RESEARCH ARTICLE

The Effect of Polyethylene Glycol-coated Gold Nanoparticle on Mice's Renal Function

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ABSTRACT

Nanotechnology is one of the newest technologies that work on adapting matter at the atomic and molecular level, and it deals with materials or components with single dimensions ranging from (1–100) nanometers. This technology is used in fields like health and food, so this experiment was designed to know this material's effect on the kidneys. Therefore, the gold nanoparticles (GNP) were prepared using a chemical method and coating with polyethylene glycol (PEG), and then a UV-visible spectrometer, Scanning electron microscopy (SEM), and zeta potential, with their sizes, were at a rate of 22.65 and 23.82 nm and after coating with PEG, the GNP volume increased to 76.50 and 80.15 nm, and when using UV, the highest absorbance was at 515.5 nm and became 530.5 nm, and using zeta potential it was -29.65 and became -9.4 millivolts. In this experiment (75), white mice were used randomly divided into three groups. The first group was the control, and the second group was given in the sub-peritoneal region 0.1 mL GNP, and it contains 0.01 IU/10 gm of mouse weight, while the third group was given in the sub-periton area. 0.1 mL of GNP + PEG containing 0.01 IU/10 gm of mice weight, and the experiment go on (60) days. It was observed from the results of the statistical analysis of renal function test, which includes urea and creatinine that there is a significant effect when injecting GNP, especially after extending the dose to (45 and 60) days, while the effect was less on both analysts when injected the mice with a coated form of GNP + PEG.

Keywords: Gold nanoparticles, Renal function, Coating with polyethylene glycol, Mice.

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INTRODUCTION

The beginning of nanomaterial precedes the naming of nanotechnology. In the twenty-first century, increasingly thought that nanotechnology had played a vital role such as metal oxide nanoparticles with a size of 1–100 nanometers represent a new way that is being developed and increasingly paid attention to adoption in research related to medical applications (Quader and Kataoka, 2017),[1] which includes Ag, Sio2, Pt, and TiO2 particles, in addition to fullerene, carbon nanotubes, and gold (Schmalz *et al*., 2017),[2] where GNP with high stability and low-ionization metal which has been used as an expensive metal since ancient times, where this metal remain recycled. Newly developed and broadly cast-off in therapeutic and industrial engineering applications due to its distinctive photosensitive possessions (Facchi *et al*., 2017),[3] its excellent photoelectronic properties have led to its usage in organic planetary cells and devices inquiries and conductive resources (Maysinger *et al*., 2015).[4] This material remained recycled in biochemical manufacturing as a catalytic agent. Michael Faraday discovered gold nanoparticles in 1857 and presented them to the Royal Society. Albert Einstein 1905 reached the existence of colloids and proved the quantitative theory of the dispersion of colloidal suspensions where he explained that the suspension atoms move a movement that explains this movement by Brownian motion (Binning and Roher, 1986).[5]

There is a slight study on the poisonousness of gold nanoparticles and their possible interactions with medications (Chen *et al*., 2018).[6] In addition, nanoparticles can have natural properties by receiving the brain, and generative structures (Ehsanifar *et al*., 2019),[7] such as carbon nanotubes might adversely affect human healthiness (Rodriguez-Yanez *et al*., 2013).[8] Nowadays, due to the widespread application of nanoparticles, there is a great understanding of their mechanism of action and the effects of these materials on everything related to human health and the environment, as the nanoparticles tin can permit during the cell membrane easily and straight pass through the blood-brain barricade

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and the blood-testis wall, consequently, it can be moves all parts of the body. It is worth noting that the toxicity of NP to cells on the one hand and the response of other cells, on the other hand, depends on the nature of the materials and doses used. Several studies have demonstrated that the solicitation of an in elevation dosage of NP is dangerous to human health (Zhou *et al*., 2012).[9]

The discovery of the safety, pharmacy, and pharmacokinetics of nanoparticles has expanded the field of nanotechnology. Silica nanoparticles have been shown to cause hepatotoxicity, cytotoxicity, and damage to the placenta. (Yamashita *et al*., 2011).[10] Furthermore, carbon nanotubes can cause pulmonary mesothelioma (Park *et al*., 2011).[11] The toxicity of nanoparticles can be reduced by using packaging materials such as polyethylene glycol, making them more biocompatible with the human body (AL-Hadedee *et al*., 2019).[12]

