The effects of intraperitoneal administration of gold nanoparticles size and exposure duration on oxidative and antioxidants levels in various rat organs

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Abstract: As one of the toxic mechanism of nanoparticles (NPs), the reactive oxygen species (ROS) generation which has been widely studied. Nevertheless, the link between GNPs and antioxidant and oxidative stress markers has not been well established. The effects of gold nanoparticles (GNPs) size and exposure duration on antioxidant and oxidative stress markers including reduced glutathione (GSH), super oxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), total antioxidant capacity and malondialdehyde (MDA) were evaluated in different rat organs. Adult male Wistar-Kyoto rats were randomly divided into 6 groups of 5 animals each. One group served as control and received vehicle only. The 10 nm GNPs were used in this study. The GNPs electron density and homogeneity in shape and size was evaluated. Dose of 50 µl of 10 nm GNPs in aqueous solution were administered to animals via intraperitoneal administration daily for exposure duration of 3 or 7 days. The rats were sacrificed 24 h after the last injection of GNPs. The specimens of liver, lung, kidney and heart were collected for biochemical analyses. The GPx, total antioxidant capacity, GSH and MDA levels significantly increased after administration of 10 nm GNPs for exposure duration of 3 and 7 days in the organs of rats compared with the control while the GR and SOD levels significantly decreased. The GNPs have the potential to interact with the biological system and cause undesirable effects. One of these damaging effects could be the disturbance in the natural balance between oxidative stress and antioxidant defense indices, which in turn can lead to various pathological effects. The changes in antioxidant and oxidative stress markers might be attributed to the production of ROS.

Keywords: Gold nanoparticles; malondialdehyde; antioxidant enzymes; superoxide dismutase; glutathione peroxidase; reduced glutathione; glutathione reductase.**;**

INTRODUCTION

The NPs have a wide range of applications due to their unique properties. The GNPs can easily enter the cells (Connor *et al*., 2005) and the demonstration that amine and thiol groups bind strongly to GNPs has enabled their surface modification for biomedical applications (Dani *et al*., 2008; Shukla *et al*., 2005; Xu and Han, 2004). The NPs possess better tissue penetration and higher biological potency due to their small sizes and large reactive surfaces (Huang *et al*., 2010).

The histological alterations induced in liver and kidney (Abdelhalim and Jarrar, 2011), heart (Abdelhalim, 2011 and 2012) and lung (Abdelhalim, 2011 and 2012) organs of the rats by administration of GNPs were size-dependent with smaller ones induced more affects and related with time exposure of GNPs. These alterations related to GNPs toxicity that became unable to deal with the accumulated residues resulting from the metabolic and structural disturbances caused by these GNPs. The histological alterations suggested that GNPs might interact with proteins and enzymes of the different rat organs interfering with the antioxidant defense mechanism and leading to ROS generation, which in turn may induce stress in the rat organs to undergo necrosis.

The GNPs were found to induce a significant oxidative stresses in the liver of rats, and it plays important roles in inflammatory, genotoxic and proliferative responses (Schins, 2003; Knaapen *et al*., 2004; Borm *et al*., 2004; Jia *et al*., 2009; Renault *et al*., 2008; Tedesco *et al*., 2008; Tedesco *et al*., 2010a,b; Cho *et al*., 2009; Murphy *et al*., 2008). However, there are contradictory data about the oxidative stress caused by GNPs. Some studies have reported that GNPs induce oxidative stress (Du *et al*., 2009; Jia *et al*., 2009; Tedesco *et al*., 2010a), while others observed no significant induction of oxidative stress or inflammatory response (Tedesco *et al*., 2008).

The recent studies have demonstrated that GNPs are potential antioxidants (Tedesco *et al*., 2010a) effective in quenching ROS, including H_2O_2 and superoxide anion radical ($O_{\cdot 2}$) in a dose-dependent manner (Xia and Nel, 2008; Tedesco *et al*., 2010b). In general, most studies have excluded the toxicity induced by 4-18 nm GNPs (Connor *et al*., 2005; Khan *et al*., 2007; Shukla *et al*.,

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2005) through the pro-apoptotic effects, oxidative stress and inflammatory response. Barathmanikanth *et al*. (2010) have described the effectual role of GNPs as an anti-oxidative agent, by inhibiting the formation of ROS, scavenging free radicals and creating a sustained control over hyperglycemic conditions.

