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Toxic effects of fabricated gold nanoparticles in albino mice

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ABSTRACT

Polyethylene glycol (PEG) is commonly used to functionalize the surface of gold nanoparticles (GNPs) in order to improve their in vivo stability. This study investigates the in vivo toxicity of GNPs coated with PEG in mice. Gold nanoparticles were fabricated by chemical method technique, it is based on reduction of HAuCl₄. GNPs were coated with Polyethylene glycol (PEG). The product was characterized by scanning electron microscope (SEM) and UV–vis absorbance spectra. Scanning electron microscopy showed formation of gold nanoparticles in spherical shapes. The average size of the gold nanoparticles was 75.2 nm and 76.6 nm for GNPs coated with PEG. UV–vis absorbance spectra results show that the optical absorption in λ 519 nm and 525.5 nm for fresh GNPs and GNPs coated with PEG respectively. Zeta potential measurements were -26.55 mV. -8.2 mV for GNPs with PEG respectively, which are sufficient to prevent nanoparticles aggregation. 45 male albino mice were randomly divided into 3 groups, injected intraperitoneally with GNPs at doses of 0.1 ml containing 0.01 IU/10g mouse weight (group A), 0.1 ml of GNPs coated with PGD containing 0.01 IU/10g of mouse weight (group B) and 0.1 ml of distilled water/10g of mouse weight (control group) every 48 hours for 2 months. Our results show that GNPs cause change in texture of spleen, brain, liver, and kidney in vivo. However, toxicity indicates that PEG coated GNPs was less than GNPs without PEG.

Key words: Gold nanoparticle, size, mice, toxicity, polyethylene glycol
INTRODUCTION
Nanotechnology and especially nanomaterials have received much consideration because their structure and properties differ appreciably from those of molecules, atoms, and bulk materials (Murphy, 2008). The extremely small size, large surface to volume ratio and increased surface activity of the nanoparticles have rendered them applicable to a widespread range of chemical and physical aspects.

The surface bioconjugation with molecular probes and the optical properties related with the localized surface plasmon resonance of GNPs have been occupying a novel platform for anobiotechnology and biomedicine (Liopo et al., 2012). The toxicity effects of NPs have been demonstrated in some cell-based assays (Tiwari et al., 2011), that may be a production of interference with the chemical probes, or differences in the innate response of particular cell types (Shaw et al., 2008). The biocompatibilities of GNPs In vivo have been widely investigated (Connor et al., 2005), the toxicity of gold-depened on the surface chemistry (Cho et al, 2010) which is the important factor (Zhang et al., 2010), the physical dimension (Cho et al., 2010).

The stability of the naked GNPs in the physiological environment haven’t a long time, even the coatin was used (Zhang et al., 2012), because of the degradation of the coating and indu the failure the formation of GNPs. An example for that the PEG coating can decrease the zeta potential of GNPs and further prevent the aggregation partially in the blood, but these coating still cannot decrease the liver and spleen accumulation and improve the metabolism of GNPs(Zhang et al., 2012). the surface charge (zeta potential) of the PGE-coated GNPs less than that the naked, thus, it be more stability in blood, it has been found 5 nm PEG-coated GNPs have long time circulation in blood, and PEG-coated GNPs can present relatively mono-disperse distribution in the physiological environment (Lipka et al., 2010).

The aim of this study synthesis and characterization of GNP and GNP-PEG, evaluates toxicity of GNP in several rat organs of animal model (Liver, heart, brain, kidney and spleen) as well as over reduce toxicity after coping with PEG.

MATERIALS AND METHODS
Synthesis of colloidal GNPs:
Preparation of GNP: A 20 ml aqueous solution containing $2.5 \times 10^{-4}$ M HAuCl$_4$ and $2.5 \times 10^{-4}$ M tri-sodium citrate was prepared in a conical flask. Next, 0.6 ml of ice cold 0.1 M NaBH$_4$ solution was added to the solution all at once while stirring. The solution turned Purple immediately after adding NaBH$_4$, indicating particle formation (Jana et al., 2001).
Capping GNPs with PEG:
In order to produce PEG capped GNPs, 16.8 μg per ml of a synthesized GNP suspension of 5,000 Mw PEG was added to the ‘as synthesized’ GNP solutions at room temperature. After the required amount of PEG was added, the solution was stirred at room temperature for 2 h to allow for complete exchange of the citrate molecules with PEG. The GNP solutions were then centrifuged using a Contifuge 17RS, Heraeus SEPATECH at 10,000 rpm for 90 min in 10 ml batches. Of the supernatant, 9.9 ml was then decanted, leaving GNPs pellet at the bottom of the centrifuge tube. The volume was then made up to 10 ml by adding 9.9 ml of DI water and agitated. This centrifugal washing process was repeated again to remove any unattached PEG or other reactants. After washing, both the citrate- and PEG-capped GNPs were dried at 60°C for 3 days. The dried samples were sonicated for 15 min in a sonic bath (Manson et al., 2011).