The renal function test includes measurement of concentration blood urea which is synthesized from protein degradation and creatinine, as it is a compound produced from the decomposition of creatine phosphate in the muscles and the skeleton.[13] It appears i n the blood with its level as a performance indicator of the glomerulus because it is responsible for filtering, and its level changes in blood in some pathological conditions, the most important of which is a renal failure (Bamanikar, 2016).[13]

MATERIAL AND METHOD

Tri-sodium citrate (BDH (England Chlorauricacid (HAuCl4) Sigma-Aldrich/USA (Sodium Borohydride) (NaBH4Sigma-Aldrich/USAPEG) molecular weight 6000 Sigma-Aldrich/USA.

Gold Nanoparticles (GNP) Preparation

Gold nanoparticles were prepared by putting 20 mL of each $2.5*10^4$ molar of HAuCL4 and $2.5*10^4$ molar trisodium citrate ($Na_3C_6H_5O_7.2H_2O$) in a conical flask and adding 0.6 mL of 0.1 molar cooled NaBH4 and put on a mixture and continued mixing until it changed color to dark violet (AL-Hadedee *et al*., 2020b).[14]

GNP Concentration Measurement

The atomic absorption of the elements was used by the flame atomic absorption (Direct Aspiration method), where the gold was converted to the atomic state by a flame, where a beam of light is shaded from a cathode bulb-made of gold into a uniform wave and then into a torch to measure the amount of light absorbed by the flame. The prepared GNP was estimated in parts per million ppm.

Polyethylene Glycol-Coated Gold Nanoparticle preparation

After the synthesis process of GNP, it was coated with (PEG) after mixing it in a ratio of 3:1 and leave the mixture for 2 hours at room temperature of 25°C. The solution was presented for cooled centrifugation at 10,000 rpm for 90 minutes so that each batch was 10 mL, after which 9.9 mL was distilled water was added. The washing process was repeated twice to ensure disposal of the residual PEG, after which it was dried at 60°C for 3 days and then exposed to a sonic bath for 15 minutes (Manson *et al*., 2011).[15]

Diagnosis and Description of GNP and GNP + PEG

Absorbance was measured with a UV-visible spectrometer over 400–900 nm wavelengths (Scanning electron microscopy) (SEM) and zeta potential at 25 ° C room temperature.

Experiment Animals and their Shelter

In the research experiment, 75 laboratory animals of the type of Swiss mice were used. The ages of these animals ranged between (8–10) weeks, and their average weight of 30 grams, obtained from the College of Veterinary Medicine, University of Baghdad. The mice were placed in special cages with 5 mice in each cage, after washing and disinfecting the cages with Sabtol (10%) and providing these cages with plastic bottles for drinking water. The cages were placed in a room designated for the laboratory animals in the animal house of the Faculty of Veterinary Medicine - University Baghdad, after fumigating it using 35 mm of formalin with 17 grams of potassium permanganate, and the walls and floors were washed with sabtol (10%). Animal feeding relied on dry fodder in too many quantities made from compressed wheat, as the bags of feed were brought from the Serum and Vaccine Institute of the Ministry of Health, and the animals were placed for two weeks in the cages prepared for them for acclimatization.

Experience Design

The experimental mice were distributed randomly and equally into three groups, with 25 mice in each group. If the groups were injected into sub-peritoneum area, every 48 hours for 2 months, and the dose was given as follows:

- The first group of mice was injected with a dose of 0.1 mL of GNP, which contained 0.01 IU/10 g of the mouse weight.
- The second group of mice was injected with a dose of 0.1 mL of PEG + GNP, containing 0.01 IU/10 gm of mouse weight.
- The third group included control mice injected with a dose of 0.1 mL of distilled water solution/10 gm of mouse weight.

Sample Collection

After the end of the experiment, blood was pull from the heart directly by heart stab and using a sterile medical syringe of (5 mL) capacity at different times (0, 15, 30, 45, 60) days, the withdrawn blood was placed in clean test tubes free of anti-clotting substance, and left for (15–20 minutes) and then placed inside a centrifuge to separate the blood serum. The serum was isolated by a fine mechanical pipette and placed in new plastic tubes to conduct biochemical tests on urea and creatinine to assess kidney function.

