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Nephrotoxicity of gold nanoparticles in rats

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Abstract

Gold nanoparticles (AuNPs) have become the object of special interest due to their biomedical applications such as cell tracking, biosensing, contrast imaging, targeted drug delivery, and tissue engineering. The aim of the present study was to investigate the nephrotoxic potential of gold nanoparticles (20 nm) on Sprague–Dawley rats. Therefore, kidney functions were assessed in addition to oxidative stress and genotoxicity parameters. Moreover histopathological examination was carried out.

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 with19.7 & 39.4 µg AuNPs/kg.b.wt of 20 nm sized AuNPs daily for 7 days, respectively. Blood and kidney tissue samples were collected from control and treated groups at four different time points (after 1, 3, 5 and 7 days from start of experiment), where five rats / group were sacrificed at each time point.

The results revealed that AuNPs exposure at 39.4 µg/kg.b.wt significantly enhanced nephrotoxicity by increasing serum urea and creatinine levels from the 1st day till the end of experiment compared to the corresponding control values and those of rats taken the therapeutic dose (19.7 µg/kg.b.wt). Moreover, intraperitoneal injection of rats with AuNPs at the toxic dose (39.4 µg/kg.b.wt) significantly reduced renal tissue GSH content and CAT activity ($P \leq 0.05$) throughout the experiment with marked increase of MDA production throughout the study when compared to control and low-dose groups.

COMET assay showed that AuNPs exposure induced renal apoptosis which was exhibited through the recorded significant decrease in 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 nephrotoxic effect of AuNPs was also confirmed by the observed renal histopathological alterations especially at the high dose.

Keywords: Gold nanoparticles (AuNPs), nephrotoxicity, renal apoptosis, MDA, GSH, CAT, rats.

Introduction

Gold nanoparticles (AuNPs) are widely used in biomedical research **(Liu and Ye, 2013)**. AuNPs of various sizes and morphologies had attracted considerable interest for medical applications for example as drugs' carrier **(Gibson et al., 2007)**, tumor-detector **(Qian et al., 2008)**, photothermal agent **(McMahon et al., 2011)**. Nevertheless, experimental use of AuNPs presented possible medical hazards as the surface to volume ratio causes 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 interactions between AuNPs and living cells **(Buzea et al., 2007)**. Because of the promising biomedical applications of such nanomaterials, assessment of their toxicity is a necessary task **(Alkilany and Murphy, 2010)**. Its 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)**.

Despite the huge potential benefit of AuNPs in the field of biomedical and industrial applications, very little is known about their *in vivo* toxicity and tissue bioavailability in animals. 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.

Therefore, this *in vivo* toxicity study was designed to investigate the potential toxic effects of AuNPs (20 nm) on Sprague–Dawley rats after ip injection of either therapeutic or toxic dose to assess its nephrotoxicity. To achieve this purpose kidney functions were evaluated in addition to oxidative stress and genotoxicity parameters of AuNPs -treated animals. Histopathological examination of kidney tissues in treated rats was carried out to confirm our results.

Materials and methods:

2.1. Animals:

Sixty adult female Sprague–Dawley rats (150–200 g) obtained from Faculty of Veterinary Medicine, Cairo University, Egypt were used in this study. They were kept under good ventilation and standard hygienic conditions with free access to food and drinking water *ad libitum*. Rats were reared and treated in accordance with the guidelines of animal bioethics committee from the faculty of Veterinary Medicine, Cairo University.

2.2. Chemicals:

Gold nanoparticles (20 nm) stabilized as a suspension in citrate buffer solution; chemical reagents used for determination of the lipid peroxidation marker (malondialdehyde, MDA), reduced glutathione (GSH) levels and test kits used for measurement of catalase activity were purchased from Sigma-Aldrich chemical co. (St. Louis, MO, USA). Urea and creatinine test kits were purchased from Biodiagnostic Co, Egypt. All the other chemicals were of analytical pure grade.

