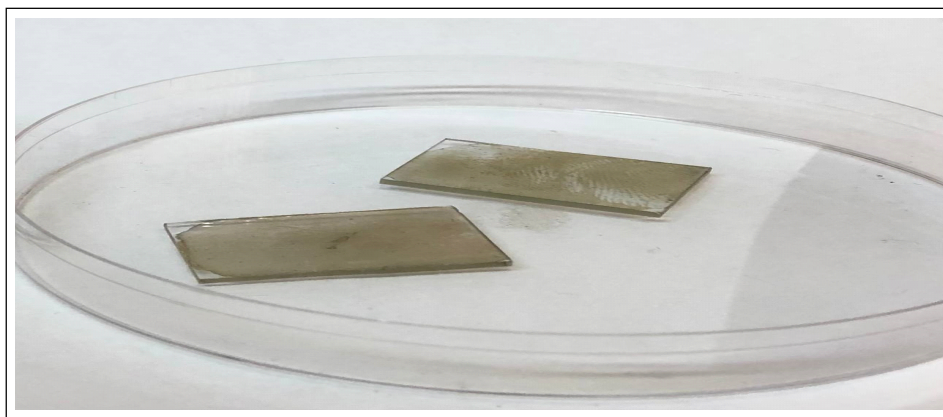


Fig 5: The glass microscope which is coated by drop casting method.

Table 1: Effect of different concentrations of silver nanoparticles (AgNPs) nanoparticles on the activities of antioxidant enzymes in rat kidney.

Parameters	Experimental groups				
	G1	G2	G3	G4	G5
SOD (U/mg protein)	62.74±2.15 ^a	52.92±1.53 ^b	46.98±1.71 ^c	44.22±1.50 ^{cd}	40.67±1.45 ^d
CAT (μmol/hr/mg protein)	51.64±1.42 ^a	44.26±1.33 ^b	40.49±1.33 ^c	36.46±0.842 ^d	31.99±0.953 ^e
GPx (U/mg protein)	24.80±0.909 ^a	21.06±0.698 ^b	18.17±0.694 ^c	16.39±0.606 ^{cd}	14.49±0.660 ^d
GR (nmol/min/mg protein)	13.60±0.286 ^a	11.51±0.401 ^b	10.80±0.390 ^b	9.51±0.269 ^c	8.61±0.258 ^c
GST(μmol/hr/mg protein)	0.498±0.017 ^a	0.411±0.013 ^b	0.384±0.011 ^{bc}	0.355±0.012 ^c	0.305±0.009 ^d

Values are expressed as means ± SE; n=7 for each treatment group. ^{abcd}Mean values within a row not sharing a common superscript letters were significantly different, $p<0.05$.

Table 2: Effect of different concentrations of silver nanoparticles (AgNPs) on the enzyme activities and protein content in rat kidney and urea and creatinine in rat serum.

Parameters	Groups				
	G1	G2	G3	G4	G5
Urea(mg/dl)	38.11±1.43 ^d	44.07±1.29 ^c	45.68±1.43 ^{bc}	49.04±1.10 ^{ab}	51.79±1.47 ^a
Creatinine(mg/dl)	0.659±0.021 ^d	0.774±0.024 ^c	0.800±0.022 ^{bc}	0.860±0.031 ^{ab}	0.909±0.024 ^a
LDH(U/mg protein)	856±31.88 ^d	957±26.89 ^c	1077±40.03 ^b	1127±36.97 ^{ab}	1205±34.35 ^a
ALP(U/mg protein)	195±6.28 ^a	173±5.62 ^b	152±5.05 ^c	136±4.78 ^d	121±4.59 ^d
Protein (mg/g tissue)	67.76±2.48 ^a	58.04±2.05 ^b	53.52±1.41 ^b	47.12±1.57 ^c	41.52±1.32 ^d

Values are expressed as means ± SE; n=7 for each treatment group. ^{abcd}Mean values within a row not sharing a common superscript letters were significantly different, $p<0.05$.

Even slight variations, the ability of cellular lipids, proteins and DNA to withstand oxidative damage can be significantly impacted by changes in the physiological concentrations of these antioxidant enzymes (Auten and Davis, 2009; Alankooshi *et al.*, 2023; Ezz *et al.*, 2023; Fadeyibi *et al.*, 2018). Through its catalytic function in dismutating superoxide radical into H_2O_2 , superoxide dismutase is essential for the detoxification of reactive oxygen species (Droge, 2002; Hameed *et al.*, 2023; Hasan *et al.*, 2022; Verma *et al.*, 2021; Hasan *et al.*, 2021).

Because of the large volume of blood that is delivered to the kidneys and its function in solute concentration, the kidneys are susceptible to toxic assault from environmental toxins. The increase in blood urea and creatinine in the SnO_2 nanoparticle-treated groups in the current study amply demonstrated renal impairment, particularly at the high doses. These results concur with earlier research where renal impairment was linked to methomyl exposure in the workplace and in experimental animal models (Mustafa, 2023; Hasan *et al.*, 2023; Hasan *et al.*, 2024; Ashtaputre *et al.*, 2014). Urea is a consequence of protein catabolism, thus liver function effects or renal dysfunction might have contributed to the increase in urea concentrations in the blood of the research animals treated with SnO_2 nanoparticles. Serum creatinine and urea levels rising suggest that the kidneys' capacity to remove waste materials from the blood and eliminate them in the urine has been compromised. A correlation between renal damage and hyperuricemia has also reported by Feig *et al.* (2006); Hasan (2024); Thakur *et al.* (2022). Possible processes that may aggravate or magnify the steady loss in renal function following injury are suggested by uric acid-mediated arteriopathy and interstitial inflammation.

CONCLUSION

It is clear that the use of silver nanoparticles at high concentrations has a toxic effect and leads to biochemical changes and disruption of enzymes, total antioxidant. Therefore, the study recommends not to be exposed to silver nanoparticles at high concentrations.

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Ethics

The study design was approved by the Institutional Ethical Committee for Animal Care and Use (code: IACUC-SCI-TU-0241).

Authors contributions

All authors are equally contributed in this study.

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Conflict of interest

The authors have not declared any conflicts of interest.

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