Data Analysis

The data was analyzed by SAS (2012) to investigate the effect of various parameters on the examined properties according to a (Totally Randomized Design-CRD). Significant differences between the means were matched with the Least Significant Difference (LSD).

RESULTS AND DISCUSSION

SEM Measurement

It is noticed from Figure 1 that GNP appeared spherical with different sizes and was at a rate of 22.65 and 23.82nm. This difference in the size of the formed GNP is due to the concentration of HAuCl4 substance, the time of formation and the concentration of the agent GNP, ChenP, the concentration of the mixed agent (Shipway *et al*.,2000).[16]

This is in agreement with what Khalida *et al.* (2012),[17] who showed that a high concentration of gold salts causes GNP to clump with the occurrence of turbidity in the solution, and its lower concentration gives smaller particle and that the difference in the size of particle in one sample indicates that it was formed at different times.After the encapsulation process, Figure 2, the size of GNP increased to 76.50 and 80.15 nm. This difference in size between GNP and PEG + GNP is due to the role of the encapsulation process, as adding the coating material will increase the diameter of GNP (Owens *et al.,* 2006).[18]

UV Absorption Spectroscopy

Figure 3 appears on the absorbance spectrum of the prepared GNP where it was 515.5 nm, and these results correspond to the characteristic of GNP, which has an absorption spectrum range between (510–550) nm due to the surface plasmon resonance, which has strong absorption, which gives a brilliant red color to GNP in Figure 4, which in turn changes according to the change in the size of these particles. The appearance of the highest absorbance value is due to the interaction between light and GNP, which changes with the location of the highest absorption (the highest value) and thus depends on the GNP's size and shape. (Harihar *et al*, 2014).[19]

After the PEG encapsulation process, the highest value of the absorption spectrum at a wavelength of 530.5 nm became Figure 3. This difference in the absorption spectrum value of samples is due to a slight change in the position of the summit, which in turn is due to the changes that occur on the surface of gold particles as a result of the envelope striate replacement with the used material PEG (Jacobsen *et al*., 1974).[20] The difference in the location of the highest absorption value of the citrate-coated. Peg-coated nanoparticles is also due to the increase in the surface of the plasmon (Oh *et al*, 2008).[21] The increase in the absorption spectrum is due to random turns of PEG material on the gold nanoparticles atoms (Hill, 2004).[22] The change in the location of the highest absorption value of gold nanoparticles after the PEG encapsulation process depends on the ratio between gold nanoparticles and the materials used in the encapsulation, and this is consistent with what (Takahashi and colleagues 2006)[23] found in their research that the dimensions of the nanomaterials will increase from 20 to 23.3 nm after using 6000 PEG Mw. At the same time, Arinda *et al*. (2011)[24] demonstrated that 5000 PEG Mw led to an increase in the dimensionality of the nanomaterials from 50 to 69 nm.

When conducting the reaction process, the absorption value appears due to the combination of electron vibrations in the conduction beam, and it is known as the surface plasmon oscillation, and thus there is a change in the location of the absorption value.

