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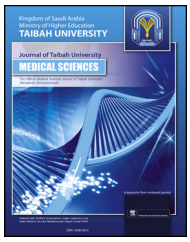

Experimental Article

Antioxidant status of rats administered silver nanoparticles orally

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الملخص

تستخدم الفضة النانوية بصورة متزايدة لأغراض الطب الحيوي بسبب إحتمال وجود خاصية مضادتها للميكروبات. ومع ذلك فإن تأثيرها على الأنظمة الخلوية لم يذكر إلا في عدد قليل من الدراسات البحثية. قمنا في بحثنا هذا بتقييم آثار هذه الجسيمات النانوية على حالة الفئران المضادة للأكسدة على 6 مجموعات من ذكور الفئران ويستار كل مجموعة تتكون من خمس فئران. وتم اعطائهم 100 و 1000 أو 5000 ملغ / كيلوغرام يوميا من الفضة النانوية من خلال قارورة عن طريق الفم لمدة 7 أو 14 أيام، وتلقت مجموعة واحدة 5000 ملغ/ كغ لمدة 21 يوما، وتلقت مجموعة مراقبة الماء المقطر فقط. تم ذبح الحيوانات بعد24 ساعة من نهاية تلقيهم العلاج، وأخذت عينات من مصل وأنسجة الفئران للدراسة. أثرت الفضة النانوية معنويا ($P < 0.05$) على زيادة تركيزات بعض الإنزيمات وخفض البعض. وهذه النتائج تشير إلى أن جزيئات الفضة قد تسبب أكسدة الدهون وتغيير الوضع المضادة للأكسدة الأنظمة الخلوية للفئران.

الكلمات المفتاحية: المضادة للمضادات الأكسدة; تأكسد الدهون; جسيمات معدنية متناهية الصغر طب النانوي; السمية

Abstract

Silver nanoparticles are being used increasingly for biomedical purposes because of their broad antimicrobial potential. Their effects on cellular systems, however, have been addressed in only a few studies. We evaluated the effects of these nanoparticles on the antioxidant status of groups of five male Wistar rats. Six groups of rats were given 100, 1000 or 5000 mg/kg daily through an oral cannula for 7 or 14 days, one group received 5000 mg/kg for 21 days, and a control group received distilled water. The animals were sacrificed 24 h after the end of treatment, and serum and tissue homogenates were prepared. Silver nanoparticles significantly ($p < 0.05$) increased the concentrations of malondialdehyde and superoxide dismutase but decreased the levels of reduced glutathione, glutathione *S*-transferase and catalase. These results indicate that silver nanoparticles may cause lipid peroxidation and alter antioxidant status in a manner that may cause oxidative stress.

Keywords: Antioxidant; Lipid peroxidation; Metal nanoparticle; Nanomedicine; Toxicity

Introduction

Nanoparticles are employed in constructing micro and nano devices used in electronics, in applications including adhesion, lubrication, stabilization and controlled flocculation of colloidal dispersions and in several other applications, including cellular delivery and imaging.[1,2] Owing to their small size and large ratio of surface area to volume, nanoparticles can interact with biomolecules and penetrate cell and nuclear membranes, causing indirect oxidative damage to DNA, inducing an inflammatory response and oxidative stress.[3] Potential risks may therefore be associated with their use. For instance, nanoparticles have been implicated in cellular injury[4] because of their ability to produce reactive oxygen species directly or indirectly.[5]

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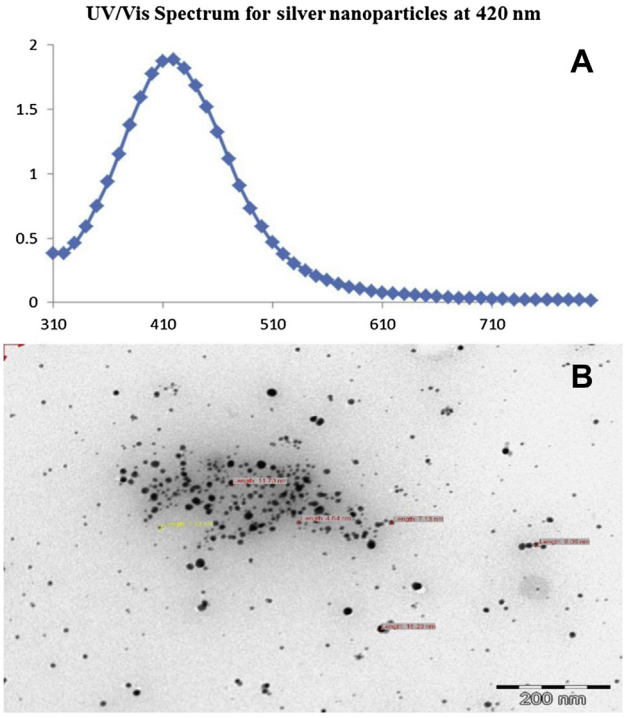

Figure 1: UV/Vis spectrum at 430 nm and transmission electron microscopy characterization of silver nanoparticles.