2.3. Animal groups and treatments:

Experimental rats were divided into three equal groups, each of 20 rats as follow: Group I: (Control group), animals were daily injected i.p with physiological saline (0.9 % NaCl) daily for 7 days; Group II: Animals were i.p injected daily with 19.7 µg AuNPs /kg.b.wt (therapeutic dose) daily for 7 days **(Stefan et al., 2013);** Group III: Animals were daily i.p injected with 39.4 µg AuNPs/kg.b.wt (double therapeutic dose) for 7 days.

2.4. Samples preparation:

At each time point, animals were anaesthetized under gentle anesthesia and sacrificed. Blood and kidney tissues samples were collected from control and treated groups at four different time points (after 1, 3, 5 and 7 days from the start of experiment). Five rats / group were sacrificed at each time point.

Blood samples were collected without anticoagulant and used after serum separation for assessment of kidney function (urea and creatinine).

In addition, kidney tissues were immediately dissected out of the body; wiped off the blood and divided into three parts. Two parts were kept in deep freezer (-20); one of them was used for assessment of GSH content, lipid peroxidation (MDA content) and catalase activity (CAT) while the other one was used for assessment of AuNPs genotoxicity using COMET assay (at the 7^{th} day). The third part was kept in formalin and subjected to histopathological examination.

2.5. Assessment of kidney functions:

Urea and creatinine levels were determined in the serum samples by the colorimetric method according to **Fawcett and Soctt (1960) and Schirmeister (1964)**, respectively.

2.6. Assessment of renal oxidative stress markers:

Lipid peroxides in the prepared renal tissue homogenates were determined chemically as thiobarbituric acid reactive substances (TBARS), according to the method of **Uchiyama and Mihara (1978).** The concentration of TBARS in the test samples was expressed as nmol/ml using serial dilutions of MDA (the standard curve).

Reduced GSH was determined in the prepared renal tissue homogenates chemically using Ellman's reagent **(Ellman, 1959)**. The concentration of GSH in the test samples was expressed as µmol/ml using the constructed using serial dilutions of GSH standard curve.

For CAT assay, tissue homogenization was carried out in 5 -10 ml cold buffer per gram kidney tissue using tissue homogenizer **(Fossati, et al., 1980)**. CAT activity was determined in the prepared kidney tissue homogenates using ready-made test kits according to the method described by **Aebi (1984).**

2.7. Assessment of renal genotoxicity using Comet assay:

Kidney tissue samples were chopped and cells were isolated in Hank's Balanced Salt Solution (HBSS) containing 20 mM EDTA and 10% DMSO. The cell suspension was prepared in HBSS solution containing 20 mM EDTA and 1% DMSO. From the suspension, the comet assay was performed as described by **Singh et al. (1988)** and **Tripathi and Jena (2009).** From the final cell-agarose suspension, 80µl was spread over the microscope slide which is pre-coated with 1% normal melting point agarose. The cells were then lysed in a buffer containing 2.5 M NaCl, 100 mM EDTA, 10 mM Tris (pH 10.0) with freshly prepared 1% Triton X-100 and 10% DMSO for 24 h at 4 °C. After lysis, slides were rinsed three times with de-ionized water to remove salt and detergent. Slides were placed in a horizontal electrophoresis unit and DNA was allowed to unwind for 20 min in alkaline solution containing 300 mM NaOH and 1 mM EDTA, pH >13. The DNA was electrophoresed for 30 min at 300 mA and 25 V (0.90 V/cm). The slides were neutralized with 0.4M Tris (pH 7.5); stained with SYBR green-I (1:10,000 dilution) for 1 hr.; covered with cover slips and stored in a humidified chamber. The fluorescence-labeled DNA was visualized using an automated imager fluorescence microscope and the images were captured with image analysis software.

2.8. Histopathological examination:

Kidney tissue samples taken from all rat groups were fixed in 10% formalin for 24 hrs then prepared, stained, and examined according to **Banchroft et al. (1996).**

2.9. Statistical analysis:

Data were expressed as means ± standard error of means (SE). Statistical analysis of the results was performed using one-way analysis of variance (ANOVA) procedure followed by Tukey-Kramer multiple comparison post-tests using the Software GRAPHPAD INSTAT (Version 2). The 0.05 level of probability was used as the criterion for significance.