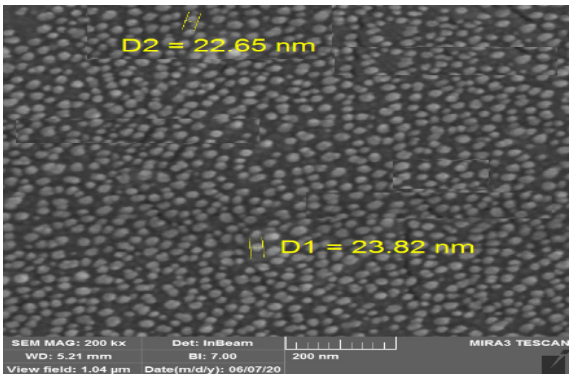

Figure 1: Size of the GNP using SEM

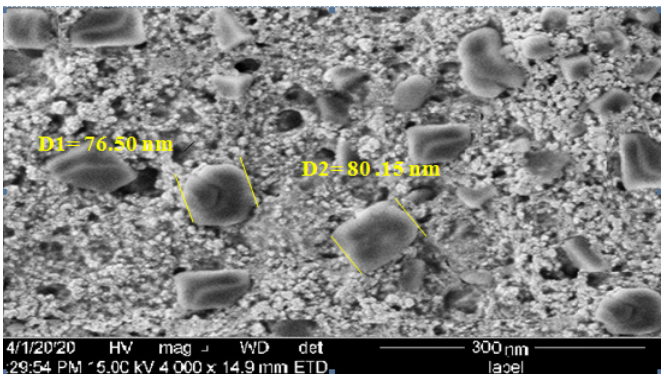

Figure 2: GNP + PEG volume using

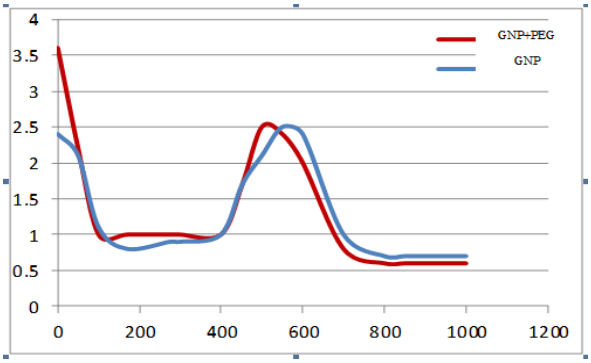

Figure 3: the UV-visible spectral absorption of GNP and) GNP + PEG)

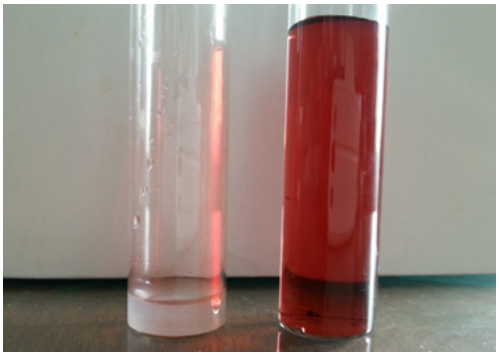

Figure 4: The color of the GNP solution

Zeta-potential

The zeta potential of the prepared GNP was measured as the zeta voltage reached 29.65-millivolts, and this confirms that the negative charge is based on citrate, which plays a role as a reducing and stabilizing factor, and that the repulsion forces between the atoms are generated due to the negative charge (Todebush *et al*., 2002).[25] If it is less than -25, it will clump because of the lack of repulsion force between the gold nanoparticles, which is insufficient to keep them apart (Ali, 2014).[26] Inside the solution. The encapsulation process also led to a change in the surface charge as it reached -9.4 millivolts for the prepared GNP + PEG about the occurrence of a change in the value of the surface charge and a reduction in the amount of oil by adding the substance PEG. The surface changes from negative charge to close to neutral when PEG is added (Etame *et al*, 2011).[27]

The Effect of GNP on Some Kidney Functions

The concentration of some non-protein nitrogenous compounds in the blood (such as urea and creatinine) indicates the efficiency of the kidney in removing these compounds from the blood through glomerular filtration and eliminating them in urine. Table 1 shows the rate of creatinine concentration (mg/dL) in the serum, where the results indicate the presence of significant differences when injecting with GNP, as the concentration of creatinine increased from 0.21 mg/dL on the first day of injection to 0.27 mg/dL while the coated nanoparticles GNP + PEG (0.25 mg/dL). The more doses are used for a longer period of time after 60 days it rose by more in case of GNP (0.3 mg/dL) while (GNP + PEG) became 0.26 mg/dL as shown in a row compared to the control group that did not show significant differences, as the creatinine concentration was 0.20 mg/dL became 0.21, 0.20 0.21, and 0.21 mg/dL, respectively, during (15,30, 45, and 60) days in a row, and this indicates that GNPs have a direct effect on kidney function,and when carrying out the encapsulation process and when injecting GNP + PEG, the creatinine concentration increased from 0.22 mg/dL to 0.22, 0.24,0.25, 0.26 mg/dL respectively during 15,30, 45, 60 days.