Silver nanoparticles have the broadest commercial applications of all nanomaterials because of their antibacterial properties.[3,6,7] Although they can act as free radical scavengers or reducing agents and quenchers of singlet oxygen formation,[8] they can also interact with cellular proteins and enzymes, with toxic effects.[9–11] However, there are no comprehensive safety or toxicity profiles of silver nanoparticles. The aim of this study was to investigate the effects of repeated administration of these nanoparticles on the antioxidant status of Wistar rats.

Materials and Methods

Preparation of nanoparticles

Silver nanoparticles were synthesized according to established protocols, with little modification. First, 100 mmol/L silver nitrate were added to a 1% (w/v) tannic acid solution (pH adjusted to 8 by addition of 150 mmol/L potassium carbonate) of polyvinylpyrrolidone with stirring.[12] A pale yellow colour revealed the presence of silver nanoparticles. The particles were filtered through a 0.22-μm filter and characterized by ultraviolet–visible (UV/Vis) spectrophotometry (Biotek Epoch, USA), inductively coupled plasma optical emission spectrometry (Cambridge, United Kingdom) and transmission electron microscopy (TEM, Brno, Czech Republic). All reagents were of analytical grade and prepared in distilled water unless otherwise stated.

Animals and treatments

Male Wistar rats weighing 190–220 g were obtained from the University of Ibadan, Ibadan, Nigeria, and housed in a well-ventilated, hygienic experimental animal house for 1 week to acclimatize them before the start of the experiment. They were fed a constant weight of commercial rat pellets and clean water.

Forty rats were grouped randomly into eight groups of five animals. One served as the control group and received distilled water. Three groups were treated with 100, 1000 or 5000 mg/kg silver nanoparticles for 7 days, and three further groups received the same concentrations for 14 days, while the eighth group received 5000 mg/kg for 21 days.

The doses were determined on the basis of a recent study in which the LD_{50} for silver nanoparticles was reported to be of >5000 mg/kg.[13] The nanoparticles were administered daily to the rats by means of a cannula. The rats were weighed daily to determine the effects of the nanoparticles on their weight and were sacrificed 24 h after cessation of treatments. The study was carried out in accordance with Institutional guidelines on the handling of animals as approved for scientific research.

Necroscopy

Animals were fasted overnight and sacrificed under slight chloroform anaesthesia 24 h after the last dose of nanoparticles. Blood samples were obtained by cardiac puncture, transferred to clean test bottles and centrifuged at 4000 × *g* for 5 min to obtain serum. The heart, liver and kidneys of each animal were removed and weighed immediately, then homogenized in ice-cold 0.25 mol/L sucrose (1:5 w/v) in a Teflon homogeniser (Sigma–Aldrich Chemie GmbH, Munich, Germany). The homogenates were then centrifuged at 4000 × *g* for 5 min (Heraeus Labofuge 300, Thermo Scientific, Hampshire, United Kingdom) to remove unbroken particulates. Tissue homogenates and serum were kept frozen until analysis.

Biochemical assays

Extracts of serum, kidney, liver and heart were analysed in a UV/Vis spectrophotometer (Shimadzu, Kyoto, Japan) for reduced glutathione (GSH), catalase, superoxide dismutase, malondialdehyde and glutathione *S*-transferase (GST). GSH was determined by the protocol described by Ellman[14] as previously reported,[15] catalase by the method described by Singha,[16] superoxide dismutase by the method described by Misra and Fridovich,[17] as previously reported by Adeyemi et al.,[15] malondialdehyde by the protocol described by Niehaus and Samuelson[18] and GST by the method described by Habig et al.[19]

Data analysis

Data were analysed on Graphpad Prism 3 (GraphPad Software Inc., San Diego, California) with one-way analysis of variance (ANOVA). Post-hoc tests were conducted with the Tukey test. Data are reported as means ± standard error of mean. Values of $p < 0.05$ were considered significant.