Characterization of gold nanoparticles instruments
The diameter and morphology of GNPs were examined by the scanning electron microscopy (SEM) type (Inspect S50, Holand), by using an accelerating voltage of 15 KV at different magnifications. Absorption peaks have been measured by UV-Vis spectrophotometer type (Cary 5000, Varian, UK). Zeta Potentials were determined by zeta potential analyzer type (Zetasizer Nano Z, Malvern, UK) with rang (-200:200mV).

Animal model and treatment
Forty five Albino Swiss male mice were used in the present study, they were obtained from balb/C selected of good healthy conditions, housed in specially prepared well condition room in the mice house of the College of Medicine, University of Tikret. The mice were placed in a plastic cage with mineral network cover, the length was 48 cm, width 15 cm and depth 8 cm, manufactured by London plastic/North Kent LTDL, received from the animal house of the College of Veterinary Medicine, University of Baghdad. The cage was cleaned every week, The mice were kept in the animal house for about two months to acclimatize them with a 25-28°C temperature with 16 hours light system, fed with pellet feed. The mice were kept under these conditions for two weeks for acclimatization until the beginning of experiments.

The 45 mice were randomly divided into three equal groups of 15 mice each designated as A, B and control respectively. They were treated intra-peritoneally for a period of 2 months, and the doses given as follows.

1. Group A received a dose of 0.1 ml of GNPs, containing 0.01 IU / 10 g of mouse weight.
2. Group B received a dose of 0.1 ml of GNPs with PEG, containing 0.01 IU / 10 g of mouse weight.
3. The control group received a dose of 0.1 ml of distilled water solution / 10 g of the mouse weight.
Histological procedures (Technique):
Liver, heart, brain, kidney and spleen of the slaughtered mice at the end of different stages of life were removed and put in formalin 10% and fixated for (24-48) hours, then transferred in to graded series of alcohol; started from 70% alcohol. The other procedures included clearing by xylene then embedding by paraffin wax using embedding system and finally the specimens were blocked by using a master block unit (Luna, 1986).

Sectioning and staining:
The tissue blocks were serially sectioned at (5µm) thickness by using a manual microtome type (As Angila scientific, Germany) in order to make histological slides ready for staining and fixing the specimen on slides by Haupts’ adhesive. All sections then immersed in Xylene for (30) min, dehydrated through graded ethanol alcohol (5-10) min for each change, and finally immersed in tap water for (3-5) min. Routine staining of sections was performed using Harris Hematoxylin and Eosin stains, then the histological slides were mounted by cover slides using Canada Balsam.

RESULTS AND DISCUSSION
Characterization of the GNPs:
UV-Vis spectrophotometer:
GNPs have optical properties characterized by their Plasmon absorbance band found to be located at 519 nm as shown in (Figure.1) compared to 525.5 nm for GNPs-PEG. The obtained result was in accordance with previously published by Huang (2006) with an absorption peak at 519 nm with Gaussian distribution indicating formation of spherical GNPs with no aggregation of a size that indicated uniformity and excellent dispersion of colloidal GNPs (Perezjuste, 2005).

Scanning electron microscopy (SEM):
The high resolution-SEM image of GNPs was synthesized by the citrate method with monodisperse spherical shape with average size of (78.3 and 72) nm, were recorded for the citrate-capped GNPs as shown in

(Fig. 1) Relative absorbance of GNPs and GNPs-PEG
GNPs were clearly evident and that particles appeared spherical and uniform in size demonstrating homogeneity.

(Figure.2) SEM Images of GNPs

Zeta Potentials:
Zeta potential measurement of the prepared colloidal GNPs by citrate methods was -26.55 mV. Negative charge result from citrate which plays the role of both a reducing and a stabilizing agent of repulsion between GNPs that forestall the aggregation of GNPs (Ali et al., 2014).
After coating GNPs with PEG the charges changed to -8.2 mV, so the PEG coating can decrease Zeta potentials and improve the monodispersity of GNPs. This result agrees with Durr et al. (2007).