Results:

3.1. Effects of gold nanoparticles on kidney function:

Fig. (1) illustrates the time course of serum urea level of rats after exposure to AuNPs (0, 19.7 and 39.4 µg/kg.b.wt). There was significant increase in urea level after exposure to AuNPs (39.4 µg/kg.b.wt) started from the 3rd day till the end of experiment when it compared with the

corresponding control values and those rats taken the therapeutic dose (19.7 µg/kg.b.wt) at P≤0.05. However, there was no significant difference in urea levels between AuNPs- therapeutic group and the control one at all the time points.

The data in **Fig. (2)** indicates that AuNPs exposure at 39.4 µg/kg.b.wt significantly enhanced nephrotoxicity by increasing creatinine release into serum especially in the high dose group compared to the control and low dose. There were no significant differences between the AuNPs-therapeutic group and the control one.

3.2. Effects of gold nanoparticles on induction of renal oxidative stress:

Exposure of rats to AuNPs ip at doses 0, 19.7 and 39.4 µg/kg.b.wt resulted in an observable dose and time-dependent increase in renal MDA level and decrease in its GSH content and CAT activity **(Figs. 3-5).**

3.3. Effects of Gold nanoparticles in induction of renal apoptosis:

The effects of AuNPs in induction of renal apoptosis were assessed by investigating DNA damage using the single cell gel electrophoresis (comet) assay and recorded in **Table (3) and Fig. (6).** The results showed that AuNPs ip injection of rats for 7 days induced significant decrease in 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 while tail %, tail length, tail DNA %, and tail moment were significantly increased. In AuNPs-treated group at the therapeutic dose, the comet assay parameters showed insignificant differences from the control one except the head diameter, tail length, and tail DNA % which were markedly changed.

3.4. Effects of gold nanoparticles on renal histoarchitecture:

Gold nanoparticles administration induced different time and dose dependent renal histopathological alterations in treated groups. In AuNPs- therapeutically treated group, focal inflammatory cells infiltration in between the degenerated tubules began to appear at the 1st day while at the 3rd day of exposure till the end of experiment; there were degeneration of tubules and atrophy of the glomeruli with dilatation of blood vessels and infiltration of focal inflammatory cells **(Fig. 7).**

In the high dose (39.4 µg/kg.b.wt) AuNPs- injected rats, the kidney tissue showed focal inflammatory cells infiltration in between the degenerated tubules at the 3rd day **(Fig. 8).** At the 5th day, there were severe congestion of blood vessels **(Fig. 9),** focal haemorrhages and inflammatory cells infiltration in between the degenerated tubules at corticomedullary junction **(Fig. 10),** and fibroblastic cells proliferation in between the atrophied tubules and congested glomeruli **(Fig. 11).** At the 7th day of AuNPs injection with the high dose, there was an infiltration of inflammatory cells in between the degenerated tubules **(Fig. 12).**

Table (3): Comet assay of kidney cells after ip injection of rats with therapeutic or double therapeutic doses of AuNPs for 7 days

Parameter \ Group		Control	AuNPs (Therapeutic dose)	AuNPs (Double therapeutic dose)
Intact cells %		83.80 ± 2.49	81.70 ± 2.6[a]	[illegible]
Tailed cells %		16.20 ± 1.19	18.30 ± 1.6[a]	[illegible]
Tailed cells	Head diameter (Px)	71.6[a] ± 1.93	64.43 ± 1.63[a]	[illegible]
	Head DNA %	92.20 ± 1.84	80.23 ± 1.13	[illegible]
	Tail DNA %	7.80 ± 0.64	10.[illegible] ± 1.03[a]	[illegible]
	Tail length (Px)	9.48 ± 0.63	10.13 ± 0.78[a]	[illegible]
	Tail moment	1.06 ± 0.08	1.1[illegible] ± 0.10	[illegible]

(a): Significantly different from control corresponding cells of group at P<0.05
(b): Significantly different from AuNPs- treated group at the therapeutic dose [illegible]