The living organisms have a large number of antioxidants and enzymes, which represent the total antioxidant activity of the system, and play a central role in preventing the oxidative stress. Therefore, quantitative measurement of the cumulative antioxidant capacity of body fluids, organs and cells, following different stimuli, may provide important biological information. The toxicological effects induced by the administration of the smaller and the exposure duration of GNs on the different rat organs have not been well documented. This study aimed to evaluate the effects of 10 nm GNPs on the oxidative stress markers and antioxidant defense indices levels in the different rat organs at the exposure duration of 3 and 7 days.

MATERIALS AND METHODS

The GNPs size

10 nm GNPs (MKN-Au-010; MK Impex Corp. MKnano 6382 Lisgar Drive Mississauga, Ontario L5N 6X1, CANADA) were purchased and used in this study. The mean size and morphology of GNPs were evaluated using the transmission electron microscopy (TEM).

Animals and treatment groups

Adult male Wistar-Kyoto rats, weighing 230±20g, obtained from the Laboratory Animal Centre, King Saud University. The animals were housed in humidity and temperature-controlled ventilated cages on a 12h day/night cycle, with free access to standard laboratory rats' diet and tap water. The animals were randomly divided into 5 groups of 5 animals each. One group served as control and received vehicle only. The remaining four groups were treated with GNPs for exposure duration of 3 and 7 days, respectively.

The gold nanoparticles and dosing

The 10 nm GNPs (MKN-Au-010 of concentration 0.01%) purchased from MK Impex Corp., Ontario, Canada. The GNPs demonstrated high electron density which highly homogeneous in shape and size. Dose of 50µl of 10 nm GNPs in aqueous solution administered to animals via intraperitoneal administration daily for 3 or 7 days. The rats were sacrificed 24 h after the last injection of GNPs. The specimens of liver, lung, kidney and heart were collected for biochemical analyses. All experiments were conducted in accordance with the guidelines approved by the Local Animal Care and Use Committee.

Chemicals

Tetraethoxypropane (TEP) and thiobarbituric acid (TBA) were obtained from Fluka (Milwaukee, USA). The Detect X® Glutathione Colorimetric Detection Kit was purchased from Arbor Assay (Michigan, USA). Other chemicals used were of analytical grade and were from Sigma (St. Louis, Missouri, USA).

The glutathione peroxidase (GPx)

(EC 1.11.1.9) was assayed using tert-butyl hydroperoxide and NADPH as substrates by Beutler method (1971). The conversion of NADPH to NADP+ was followed by recording the changes in absorbance at 340 nm, and the concentration of NADPH was calculated using a molar extinction coefficient of $6.22\times103\ M^{-1}\cdot cm^{-1}$. The activity expressed as U/mg. One unit of activity defined as the amount of enzyme that catalyzes the conversion of one µmole of NADPH per minute under standard conditions.

The glutathione reductase (GR)

(EC 1.6.4.2) activity measured according to the method of Goldberg and Spooner (1983), in 0.1M phosphate buffer, pH 7.4 with 0.66mM GSSG and 0.1mM NADPH by recording the decrease of absorbance at 340nm. The activity of this enzyme was expressed as U/mg, one unit of GR activity been defined as one µ mole of NADPH per minute under standard conditions. All enzymatic activities were calculated as specific activities (units/mg protein) expressed as percentage of controls.

The total antioxidant capacity

Total Antioxidant Status (TAS) measured using a kit manufactured by Randox Laboratories Ltd. (Cat. No. NX2332). Assays performed at 37°C using a CobasFara centrifugal analyser (Roche, Switzerland) with readings taken at 600 nm. The test requires 5µl of sample, with a read time of 3 minutes.

The reduced glutathione (GSH)

The cellular lysate, deproteinized with 5% sulfosalicylic acid, analyzed for total glutathione and oxidized glutathione (GSSG) using the Detect X® Glutathione colorimetric detection kit and following manufacturer's instructions. The reduced glutathione (GSH) concentration is obtained by subtracting the GSSG level from the total glutathione. The total and GSH levels were calculated as nmoles/mg protein.

