STUDIES ON POTENTIAL GOLD NANOPARTICLES TOXICITY IN RATS

By

FARAG YOUNES MOHAMED
B.V.Sc., Faculty of Veterinary Medicine, Omar El- Mokhtar University, Libia, 2001

THESIS
Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

In

Veterinary Medicine
(Forensic Medicine and Toxicology)

Department of Toxicology and Forensic Medicine
Faculty of Veterinary Medicine
Cairo University
EGYPT

2015

APPROVAL SHEET

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APPROVAL COMMITTEE

Dr. Hesham Saad EL-Sabbagh ………………………………

Professor and Head of Forensic Medicine and Toxicology department,
Fac. Vet. Med., EL- Sadat City University

Dr. Ali Hosni Hassan Nounou ………………………………….

Professor of Toxicology and Forensic Medicine,
Fac.Vet.Med., Cairo University

Dr. Mohamed Sayed Hassan …………………………………

Professor of Toxicology and Forensic Medicine,
Fac.Vet.Med., Cairo University

Dr. Ashraf Mohammed Hassan Morgan ……………………….

Professor of Toxicology and Forensic Medicine,
Fac.Vet.Med., Cairo University

Date: / /

SUPERVISION SHEET

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SUPERVISION COMMITTEE

Dr. Mohamed Sayed Hassan

Professor of Toxicology and Forensic Medicine, Fac.Vet. Med., Cairo University

Dr. Ashraf Mohammed Hassan Morgan

Professor of Toxicology and Forensic Medicine, Fac.Vet. Med., Cairo University

Dr. Adel Fathy Tohamy

Lecturer of Toxicology and Forensic Medicine, Fac.Vet. Med., Cairo University

Name of Candidate: FaragYounis Mohamed **Degree:** M. Sc.

Thesis Title: Studies on potential gold nanoparticles toxicity in rats

Supervisors: Dr.Mohamed Said Hassan

Dr. Ashraf Mohammed Hassan Morgan

Dr. Adel FathyTohamy

Department: Toxicology and Forensic Medicine **Approval:** 27/8/2015

ABSTRACT

The production and use of nanomaterials, which continue to grow, have given rise to many concerns and debates among public, scientific and regulatory authorities regarding their fate in biological systems. The aim of the present study was to investigate the hepato- and nephro- toxic potential of gold nanoparticles (20 nm) on Sprague–Dawley rats. Adult female rats were divided into three equal groups of 20 animals each. Group I: (Control group), animals were injected i.p with physiological saline (0.9 % NaCl) daily for 7 days; the other two groups were treated with 19.7 & 39.4 µg AuNPs/kg.b.wt of 20 nm sized AuNPs daily for 7 days, respectively. The liver and kidney functions were assessed in addition to oxidative stress and genotoxicity parameters. Moreover histopathological examination was carried out at four different time points (after 1, 3, 5 and 7 days from start of experiment). The results revealed that AuNPs exposure at 39.4 µg/kg.b.wt significantly enhanced hepatotoxicity and nephrotoxicity by increasing serum ALT, ALP activity; urea, creatinine and tissue MDA levels and significantly reduced hepatic and renal tissue GSH content and CAT activity ($P \leq 0.05$). COMET assay of hepatic and renal tissues showed that AuNPs exposure significantly decreased the intact cells %, head diameter, and head DNA % in the double therapeutic dose of AuNPs-treated group compared to the control and therapeutic dose of AuNPs-treated groups. The hepato- and nephro- toxic effect of AuNPs was also confirmed by the observed liver and kidney histopathological alterations especially at the high dose. We can conclude that AuNPs had severe hepato and nephro-toxic effects in rats when given at the high (double therapeutic) dose.

Keywords: Gold nanoparticles (AuNPs), hepatotoxicity, nephrotoxicity, oxidative stress,genotoxicity, rats.

ACKNOWLEDGMENT

First of all, prayerful thanks to our merciful God who gives me everything I wish.