Histopathological examination
In comparison with the control group, the following histological alterations were detected in the liver tissue of GNPs-treated mice. These histological alterations in the liver revealed amyloid like subsrance deposition in the wall of sinusoids, necrosis of hepatocytes with division hepatic nuclei and neutrophils aggregation in sinusoids as shown in (Figure.3), in other section increase thickness of capsular layer due to polymorphonuclear (PMN) cells, and mononuclear cells infiltration and fibrosis were the main lesions in the liver as shown in (Figure.4), in other animal (group one) ,the liver expressed cell necrosis and division of nuclei of hepatocytes as demonstrated in (Figure.5). As well as aggregation of macrophages in one side of congested blood vessels with necrosis of hepatocyte and inflammatory cells in dilated sinusoids as shown in (Figure.6) with a granulomatous section consisting of aggregation of macrophages ,neutrophils and lymphocytes in liver parenchyma and infiltration in the adjacent tissues were recorded (Figure.7) in addition to neutrophils and mononuclear cells aggregation in dilated sinusoids (Figure.8).
The increase in GNPs levels might be an indication of injured hepatocytes due to GNPs toxicity that became unable to deal with the accumulated residues resulting from metabolic and structural disturbances caused by these
NPs (Abdelhalim & Jarrar, 2011). The spleen showed marked depletion of white pulp and fibrin networks deposition in white pulp with inflammatory cells infiltration (Figure 9) together with proliferation of fibrous connective tissue around hyperatrophy muscular layer of central arteries with depletion of white pulp (Figure, 10). In the other section, proliferation of megakaryocytes were seen (Figure, 11), and amyloid like substance deposition around white pulp as shown in (Figure, 12). The heart showed few mononuclear cells infiltration in epicardium as demonstrated in (Figure, 13). The main lesions in the kidney characterized by mononuclear cells aggregation around congested blood vessels and glomerula with acute cellular degeneration of epithelial cells of renal tubules as shown in (Figures, 14 and 15). Yu et al (2007) had shown that, when the rate have been exposed to GNP’s (30 – 110) nm for up to 15 days, the accumulation can be in the lungs, olfactory bulb, spleen, oesophagus, tongue, kidney, heart, septum and blood (Yu et al., 2007). However, the engineered gold nanomaterials of (5-8) nm were found retained in rat’s lungs before translocating into other tissues. These result also in agreement with result reported by Lasagna-Reeves et al (2010), who determine a significant amount of GNP’s in the liver, blood, brain, kidney, spleen and lungs (Lasagna-Reeves et al., 2010). Sadauskas et al (2009) shows that GNP’s bio-distribution vary with different sizes due to the availability of atoms ready to take part in various chemical reactions (Sadauskas et al., 2009).

(Fig:3) Histopathological section in the liver of animal in group one post-treatment with GNP’s shows amyloid like substance deposition in the wall of sinusoids, necrosis of hepatocytes with division of hepatic nuclei and neutrophil’s aggregation in sinusoids.
(Fig 4). Histopathological section in the liver of animal in group one post-treatment with GNPs shows increased thickness of capsular layer due to PMN and mononuclear cells infiltration and fibrosis.

(Fig 5). Histopathological section in the liver of group one post-treatment with GNPs shows single cell necrosis and division of nuclei of hepatocytes.

(Fig 6). Histopathological section in the liver of group one post-treatment with GNPs shows aggregation of macrophages in one side of congested blood vessels with necrosis of hepatocytes and inflammatory cells in dilated sinusoids.
Histopathological section in the liver of group one post-treatment with GNPs shows granulomatous section consisting from aggregation of macrophages, neutrophils and lymphocytes in liver parenchyma and infiltration in the adjacent tissues.

Histopathological section in the liver of animal in group one post-treatment with GNPs shows inflammatory cells particularly neutrophils and mononuclear cells aggregation in dilated sinusoids with amyloid-like substance deposition around sinusoids.

Histopathological section in the spleen of animal in group one post-treatment with GNPs shows marked depletion of white pulp and fibrin networks deposition in white pulp with inflammatory cells infiltration.
(Fig: 10). Histopathological section in the spleen of animal in group one post-treatment with GNPs shows proliferation of fibrous connective tissue around hyperatrophy muscular layer of central arteries with depletion of white pulp.

(Fig: 11). Histopathological section in the spleen of animal in group one post-treatment with GNPs shows proliferation of megakaryocytes.