The super oxide dismutase (SOD)

(EC 1.15.1.1) activity was measured according to the spectrophotometric method of Paoletti *et al*. (1986), based on NADPH oxidation by the superoxide anion generated from molecular oxygen by a purely chemical reaction in the presence of EDTA, manganese (II) chloride and mercaptoethanol. The decrease in absorbance at 340 nm due to NADPH oxidation was followed for 10 min. A control was run with each set of three duplicate samples

and the percent inhibition was calculated as (sample rate)/(control rate) × 100. One unit (U) of activity was defined as the amount of enzyme required to inhibit the rate of NADPH oxidation of the control by 50 %.

The malondialdehyde (MDA)

As an *in vitro* marker of lipid peroxidation, was assessed by a method described by Del Rio *et al*. (2003). To 200µl of sample with a protein concentration of 2mg/ml, 700µl of 0.1M HCl was added and the mixture was incubated for 20 min at room temperature. Then, 900µl of 0.025 M thiobarbituric acid (TBA) was added and the mixture was incubated for 65 min at 37°C. Finally, 400 µL of 10mM PBS was added. The fluorescence of MDA was recorded using a 520/549 (excitation/emission) filter. A calibration curve with MDA in the range 0.05–5 µM was used to calculate the MDA concentration. The results were expressed as nmoles of MDA/mg protein.

RESULTS

Size and morphology of GNPs

The 10 nm GNPs showed spherical morphology with a narrow particle size distribution when dispersed in the solution. The mean size for GNPs was calculated from the TEM images. The mean measured size was 9.45±1.33 nm for 10 nm GNPs images (fig. 1). The high electron density and homogeneous shape and size of GNPs make them highly conspicuous under the TEM (Abdelhalim and Mady, 2011; Abdelhalim and Jarrar, 2011a; Abdelhalim and Jarrar, 2011b).

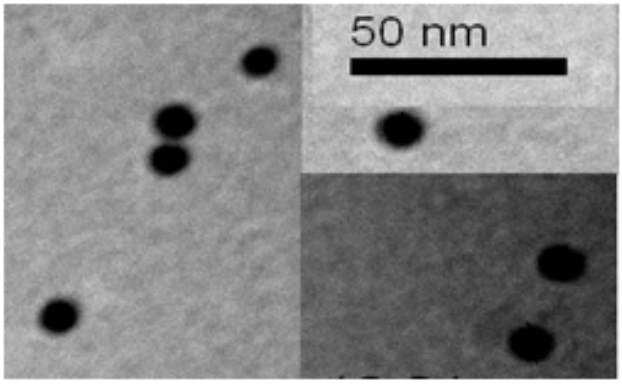

Fig. 1: TEM images for GNPs samples (Abdelhalim and Mady, 2011).

The GNPs have a wide range of applications in the various biomedical fields. It is therefore essential to study their interaction with the biological system. In the present study, the effect of GNPs on the oxidative stress and antioxidants levels in the various rat organs studied. The Gpx level significantly increased in the rat liver organ after exposure duration of 3 and 7 days compared with the control (fig. 2).

The GR is the substrate of GPx, it significantly increased which leads to decrease in GR. The level of GR significantly decreased after intraperitoneal administration of 10 nm GNPs for exposure duration of 3 and 7 days in liver, kidney and lung organs of rats compared with the control (fig. 3). The GR catalyzes the reduction of oxidized glutathione (GSSG) to GSH. This enzyme is found in many rat organs, enables the cell to sustain adequate levels of cellular GSH. The GSH is a substrate for the GPx, which provide a mechanism for the detoxification of peroxides, and glutathione S-transferases, which are involved in the conjugation and elimination of xenobiotics from the organism. The GSH also acts as an antioxidant and reacts with the free radicals and organic peroxides.