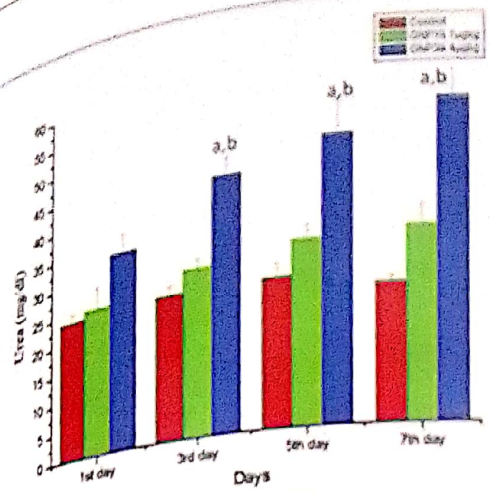

Fig.(1): Effects of gold nanoparticles on serum urea level (mg/dl)

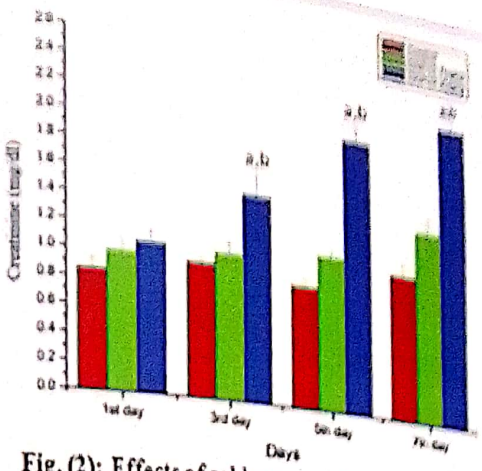

Fig. (2): Effects of gold nanoparticles on serum creatinine (mg/dl)

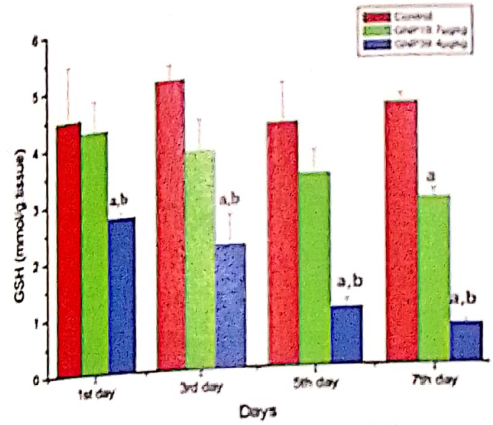

Fig (3): Effects of gold nanoparticles on kidney GSH content (mmol/g tissue)

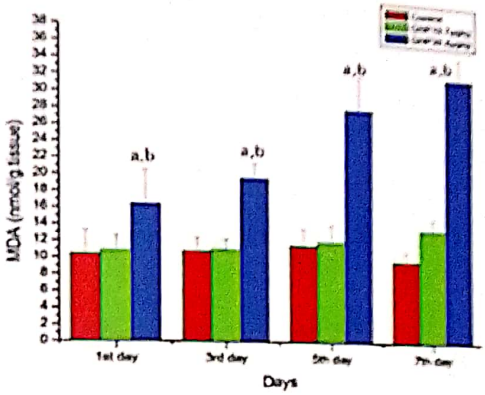

Fig. (4): Effects of gold nanoparticles on kidney MDA content (nmol/g tissue)

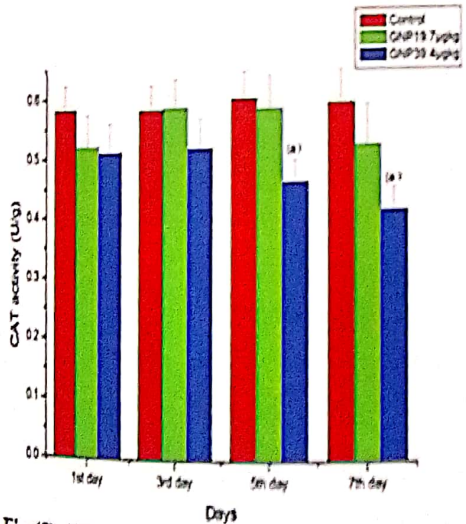

Fig.(5): Effects of gold nanoparticles on kidney CAT activity (U/g tissue)

(a): Significantly different from corresponding control group at P≤0.05.
(b): Significantly different from AuNPs- treated group at the therapeutic dose(19.7 µg/kg.b.wt) at p≤0.05.