I wish to express my deepest thanks and gratitude to ***Prof. Dr. Mohamed Sayed Hassan,*** *Professor of Toxicology and Forensic Medicine, Faculty of Veterinary Medicine, Cairo University, for his enthusiastic kind scientific supervision of this thesis, meticulous help, kind encouragement, unlimited noteworthy suggestion and valuable guidance and continuous interest which played an important role in the completion of this work. I am very proud to be one of his students and I will always remember his kindness and generosity.*

My sincere thanks to ***Prof. Dr. Ashraf Mohamed Morgan****, Professor of Toxicology and Forensic Medicine, Faculty of Veterinary Medicine, Cairo University, who devoted the time and energy to help me through his supervision, indispensable support, sincere cooperation, wrathful advices and offering his maximal effort during the whole thesis writing and revision.*

My sincere thanks and appreciation to ***Dr. Adel Fathy Tohamy,*** *Lecturer of Toxicology and Forensic Medicine, Faculty of Veterinary Medicine, Cairo University; for suggesting the research point, teaching me the practical skills, scientific meticulous supervision, valuable comments, kind constructive help and support, sincere cooperation and wrathful advices. Also, solving the daily problems during my experimental study and for his extensive efforts and valuable comments during writing of this thesis.*

Finally, but not lastly, I express a heartfelt appreciation to all the staff members of Toxicology and Forensic Medicine Department for their kind support and encouragement.

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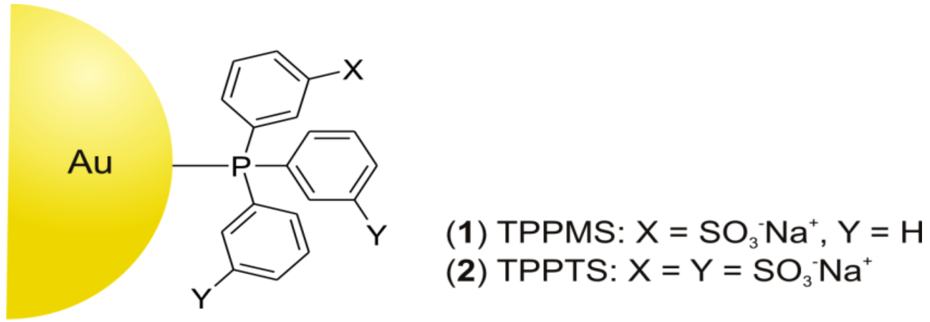
INTRODUCTION

Nanoparticles, also called ultrafine particles, are defined as particles sized between 1 and 100 nanometers (10^{-9}m) and form a bridge between bulk materials and atomic or molecular structures **(Granqvist*et al*., 1976).Schmid (1992)** defined nanoparticles as particles with a diameter less than 100 nm. It is a relatively broad definition compared to cluster and colloid. Colloids are used to name those nanoparticles of a size larger than 10 nm. Clusters are usually applied to nominate those particles with a diameter less than 10 nm, which have well-defined chemical and structural properties including number of atoms, ligands, crystal arrangement and atom-ligand coordination. They occur in nature in the context of volcanic eruptions or any natural or anthropogenic combustion process. Man-made nanoparticles may appear for example as globular carbon molecule (fullerene or “buckyball”), as branched ribbons (dendrimers) or as nanotubes **(Nasterlack andGroneberg, 2011).**

Gold nanoparticles (AuNPs) consist of a core of gold atoms and ligand layers connected through a bond as shown in figure 1. The parameters used to describe the atom core include the number of atoms, atomic arrangement and diameter. In most of the cases when it is not specifically emphasized, the size of the nanoparticle describes the diameter of the atom core. Naked nanoparticles are not stable in solutions. Therefore, to avoid aggregation, AuNPs are further stabilized with one or more ligand layers. The surface modification, on one side stabilizes the nanoparticle and on the other side, gives those metallic atoms different properties depending on the position of the atoms and their ligand binding (**Schmid, 1992**).



Model of Au cluster with the triphenylphosphine derivative ligand.

The gold nanoparticle consists of a core of gold atoms (Au), and ligands connected through gold-phosphor bonds.