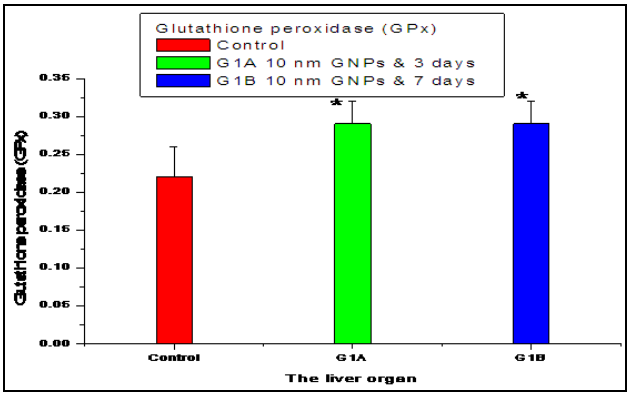

Fig. 2: The GPx levels after intraperitoneal administration of 10 nm GNPs for exposure duration of 3 (G1A) and 7 (G1B) days in the rat liver organ

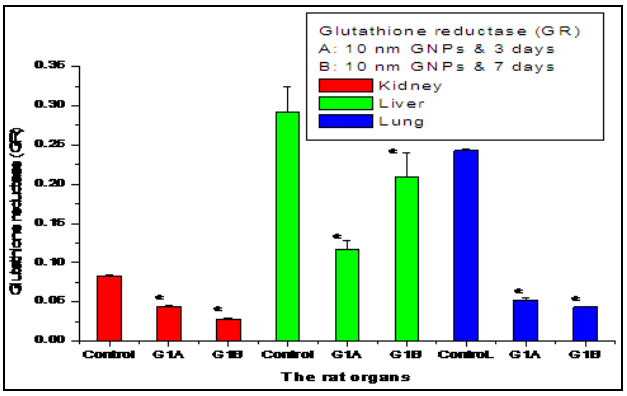

Fig. 3: The glutathione reductase (GR) levels after intraperitoneal administration of 10 nm GNPs for exposure duration of 3 (G1A) and 7 (G1B) days in the rat liver, kidney and lung organs

The level of total antioxidant capacity in rat kidney, liver and lung organs significantly increased after intraperitoneal administration of 10 nm GNPs for exposure duration of 3 and 7 days compared with the control (fig. 4). The total antioxidant capacity level gives information on the overall status of antioxidants within the biological sample (Rosenkranz, 2010). Recently, the protective effect of 13 and 50 nm GNPs on joint swelling

in a rat arthritis model has attributed to increased level of antioxidant enzyme catalase by GNPs (Leonavicˇien_e *et al*., 2012). Moreover, there is a plea for developing new artificial antioxidant based on enhanced radical-scavenging activity by antioxidant-functionalized GNPs (Yin, 2007). To cope with the elevated oxidative stress, the cells mount protective or injurious responses. For instance, the cells activate enzymatic and non-enzymatic antioxidant defense mechanisms like GPx, catalases, SOD, etc (Huang *et al*., 2010). The GSH level significantly increased in the rat liver, lung heart and kidney organs after intraperitoneal administration of 10 nm GNPs for exposure duration of 3 and 7 days compared with the control (fig. 5).

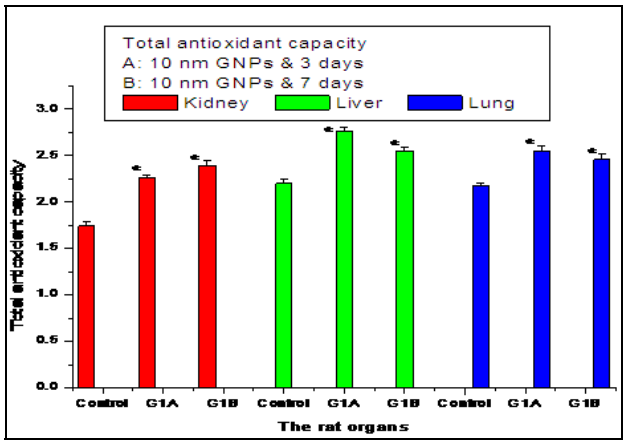

Fig. 4: The total antioxidant capacity levels after intraperitoneal administration of 10 nm GNPs for exposure duration of 3 (G1A) and 7 (G1B) days in the rat liver, kidney and lung organs.