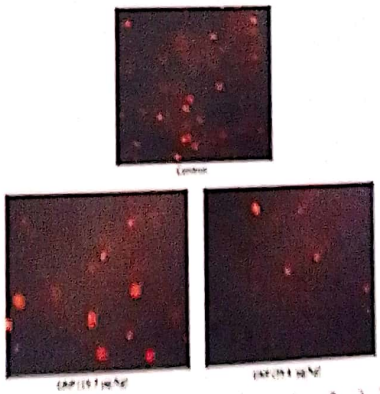

Fig.(6): Comet assay of kidney cells after ip injection of rats with therapeutic (19.7 µg/kg.b.wt) or double therapeutic doses (39.4 µg/kg.b.wt) of AuNPs for 7 days showing high molecular weight, unbroken DNA remains in the head (control), while smaller broken pieces of DNA migrate out to take the form of a comet (AuNPs-treated rats).

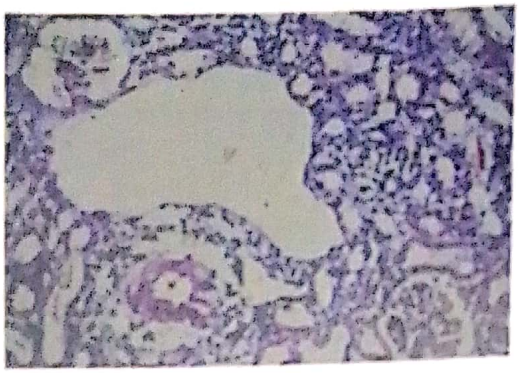

Fig. (7): Kidney of a rat treated with AuNPs at a dose of 19.7 µg/kg b.wt for 5 days showing focal inflammatory cells infiltration (m) in between the degenerated tubules and atrophied glomeruli with dilatation of blood vessels(v) (H& E, X 40).

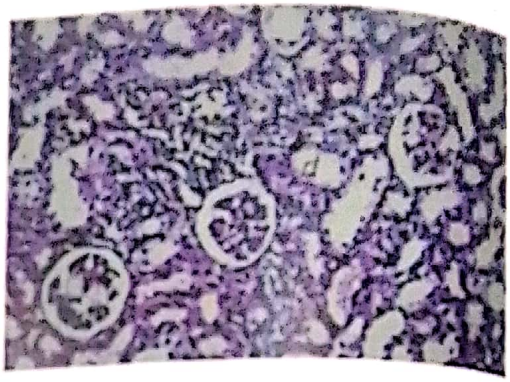

Fig. (8): Kidney of a rat treated with AuNPs at a dose of 39.4 µg/kg b.wt for 3 days showing focal inflammatory cells infiltration in between the degenerated tubules (H& E, X 40).

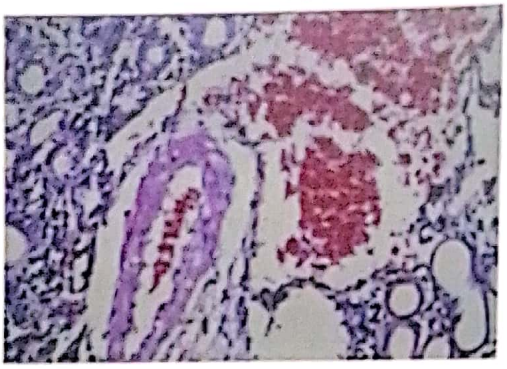

Fig. (9): Kidney of a rat treated with AuNPs at a dose of 39.4 µg/kg b.wt for 5 days showing severe congestion of blood vessels (H& E, X 40).