Blood Urea Nitrogen Concentration

Table 2 shows the average concentration of blood urea in (mmol/L), where the results indicate the presence of significant differences when injecting GNP, as the concentration of blood urea increased from (4.28 mmol/L) on the first day of injection to 5.29, 7.41, 8.68, 9. 37 mmol/L during 15, 30, 45, 60 days respectively compared to the control group that did not show any significant differences, as the blood urea nitrogen concentration was 4.22 mmol/L/liter became 4.24, 4.25, 4.25, and 4.26 mmol/L during 15, 30, 45, 60 days, respectively, and this indicates that GNPs have a direct effect on kidney function, while when carrying out the encapsulation process and injecting GNP + PEG, the blood urea nitrogen concentration increased from 4.28 mmol/L to 4.30, 5.30, 6.36, 7.40 mmol/L during 15, 30, 45, and 60 days, respectively.

When the NPs are administered, they will arrive at the circulating blood then be spread to all tissues of the body through the bloodstream that contains platelets and proteins, and its movement is dependent on the mass and charge; the GNP will go through adsorption otherwise shading by serum proteins. As a dynamic and semi-transitional barrier that regulates the transfer of fluids and large particles between inside and outside the blood vessels, if the size of these particles is more than the diameter of the intact vascular endothelium, they will suffer a long rotation due to the slow transfer through the vascular endothelium. The kidneys can quickly remove particles from the blood vessels, which is a multi-faceted process that folds over glomerular separation, tubular exudation and lastly removal of particulate matter through diuresis (Longmire, 2008).[28]

Histological studies of the kidneys showed the occurrence of histopathological changes as a result of the accumulation of GNP and GNP + PEG, including changes in the shape of the glomerulus, abnormalities in the proximal and distal tubules, necrosis and infiltration of inflammatory cells, as well as congestion in the cortex and pulp (Ibrahim, 2018).[29] Creatinine is a significant indicator of kidney function and is a metabolic waste product that is chiefly formed in the muscles and enters the bloodstream then excreted in the urine via the kidney. Its concentration in the blood depends on the filtration rate through the glomeruli, but the level of creatinine in the blood does not correspond to its clearance in early renal failure (not compensated). The creatinine clearance rate increases more than 50% of the usual range, creatinine in the blood starts

Table 1: Effect of GNP and GNP + PEG on Creatinine Concentration (mg/dL)) in Mice's Serum.

Group / *Days/period*		*Control*	*GNP*	*GNP+PEG*	*Value LSD*
Before treatment	0	0.20	0.20	0.22	0.0487 NS
During treatment	15	0.20	0.22	0.22	0.0451 NS
	30	0.21	0.25	0.24	0.0488 NS
	45	0.21	0.27	0.25	0.063 *
	60	0.21	0.30	0.26	0.078 *
LSD	-	0.066 NS	0.081 *	0.057 NS	—

* ($P \leq 0.05$)

Table 2: GNP and gNP + PEG effect on blood urea nitrogen concentration (mmol/l) in rat serum

Group / *Days/period*		*Control*	*GNP*	*GNP +PEG*	*Value LSD*
Before treatment	0	4.22	4.28	4.28	0.815 NS
During treatment	15	4.24	5.29	4.30	0.822 *
	30	4.25	7.41	5.30	1.029 *
	45	4.25	8.68	6.36	1.427 *
	60	4.26	9.37	7.40	1.844 *
LSD	—	0.173 NS	1.437 *	1.508 *	---

to increase quickly, so after the creatinine level in the blood stays considerably higher than usual, the kidney function is highly impaired. This was observed in the results of the study. (Zhang *et al*., 2011).[30] While administering 60 nm GNPs caused alteration in creatinine concentration partially. However, at 5 and 10 nm GNPs, some kidney damage occurred by reducing the level of total protein and globulin, and toxicity may be a result of PEG metabolism, and this is in agreement with Cho *et al*.,[31] whose results showed that the size of 13.5 nm of PEG causes serious toxicity while the concentration of 5.30 nm is of less toxicity and when giving a volume of 100 nm of PEG leads to a defect in kidney function. Our current study coated GNP + PEG results had a significantly less effect on urea and creatinine compared to injected GNPs alone. The elevation of blood urea also confirms an imbalance in the kidneys and metabolism because urea acts as a carrier of nitrogenous waste products, which affects the system of nephrons that re-absorb water and ions that are excreted into urine through the kidneys (Doudi *et al.,* 2013).[32]

CONCLUSION

It was observed from the results of the statistical analysis of renal function test, which includes urea and creatinine, that there is a significant effect when injecting gold nanoparticles GNP, especially after extending the dose to 45 and 60 days. In contrast, the effect was less on both analysts when injected the mice with a coated form of gold nanoparticles coating with polyethylene glycol (GNP+PEG).