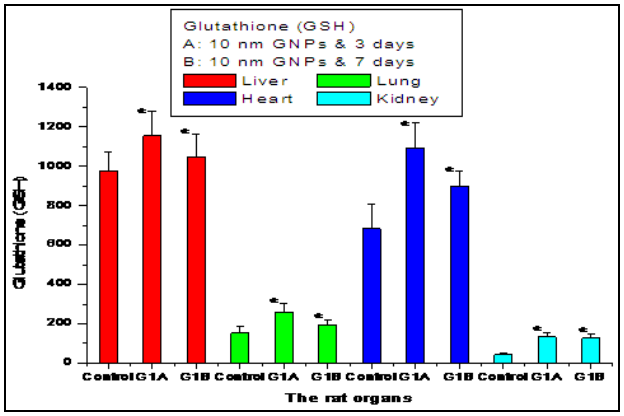

Fig. 5: The GSH levels after intraperitoneal administration of 10 nm GNPs for exposure duration of 3 (G1A) and 7 (G1B) days in the rat liver, lung, heart and kidney organs.

The level of superoxide dismutase (SOD) significantly decreased in rat kidney liver and lung organs after intraperitoneal administration of 10 nm GNPs for exposure duration of 3 and 7 days compared with the control (fig. 6). The SOD catalyzes the dismutation of the superoxide anion ($O_2^{\cdot-}$) into hydrogen peroxide and molecular oxygen, is one of the most important antioxidative enzymes. In order to determine the SOD activity, several direct and indirect methods have developed.

The malondialdehyde (MDA: lipid peroxidation) values significantly increased in rat liver, lung, heart and kidney organs after intraperitoneal administration of 10 nm GNPs for exposure duration of 3 and 7 days compared with the control (fig. 7). This indicates the increased production of free radicals or ROS in these organs, which became concomitant with the increased production of MDA. Haseeb *et al*. (2012) found the same result for MDA in the rat liver organ while for the rat heart and lung organs there is no significant change.

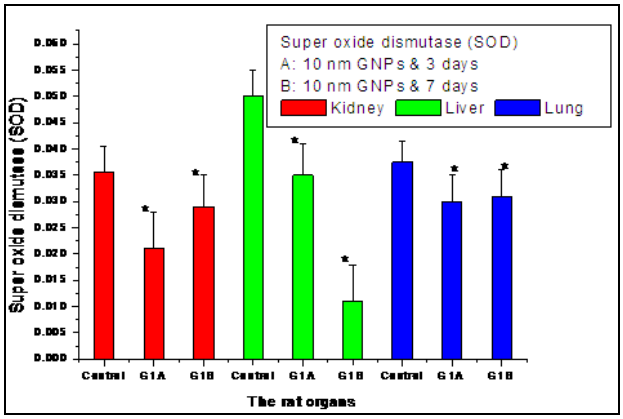

Fig. 6: The superoxide dismutase (SOD) levels after intraperitoneal administration of 10 nm GNPs for exposure duration of 3 (G1A) and 7 (G1B) days in the rat kidney, liver and lung organs.

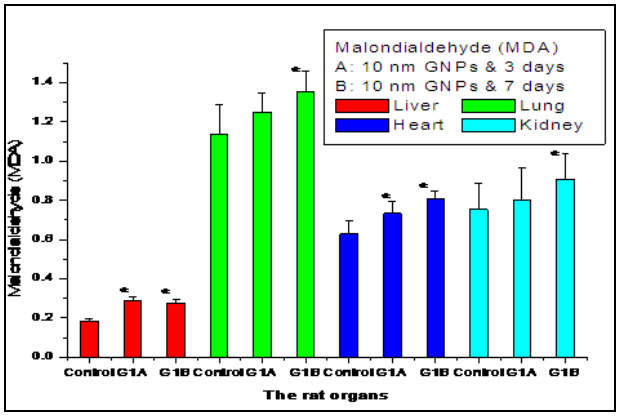

Fig. 7: The malondialdehyde (MDA) levels after intraperitoneal administration of 10 nm GNPs for exposure duration of 3 (G1A) and 7 (G1B) days in the rat liver, lung, heart and kidney organs.