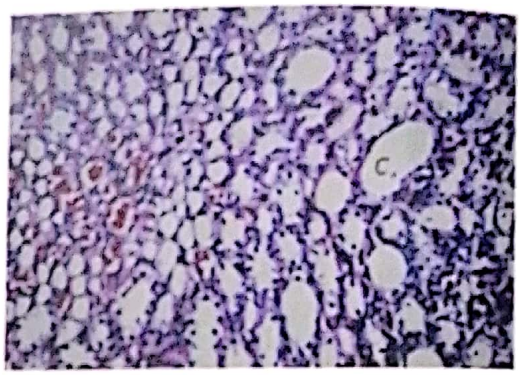

Fig. (10): Kidney of a rat treated with AuNPs at a dose of 39.4 µg/kg b.wt for 5 days showing focal haemorrhages (h) and inflammatory cells infiltration in between the degenerated tubules at corticomedullary junction (c) (H& E, X 40).

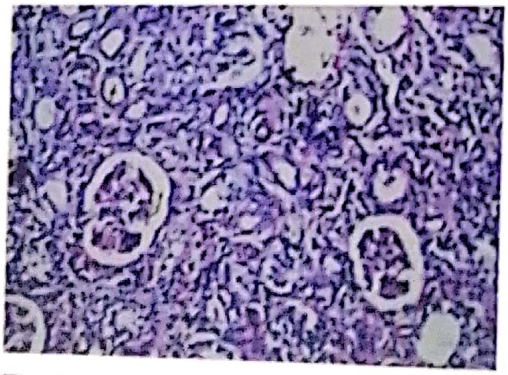

Fig. (11): Kidney of a rat treated with AuNPs at a dose of 39.4 µg/kg b.wt for 5 days showing focal inflammatory cells infiltration with fibroblastic cells proliferation in between the atrophied tubules and congested glomeruli (H& E, X 40).

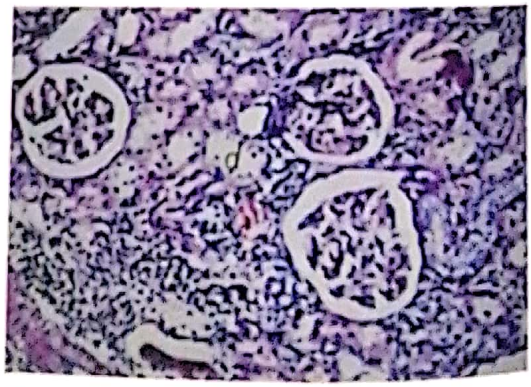

Fig. (12): Kidney of a rat treated with AuNPs at a dose of 39.4 µg/kg b.wt for 7 days showing inflammatory cells infiltration in between the degenerated tubules (H& E, X 40).

Discussion

Some literatures suggesting that AuNPs themselves might be inherently toxic **(Alkilany and Murphy, 2010), while** AuNPs chemical reactivity becomes important, and oxidative damage to cells is possible **(Fadeel and Garcia-Bennett, 2010).** Our results summarized that AuNPs *ip* injection of rats daily at 19.7 (therapeutic dose) or 39.4 μg/kg.b.wt (double of therapeutic dose) for 7 days was nephro-; and geno- toxic in a time and dose dependent manner as indicated by disturbed kidney functions; renal tissues oxidative stress markers with increased % of DNA damage (DNA strand breaks indicating cellular apoptosis) and histopathological alterations. In coincidence with this finding, **Abdelhalim and Jarrar (2011); Jung *et al.* (2012); Painoa *et al.* (2012) and Abdelhalim and Abdelmottaleb (2013) and Shrivastava *et al.* (2014)** confirmed the nephro-; and geno-toxic effects of AuNPs in experimental animals at different higher doses than therapeutic one which was dependent on the size of AuNPs and duration of exposure. They increased BUN and creatinine levels; tissue MDA and decreased renal GSH content and CAT activity.

Since, membrane phospholipids are major targets of oxidative damage, lipid peroxidation is often the first parameter analyzed for proving the involvement of free radical damage. Lipid peroxidation produces a progressive loss of cell membrane integrity, impairment in membrane transport function and disruption of cellular ion homeostasis **(Bano and Bhatt 2007).** The increased MDA levels following AuNPs exposure in our investigation could be attributed to increased lipid peroxidation due to increased free radical production **(Chuang et al., 2014).**

The recorded AuNPs- induced renal oxidative stress was reflected as disturbance in kidney function and histological structure and induction of renal cell apoptosis. Gold nanoparticles administration induced different time and dose dependent renal histopathological alterations.