REFERENCES

1. Quader S, Kataoka K. Nanomaterial-enabled cancer therapy. MolTher 2017;25(7):1501–1513 PubMed PMID: 28532763. Pubmed Central PMCID: PMC5498831. Epub 2017/05/24.eng
2. Schmalz G, Hickel R, van Landuyt KL, Reichl FX (2017) Nanoparticles in dentistry. Dent Mater 33(11):1298-1314 PubMed PMID: 28951037.
3. Facchi DP, da Cruz JA, Bonafe EG, Pereira AGB, Fajardo AR, Venter SAS et al. Polysaccharide-based materials associated with or coordinated to gold nanoparticles: synthesis and medical application. Curr Med Chem 2017;24(25):2701-2735 PubMed PMID: 28294043. Epub 2017/03/16.Eng
4. Maysinger D, Ji J, Hutter E, Cooper E. Nanoparticle-based and bioengineered probes and sensors to detect physiological and pathological biomarkers in neural cells. Front Neurosci 2015;9:480 PubMed PMID: 26733793. Pubmed Central PMCID: PMC4683200. Epub 2016/01/07.Eng
5. Binnig, G.and Rohrer, H. "Scanning tunneling microscopy". IBM J. Res. Dev. 1986;30(4):355-369.
6. Chen S, Li R, Li X, Xie J. Electrospinning: an enabling nanotechnology platform for drug delivery and regenerative medicine. Adv Drug Deliv Rev 2018;132:188-213 PubMed PMID: 29729295. Epub 2018/05/08.Eng
7. Ehsanifar M, Tameh AA, Farzadkia M, Kalantari RR, Zavareh MS, Nikzaad H et al. Exposure to nanoscale diesel exhaust particles: oxidative stress, neuroinflammation, anxiety and depression on adult male mice. Ecotoxicol Environ Saf 2019;168:338-347. PubMed PMID: 30391838. Epub 2018/11/06 Eng
8. Rodriguez-Yanez Y, Munoz B, Albores A. Mechanisms of toxicity by carbon nanotubes.ToxicolMech Methods 2013;23(3):178-195 PubMed PMID: 23193995. Epub 2012/12/01.Eng
9. Zhoua H, Yana H, Senpanb A, Wicklineb SA, Panb DG,; Lanzab M, Phama CTN,; Lanza GM, and Pham C T N. Suppression of inflammation in a mouse model of rheumatoid arthritis using targeted lipase-labile fumagillinprodrugnanoparticles; 2012;33(33): 8632-8640.
10. Yamashita K, Yoshioka Y, Higashisaka K, Mimura K, Morishita Y, Nozaki M et al. Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. Nat Nanotechnol 2011;6(5):321-328 PubMed PMID: 21460826. Epub 2011/04/05.Eng
11. Park EJ, Roh J, Kim SN, Kang MS, Han YA, Kim Y et al. A single intratracheal instillation of single-walled carbon nanotubes induced early lung fibrosis and subchronic tissue damage in mice. Arch Toxicol 2011;85(9):1121-1131 PubMed PMID: 21472445. Epub 2011/04/08.Eng
12. Al-Hadedee L.Th., hasan S. K. and Allaith.Sh.A. nanotechnology in detecting contamination and spoilage of food. Plant Archives 2019a;19(1):1334-1340.
13. Bamanikar, S.A, Bamanikar, A. A. &Arora, A. Study of Serum urea and Creatinine in Diabetic and non-diabetic patients in in a tertiary teaching hospital. Journal of Medical Research, 2016; 2(1):12-15.
14. AL-Hadedee L.Th., AL Sadoon T.H and Chechan.R.A. biocompatibility of nanoparticles and some of it's applications. Biochem.Cell. Arch. 2020b:20(1)131-136.
15. Manson, J., Kumar, D., Meenan, B. J., & Dixon, D. (2011). Polyethylene glycol functionalized gold nanoparticles: the influence of capping density on stability in various media. Gold bulletin, 44(2), 99-105.
16. Shipway, A. N., Katz, E., &Willner, I. Nanoparticle arrays on surfaces for electronic, optical, and sensor applications. Chem Phys Chem, 2000;1(1):18-52.
17. Khalida S. M., Al-Attabi, H. D., Abbas, Z. M., &Yusr, H. A. (2012).Comparative study on methods for preparation of gold nanoparticles.Green and Sustainable Chemistry, 2(01), 26.
18. Owens DE, & Peppas NA. Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles.International journal of pharmaceutics, 2006;307(1), 93-102.
19. Harihar NV, Singh P, & Chavan RM. Gold nanoparticle: synthesis and characterization. Veterinary World, 2014;7(2):72-77.
20. Jacobsen NW, & Dickinson RG. Spectrometric assay of aldehydes as 6-mercapto-3-substituted-s-trizolo(4,3-b)-tetrazines. Analytical Chemistry, 1974;46(2):298-299.
21. Oh, K. S., Kim, R. S., Lee, J., Kim, D., Cho, S. H., & Yuk, S. H. Gold/chitosan/pluronic composite nanoparticles for drug delivery.Journal of applied polymer science, 2008;108(5), 3239-3244.
22. Hill, R. J. Hydrodynamics and electrokinetics of spherical liposomes with coatings of terminally anchored poly (ethylene glycol): numerically exact electrokinetics with self-consistent mean-field polymer. Physical Review E, 2004;70(5):051406.
23. Takahashi, H., Niidome, Y., Niidome, T., Kaneko, K., Kawasaki, H., & Yamada, S. Modification of gold nanorods using phosphatidylcholine to reduce cytotoxicity.Langmuir, 2006;22(1):2-5.
24. Arnida A. Ray A, Peterson CM, & Ghandehari H. Geometry and surface characteristics of gold nanoparticles influence their biodistribution and uptake by macrophages. European Journal of Pharmaceutics and Biopharmaceutics, 2011;77(3): 417-423.