The increase in total antioxidant capacity may eliminate the increase in the free radical generation indicated by MDA. If the increases in both antioxidants and oxidants are balanced, it is not possible to say that oxidative stress

exists. This idea is true if we studied the effects of both total antioxidant capacity and MDA together, but we studied the effects of each marker in different rats' organs separately. The significant increase in total antioxidant capacity level, produced in several rat organs, after intraperitoneal administration of 10 nm GNPs for exposure duration of 3 and 7 days compared with the control might indicate decreased production of free radicals or ROS within the biological sample for this short period, while it may induce the opposite for a long period.

DISCUSSION

The GPx plays a crucial role in protecting cells from the damage induced by the free radicals, which formed by peroxide decomposition. The lipid components of the cell are especially susceptible to reactions with free radicals, resulting in lipid peroxidation. The GPx enzymes reduce peroxides to alcohols using glutathione, thus preventing the formation of the free radicals.

The cellular GPx is present in all organs; however, various diseases may influence its level. An increase in the level of glutathione peroxidase has observed in reticulocytes of diabetic rats. The level returned to normal after administration of insulin. A decrease in the level of the enzyme has observed in patients suffering from diseases associated with extreme hemolytic crisis or hairy cell leukemia.

The GPx enzymes catalyze the reduction of hydrogen peroxide (H_2O_2) and a wide variety of organic peroxides (R-OOH) to the corresponding stable alcohols (R-OH) and water using cellular glutathione as the reducing reagent. The enzyme then uses the reduced glutathione as a hydrogen donor to regenerate the selenocysteine. The GPx enzymes also exist as non-selenium containing enzymes.

The NPs have the potential to interact with the biological system and cause undesirable effects. One of these damaging effects could be the disturbance in the natural balance between oxidative stress and antioxidant defense indices, which in turn can lead to various pathological effects. The oxidative stress has identified as a likely mechanism of nanoparticle toxicity (Li *et al*., 2008).

The liver may be susceptible to lipid peroxidation than other organs. The GNPs taken up by the Kupffer cells of the liver and their bioaccumulation regulated by the reticuloendothelial system (Abdelhalim and Jarrar, 2011). In comparison with the control rats, the exposure to GNPs doses has produced inflammatory cell infiltration, Kupffer cells hyperplasia, central veins intima disruption, hepatic strands dilatation and occasional fatty change together with a loss of normal architecture of the hepatic strands (Abdelhalim and Jarrar, 2011).

Haseeb *et al*. (2012) have found the same result in most organs of the rats. The GSH is one of the primary cellular antioxidant defenses against oxidative stress. The cysteine amino acid in glutathione can function as a thiol reducing agent, thus buffering cellular oxidants. Glutathione homeostasis predominantly regulated by a complex cycle of synthesis and catabolism that occurs in the liver, lung and kidney. Under physiological conditions, glutathione reductase rapidly reduces any oxidized glutathione (GSSG) to its thiol form (GSH), so that under normal conditions more than 98% of intracellular glutathione is GSH (Deleve and Kaplowitz, 1991).

In the present study, nanoparticle treatment caused a significant increase in the reduced glutathione, which may be due to increase in lipid peroxidation in the liver and a preventive measure in lungs and kidneys. The GSH has a prominent role in resistance to chemotherapy (Chen *et al*., 1998). The GSH and its associated enzymes play a critical role in the cell susceptibility to the cytotoxic effect of alkylating agents, doxorubicin, cisplatin and nitrosoureas (Tew, 1994). It has shown that for these drugs, the increased cellular GSH levels can confer cells resistance and decrease cellular GSH levels which can sensitize cells to the killing effects (Chiba *et al*., 1996; Yang *et al*., 2000).

The results of this study showed that the intraperitoneal administration of 10 nm GNPs caused oxidative stress in several rat organs. Recently, Lasagna-Reeves *et al*. (2010) have showed a low level of toxicity at the dose range 320-3200lg/kg/day. Zhang *et al*. (2010) have noticed that the intraperitoneal administrations of GNPs are less toxic than the oral administration at the dose of 1100 lg/kg. The NPs size is a key factor in the biological responses to NPs; the smaller NPs tend to be more toxic than the larger ones (Abdelhalim and Jarrar, 2011). The exposure of GNPs (5.3±1 nm) has produced oxidative stress within 24h in Mytilusedulis (Tedesco *et al*., 2010). At sizes larger than 5 nm, the general assumption is that gold is chemically inert like the bulk. However, the chemical reactivity of GNPs for diameters less than 3 nm is most likely different than larger GNPs (Tsoli *et al*., 2005).