(Fig: 12). Histopathological section in the spleen of animal in group one post-treatment with GNPs shows amyloid like substance deposition around white pulp.
(Fig:13). Histopathological section in the heart of group one post-treatment with GNPs shows few mononuclear cells infiltration in the

(Fig:14). Histopathological section in the kidney of animal post-treatment with GNPs shows a mononuclear cells aggregation around congested bloodvessels

(Fig:15). Histopathological section in the kidney of animal in group one post-treatment with GNPs shows a mononuclear cells aggregation around congested bloodvessels and around glomerula with acute cellular degeneration of epithelial cells of renal tubules
Partial surface composition coupled with size of the NP is accountable for toxic effects. However, other studies by Bar-Ilan et al (2009) demonstrated that zebra-fish embryo toxicity depends more on its surface chemical composition rather than on particle size (Bar-Ilan et al, 2009). This implies that surface functionalization or coatings of GNPs has a very huge impact on the toxicity of nanomaterials. However, GNP coated with PEG appeared low impact on tissue liver, brain, kidney, spleen and heart. The changes in kidney was atrophy of glomerular tuft with vacuolar degeneration of epithelial cells of renal tubules as shown in (Figure.16) and marked acute cellular degeneration characterized by vacuolar degeneration of epithelial cells of renal tubules as shown in (Figure.17). The changes in heart it recorded neutrophils and mononuclear cells in congested blood vessels and edema between cardiac muscle as shown in (Figure.18), while the changes in spleen expressed congested of red pulp with depletion of white pulp as shown in (Figure.19). The brain revealed inflammatory cells in congested blood vessels in their parenchyma of cerebela as shown (Figure.20), and central chromatolysis characterized by round cell body of purkinje with disappear of their nuclei as shown in (Figure.21). In other animal (group two), inflammatory cells in congested blood vessels in pia mater were seen in (Figure.22) and edema and congested blood vessels in the granular layer as shown in (Figure.23), in addition, to perivascular edema, proliferation of astrocytes and oligodendroglial cells as shown in (Figure, 24). Perineuronal edema, proliferation of oligodendroglial cells and proliferation of astrocytes with ALzheimer typell cells were observed in (Figure.25). The liver showed marked inflammatory cells particularly mononuclear cells aggregation around central veins with vacuolar degeneration of hepatocytes (Figure.26). This is due to the fact that in vivo biodistribution pattern of nanoparticles depends on particle size and surface engineering. Surface PEG reduces the uptake rate by macro- phages and prolongs the circulation half-life (Varna, 2012).

(Fig.16) Histopathological section in the kidney of animal at 60 day post-treatment with GNPs - PEG shows atrophy of glomerular tuft with vacuolar degeneration of epithelial cells of renal tubules
(Fig. 17) Histopathological section in the kidney of animal at 60 day post-treatment with GNPs-PEG shows marked acute cellular degeneration.

(Fig. 18) Histopathological section in the heart of animal at 60 day post-treatment with GNPs-PEG shows neutrophils and mononuclear cells in congested blood vessels between cardiac muscle.

(Fig. 19) Histopathological section in the brain of animal at 60 day post-treatment with GNPs-PEG shows inflammatory cells in congested blood vessels in their parenchyma of cerebela.
(Fig. 20) Histopathological section in the spleen of animal at 60 day post-treatment with GNPs - PEG shows congested of red pulp

(Fig. 21) Histopathological section in the cerebelum of animal at 60 day post-treatment with GNPs - PEG shows central chromatolysis characterized by round cell body of purkinje with disappear of their nuclei

(Fig. 22) Histopathological section in the brain of animal at 60 day post-treatment with GNPs - PEG shows inflammatory cells in congested blood vessels in pia mater
**Fig. 23** Histopathological section in the cerebellum of animal at 60 day post-treatment with GNPs - PEG shows edema and congested blood vessels in the granular layer.

**Fig. 24** Histopathological section in the cerebella of animal at 60 day post-treatment with GNPs - PEG shows perivascular edema, proliferation of astrocytes and oligodendroglial cells.

**Fig. 25** Histopathological section in the cerebella of animal at 60 day post-treatment with GNPs - PEG shows perineuronal edema, and proliferation of oligodendroglial cells, proliferation of astrocytes with Alzheimer type II cells.
Histopathological section in the liver of animal at 60 day post-treatment with GNPs - PEG shows marked inflammatory cells particularly mononuclear cells aggregation around central veins with vacuolar degeneration of hepatocytes.

CONCLUSIONS

Our results might indicate that GNPs are mostly taken up and accumulate in organs (Liver, heart, brain, kidney and spleen) and the toxic effects for GNP more than toxic effects for GNP-PEG on all organs especially in the spleen because of PEG is commonly used to functionalize the surface of GNP in order to improve their in vivo stability.

REFERENCES


Lasagna-Reeves C, Gonzalez-Romero D, Barria MA, Olmedo I, Clos A, Ramanujam VMS, Soto C. (2010). Bioaccumulation and

Liopo A V, Conjusteau A, and Oraevsky AA (2012). PEG-coated gold nanorod monoclonal antibody conjugates in preclinical research with optoacoustic tomography, photothermal therapy, and sensing. Paper presented at the SPIE 8223, San Francisco, California, USA.