25. Todebush, M. P., Camden J., Roden J. and Schatz G.C. (2002). theoretical Analysis of Gold Nano particles. Department of Chemistry. Northwestern University.
26. Ali MA, Eldin TAS, Moghazy GE, Tork IM, & Omara II. Detection of E. coli O157: H7 in feed samples using gold nanoparticles sensor. Int. J. Curr. Microbiol. App. Sci, 2014;3(6): 697-708.
27. Etame AB, Smith CA, Chan WC, & Rutka JT. Design and potential application of PEGylated gold nanoparticles with size-dependent permeation through brain microvasculature. Nanomedicine: Nanotechnology, Biology and Medicine, 2011;7(6):992-1000.
28. Longmire,M., Peter L. Choyke, M.D., and Hisataka Kobayashi, M.D., Ph.D.(2008). Clearance Properties of Nano-sized Particles and Molecules asImaging Agents: Considerations and Caveats. Journal of Nanomedicine (Lond). 3(5): 703-717
29. IbrahimK.E., Mohsen Ghaleb Al-Mutary, Amel Omer BakhietandHaseeb Ahmad Khan. Histopathology of the Liver, Kidney, and Spleen of Mice Exposed to Gold Nanoparticles : journal molecules 2018;10:3390
30. Zhang, and Fei-Yue Fan(2011). Size-dependent in vivo toxicity of PEG-coated gold nanoparticles. Int J Nanomedicine. 6: 2071-2081.
31. Cho WS, Cho M, Jeong J, et al. Acute toxicity and pharmacokinetics of 13 nm-sized PEG-coated gold nanoparticles. ToxicolApplPharmacol. 2009;236:16-24.
32. DoudiMonir, MahbubehSetorki. The effect of gold nanoparticle on renal function in rats. Journal of nanomedicine. 2013;1:3.