Abdelhalim and Jarrar (2011) have reported that the exposure to intraperitoneal administration of 10 nm GNPs for exposure duration of 3 and 7 days produced the following histological alterations in the different rat organs: 1) In the liver: the hepatocytes, portal triads and the sinusoids, which were mainly vacuolar to hydropic degeneration, cytopasmic hyaline vacuolation, polymorphism, binucleation, karyopyknosis, karyolysis, karyorrhexis and necrosis (Abdelhalim and Jarrar, 2011); 2) In the kidney: cloudy swelling, vacuolar degeneration, hyaline droplets and casts, anisokaryosis, karopyknosis, karyorrhexis and karyolysis. The glomeruli showed moderate congestion with no hypercelluraity, mesangial

proliferation or basement membrane thickening (Abdelhalim and Jarrar, 2011); 3) In the heart: congested heart muscle with prominent dilated blood vessels, scattered and extravasations of red blood cells, focus of muscle hyalinosis, disturbed muscle fascicles, dense prominent focus of inflammatory cells infiltrate by small lymphocytes and few plasma cells (Abdelhalim and Jarrar, 2011; Abdelhalim, 2011 and 2012).

The aggregation of NPs could influence their ability to interact with or enter cells, thus adds complexity to the system (Alkilany and Murphy, 2010). The organ distributions of GNPs are size-dependent (Abdelhalim and Jarrar, 2011) while the smaller GNPs of 5–15 nm have wider organ distribution than that of large GNPs of 50–100 nm (de Jong *et al*., 2008; Semmler-Behnke *et al*., 2008; Chen *et al*., 2009). It has found that GNPs with a long blood circulation time can accumulate in the liver and spleen, and significantly affect the gene expression (Balasubramanian *et al*., 2010; Cho *et al*., 2009). Thus, the hepatotoxicity of GNPs may attribute to accumulation of NPs in the liver.

The liver and spleen are considered two organs for the biodistribution and metabolism of GNPs (de Jong *et al*., 2008; Semmler-Behnke *et al*., 2008; Chen *et al*., 2009; Balasubramanian *et al*., 2010). It thought that NPs should have final hydrodynamic diameters 65.5 nm to excrete from the rat body through kidneys (Choi *et al*., 2007). If GNPs are larger than this renal filtration cutoff, they not excreted in urine; instead, they eliminated from the blood by the reticuloendothelial system and thus tend to accumulate in the spleen and liver (de Jong *et al*., 2008; von Maltzahn *et al*., 2009).

CONCLUSIONS

The NPs have the potential to interact with the biological system and cause undesirable effects. One of these damaging effects could be the disturbance in the natural balance between oxidative stress and antioxidant defense indices, which in turn can lead to various pathological effects. The oxidative stress has identified as a likely mechanism of NPs toxicity.

The GPx, total antioxidant capacity, GSH and MDA levels significantly increased after intraperitoneal administration of 10 nm GNPs for exposure duration of 3 and 7 days in the organs of rats compared with the control. The GPx enzymes might reduce peroxides to alcohols using glutathione, thus preventing the formation of the free radicals, and the GPx enzymes might catalyze the reduction of hydrogen peroxide (H_2O_2) and a wide variety of organic peroxides (R-OOH) to the corresponding stable alcohols (R-OH) and water using cellular glutathione as the reducing reagent. The GSH is a substrate for the GPx, which provide a mechanism for the detoxification of peroxides, and glutathione S-transferases, which are involved in the conjugation and elimination of xenobiotics from the organism.

The level of GR and SOD significantly decreased after intraperitoneal administration of 10 nm GNPs for exposure duration of 3 and 7 days in the organs of rats compared with the control. The SOD catalyzes the dismutation of the superoxide anion ($O_2.^-$) into hydrogen peroxide and molecular oxygen, is one of the most important antioxidative enzymes. The GR catalyzes the reduction of GSSG to GSH. This enzyme is found in many rat organs, enables the cell to sustain adequate levels of cellular GSH.

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