The nephrotoxicity induced by AuNPs may be attributed to their cytotoxic and genotoxic effects. In addition, it may be due to the observed AuNPs - induced severe pathological alterations in liver and kidney tissues especially at the high dose. As a metal, the observed AuNPs- induced cytotoxicity and genotoxicity with the recorded biochemical and pathological alterations may be related to the generation of organic radicals and reactive oxygen species **(Chuang *et al.*, 2014).** This could be confirmed by the recorded oxidative stress (increased MDA level with decreased GSH content and CAT activity).

It has been shown experimentally that gold nanorods can enter mammalian cells **(Alkilany and Murphy, 2009; Hauck *et al.*, 2008; Takahashi *et al.*, 2008).** It was generally accepted that larger particles (500 nm) enter cells via phagocytosis while receptor-mediated endocytosis (RME) was suggested as the primary mechanism of cell uptake for nanoparticles with dimensions less than 100 nm **(Conner and Schmid, 2003). Oyelere *et al.* (2007)** qualitatively showed that gold nanoparticles could enter the nucleus if the particles were functionalized with a nuclear targeting agent.

Once AuNPs are taken inside the cell, they produce ROS and generate oxidative stress, which is responsible for many deleterious effects in the cell including DNA damage, lipid peroxidation and protein modification **(Satoh, 1978).** In addition, these metallic nanoparticles or their radical metabolites may be interacted with the mitochondrion as that reported by **Yu *et al.* (2009),** which may lead to uncoupling between oxidative phosphorylation and electron transport in respiratory chain **(Michałowicz and Duda, 2006).**

In vivo, **Tedesco *et al.* (2010)** concluded that small AuNPs seemed to have greater effects on thiol-containing protein profiles in Mytilus edulis than Cd as a well-known environmental pollutant. In particular, their work showed that AuNP (5 nm) caused significantly greater oxidative stress and cytotoxicity effects than AuNPs of larger average diameters **(Pan *et al.*, 2007; Tedesco *et al.*, 2008, 2010).** Reactive oxygen species production could result from the proportionately high surface area of AuNPs **(Nel *et al.*, 2006).** Gold nanoparticles (5 nm) are known to catalyze NO production from

endogenous S-nitroso adducts with thiol groups in blood serum (**Jia *et al.*, 2009**). NO reacts rapidly with superoxide producing peroxynitrite which can interact with lipids, DNA, and proteins via direct oxidative reactions or via indirect, radical-mediated damage (**Senaratne *et al.*, 2006**).

In vitro, AuNPs exposure most likely cause cytotoxicity that is associated with oxidative stress, endogenous ROS production; depletion of the intracellular antioxidant pool and mitochondrial damage (**Yu *et al.*, 2009**). **Li *et al.* (2010)** showed that AuNPs, which were taken up by MRC-5 human lung fibroblasts *in vitro*, induced autophagy concomitant with oxidative stress. AuNP treated cells also generated significantly more lipid hydroperoxides, a positive indication of lipid peroxidation. In addition, AuNP treatment also induced upregulation of antioxidants, stress response genes and protein expression.

In agreement with our results, **Shrivastava *et al.* (2014)** also demonstrated significant increase in reactive oxygen species (ROS) and depletion of antioxidant enzyme status in erythrocytes and tissues of mice after 14 days oral administration of AuNPs. Hepatic and renal toxicity was evident from liver and kidney function tests. Inflammatory markers, interleukin-6 and nitric oxide synthase were increased in plasma following exposure to these NPs. Toxic potential of these NPs was further confirmed by increased 8-hydroxy-2'-deoxyguanosine levels in urine, a biomarker of DNA damage. They concluded that oxidative stress was the major mechanism responsible for the toxic manifestations induced by AuNPs.

The increased renal cell's DNA damage and the consequently observed increased apoptosis could be attributed to the AuNPs-induced oxidative stress especially GSH depletion as recorded by **Gao *et al.* (2011)** and **Shrivastava *et al.* (2014)**.