For gold has traditionally been considered inertand biocompatible, its physicochemical properties and high surface area, gold nanoparticles (AuNPs) are more and more used in biomedical research **(Liu and Ye, 2013).** AuNPs of various sizes and morphologies had attracted considerable interest for medical applications for example as carrier for drugs such as paclitaxel **(Gibson *et al.*, 2007),** as tumor-detector **(Qian*et al.*, 2008),** photothermal agent or radiotherapy dose enhancer **(Hainfeld*et al.*, 2010; and McMahon et al., 2011).** Nevertheless, experimental use of AuNPs presented possible medical hazards as the surface to volume ratiocauses catalytic properties and can make particles very reactive **(Slocik*et al.*, 2013).**

Furthermore, Nanoparticles easily pass cell membranes and can interact with intracellular metabolism **(Hanley *et al.*, 2009).** As nano-scale gold-particles may exhibit size-related properties that differ significantly from the known properties of non-nano-scaled gold-particles, one cannot predict reliably the nature of AuNPs and a biologic system and interactions between AuNPs and living cells**.** Beside the size, further potentially toxic features of AuNPs depend on charge and surface-chemistry **(Buzea *et al*., 2007)**.

For metallic nanoparticles that can support photothermal effects, like gold nanorods, the dissipation of absorbed light energy as heat can be employed to actively release drugs, in addition to any passive release that might occur from the nanoparticle surface. For these reasons, gold nanoparticles have had some success as drug delivery vehicles in laboratory settings**,** although their fate *in vivo* after delivery is frequently not measured **(Kim *et al*., 2009)**.

Gold nanorods can be coated with thermo-sensitive shell such as a poly (N-isopropylacrylamide) hydrogel, in which the drug molecules can be dispersed**.** Irradiating gold nanorods with near-infrared (NIR) light generates heat, which induces a phase transition to the expanded form in the hydrogel and thus releases the drug **(Kawano *et al*., 2009).** The generated heat can also be used to increase the efficacy of drug molecules that have been dosed to a certain localized area. More specifically, the effects of heat on chemotherapeutic agents can be synergistic in that the results are quite often more than the sum of the two individual treatments. This effect was demonstrated using cisplatin drugs coupled with localized heating from gold nanorods. It is still unclear what the exact mechanism is for the thermal enhancement of drug treatment efficiency, although “improved membrane permeability" is a general notion that exists in the literature **(Hauck *et al.*, 2008).**

Because of the promising biomedical applications of such nanomaterials, assessment of their toxicity is a necessary task **(Alkilany and Murphy, 2010).** For very small gold nanoparticles (4 nm diameter), their chemical reactivity becomes important, and oxidative damage to cells is possible **(Bhattacharya and Mukherjee, 2008; Aillon *et al.*, 2009; and Fadeel and Garcia-Bennett, 2010).** Thus, it is possible to consider that nanoparticles themselves might be "drugs" in that they might exert toxic effects at sufficiently high doses. However, when gold nanorods are discussed as therapeutic agents, workers in the field generally mean the photothermal properties of the gold nanorods was used to irreversibly damage cells upon illumination **(Huang *et al.*, 2006; Pissuwon*et al.*, 2007; and Alkilany and Murphy, 2010)**.

Despite the huge potential benefit of AuNPs in the field of biomedical and industrial applications, very little is known about their toxicity and tissue bioavailability in animals (*in vivo*). Although AuNPs were recognized as being nontoxic **(Merchant, 1998; Connor *et al.*, 2005; and Shukla *et al.*, 2005),** there have been still some reports suggesting that gold nanoparticles themselves might be inherently toxic **(Goodman *et al.*, 2004; Pernodet*et al.*, 2006; Chithrani and Chan, 2007; Pan *et al.*, 2007; and Alkilany and Murphy, 2010).** This has been shown to depend on the physical dimension, surface chemistry, and shape of the AuNPs. Nanoparticles could also be identified as foreign by the immune cells, causing the cells to react against either surface or core components to mount an inflammatory response, which involves secretion of signaling molecules (known as cytokines) to attract more cells to destroy the foreign substances. However, many of these molecular events are still poorly understood, partly because most papers addressing AuNPs toxicity have only used *in vitro* models **(Dobrovolskaia and McNeil, 2007; and Stern and McNeil, 2008)**.

Therefore, this *in vivo* toxicity study was designed to investigate the potential toxic effects of gold nanoparticles on Sprague–Dawley rats after injection ip with either therapeutic or toxic dose to assess their hepato- and nephrotoxicity. To achieve this purpose liver and kidney functions were evaluated in addition to oxidative stress and genotoxicity parameters in both liver and kidney of gold nanoparticles-treated animals. Histopathological examination was carried out to confirm our results.