Conclusion:

The study, could conclude that AuNPs had severe nephrotoxic effects in rats when given at the high (double therapeutic) dose. However, at the low (therapeutic) dose, these effects were minimum and only observed at the last 2 time points of exposure. At the high dose, they significantly increased serum urea, creatinine levels and renal tissue's MDA content. Moreover, they significantly decreased renal tissue's GSH content and CAT activity with different time and dose dependent tissue's pathological alterations and cellular apoptosis. All these effects were reflected by kidney dysfunction, oxidative stress as well as cellular lipid peroxidation, apoptosis and histopathological alterations.

From our results, we recommend that usage of gold nanoparticles must not exceed their therapeutic dose to minimize their potential nephrotoxicity and co-administration of an antioxidant to alleviate their oxidative stress properties.

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الملخص العربى

اصبحت جزيئات الذهب متناهية الصغر موضع اهتمام خاص ، بسبب التطبيقات الطبية الحيوية مثل تتبع الخلية، المجسات الحيوية، والتصوير الطبى ، تقديم الأدوية المستهدفة، وهندسة الأنسجة. وكان الهدف من هذا البحث دراسة التسمم الكلوى المحتمل لجزيئات الذهب متناهية الصغر (20 نانومتر) على الجرذان. لذلك، تم تقييم وظائف الكلى بالإضافة إلى الضغط التأكسدى والسمية الجينية. وعلاوة على ذلك تم إجراء فحص الأنسجة.

تم تقسيم إناث الجرذان البالغات إلى ثلاث مجموعات متساوية من 20 حيوانا لكل منها. المجموعة الأولى: (مجموعة ضابطة)، تم حقن الحيوانات بها داخل البريتون بمحلول ملحي فسيولوجي (0.9٪ كلوريد الصوديوم) يوميا لمدة 7 أيام. و حقنت المجموعتين الأخرتين ب 19.7 و 39.4 ميكروجرام/كيلوجرام من جزيئات الذهب متناهية الصغر(20 نانومتر) فى البريتون يوميا لمدة 7 أيام، على التوالي. تم جمع عينات من الدم والنسجة الكلى من المجموعة الضابطة والمجموعتين المعالجتين خلال أربع نقاط زمنية مختلفة (بعد 1 و 3 و 5 و 7 أيام من بداية التجربة)، حيث تم ذبح خمسة جرذان لكل مجموعة في كل نقطة زمنية.

وكشفت النتائج أن التعرض لجزيئات الذهب متناهية الصغر بجرعة 39.4 ميكروجرام / كيلوجرام ، تعزز التسمم الكلوي عن طريق زيادة مستويات اليوريا والكرياتينين في الدم من اليوم الاول حتى نهاية التجربة مقارنة مع قيم المجموعة الضابطة المناظرة والمجموعة المعالجة بالجرعة العلاجية (19.7 ميكروجرام / كيلوجرام). وعلاوة على ذلك، حقن الجرذان داخل البريتون بالجرعة السامة من جزيئات الذهب متناهية (39.4 ميكروجرام / كيلوجرام) أدى إلى انخفاض ملحوظ للجلوتاثيون ونشاط انزيم الكاتاليز فى الأنسجة الكلوية في كافة مراحل التجربة مع زيادة ملحوظة فى تراكم المادة الفعالة لحمض الثيوباربيتوريك طوال فترة الدراسة مقارنة بالمجموعة الضابطة والمجموعة ذات الجرعة المنخفضة.

وأظهر الفحص المذنب للحامض النووى (دى ان اى) أن التعرض لجزيئات الذهب متناهية الصغر يسبب موت خلايا الكلى المبرمج، الذي اثبت من خلال انخفاض كبير في نسبة الخلايا السليمة، وقطر الرأس، ونسبة الحامض النووى فى الرأس في المجموعة المعالجة بالجرعة المضاعفة للجرعة العلاجية من جزيئات الذهب متناهية الصغر مقارنة بالمجموعة الضابطة ومجموعة الجرعة العلاجية. وقد تأكد التأثير الكلوى السام لجزيئات الذهب متناهية الصغر من خلال التغيرات الهستوباثولوجية المسجلة وخاصة في الجرعة العالية.