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Original article

Bio-functionalized gold nanoparticles: Benign effect in Sprague-Dawley rats by intravenous administration

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ABSTRACT

Gold nanoparticles offer a great promise in clinical research. Despite various applications of the metal nanoparticles it is challenging to implement *in vivo* in clinical applications. This aspect is deprived of understanding the biological mechanisms that occurs in the cells. In this report we have evaluated application of AuNP on the safety profile at different doses (100, 200, and 500 μg/kg Bwt/day) on intravenous administration in rats regularly for 28 days. The study was performed based on the OECD test guideline 407. No clinical signs and mortalities were observed in any groups of rat treated with AuNP. No evidence of toxicity was observed in any of the diverse studies performed which is noteworthy. The study includes survival, behavior, animal weight, organ morphology, blood biochemistry and tissue histology. The results indicate that tissue accumulation pattern of gold nanoparticles depends on the surface, size and doses of the nanoparticle. The accumulation of the particles does not produce subacute physiological damage.

1. Introduction

Noble metal based nanoparticles are being produced using different physical and chemical methods, their applications in healthcare and medicine is increasing continuously. Human safety trepidations are gaining attention, which makes it necessary to understand the toxicity of these noble metals nanoparticles. The life care scientists has stressed upon the making of gold nanoparticles on bio-based modus as a need for immediate use in medicine, healthcare, in biolabelling, targeted drug-delivery, hyperthermia, and biosensors etc (El-Sayed et al., 2005; Chah et al., 2005; Huff et al., 2007; Brown et al., 2010; Giljohann et al., 2010; AnaMourato et al., 2011). However, for *in vivo* application study of all the parameters to ensure safety is mandatory for their internal application. The toxicity of gold nanoparticles have been extensively studied and reported. The over-all toxicity of gold nanoparticles depends on their intrinsic properties which in turn depend vitally on particle size, shapes and surface chemistry (such as coating) (Murphy et al., 2008; Chen et al., 2009a, 2009b). Nanoparticles in medicine have fascinated little attention due to basic chemical constituents of NPs which cause uneven biodistribution and toxic profiles (Yang et al., 2017). The biosafety of metallic gold is well understood and has been used *in vivo* since the 1950s. Functionalized gold nanoparticles show obvious toxicity *in vivo*. There is a need of *in vivo* toxicity analysis before introducing nanoparticles in medical field including drug delivery and treatment of various organ disorders (Aravinthan et al., 2016). Elaborate studies have been done to understand the cytotoxicity of gold nanoparticles which were nontoxic compared to ionic gold showing obvious cytotoxicity (Goodman et al., 2004). Similar results were also reported with functionalized gold nanoparticles *in vitro* (Pan et al., 2007). The gold nanoparticles administered through different routes. The nanoparticles with oral route offered highest toxicity and those used in tail vein injection show least

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toxicity (Zhang et al., 2010). Toxicological reports of gold nanoparticles in animal models were used as a novel agent to characterize the toxicity of gold nanoparticles (Goel et al., 2009; Samberg et al., 2010; Lasagna-Reeves et al., 2010). The *in vitro* models cannot imitate the intricacy of an *in vivo* system to provide significant pharmacological statistics about the response of a physiological system. However, there is inadequate amount of *in vivo* experiment which can support the *in vitro* mechanistic studies. GNP cannot be transformed into the clinic unless we know the experimental limitations like biological response, physical action and their inadequate mechanistic knowledge (Rosa et al., 2017).

In recent times, the increased toxicity of nanoparticles due to their tiny physical dimensions has been widely recognized. Carbon black is nontoxic but nanoforms of carbon and fullerene are highly toxic (Fiorito et al., 2006). Similarly, higher toxicity of titanium oxide nanoparticles has been reported (Chen et al., 2009a, 2009b). Many inorganic metals proved safe in bulk forms which were toxic on nanoscale (Jaclyn et al., 2011; Schrand et al., 2010). This undesired effect might be because of enhanced surface area and surface to volume ratio. The organ distributions of gold nanoparticles have demonstrated that it is size dependent. The smallest particles shows the most widespread organ distribution including blood, heart, lungs, liver, spleen, kidney, thymus, brain, and reproductive organs. The study showed smaller AuNP had wider organ distribution than the larger particles (Khan et al., 2012). It was found that AuNP with prolong blood circulation time can accumulate in the liver and spleen effecting the gene expression (Wim et al., 2008). The 20 nm poly ethylene glycol (PEG) coated gold nanoparticle was more stable and less toxic than PEG gold nanoparticles of higher size (Balasubramanian et al., 2010). Advances in biomolecular functionalization of AuNP have led to a vibrant expansion in their potential biomedical applications (Zhang et al., 2011). The therapeutic use of nanomaterial with Au containing drugs has improved the actions by reducing toxicity. The progress of biofunctionalized noble metal nanoparticles as therapeutic agents has generated great interest in current research due to higher biocompatibility and biosensitivity (Khlebtsov and Dykman, 2011). Noble metal nanoparticles in pharmaceutical and medicine have shown promising results in preclinical studies as therapeutics carriers in drug delivery systems (Liu and Han 2005). The balance between therapeutic properties and development of adverse effects were not well established (Carneiro et al., 2016). The *in vitro* anti-proliferative effect of biofunctionalized AuNP was evaluated (Raghunandan et al., 2011; Parveen and Rao 2014). However, *in vivo* toxicological investigations and biological changes are necessary to prove the required safety of nanoparticles. An attempt was made to understand the effect of biofunctionalized AuNP synthesized using clove bud extract by repeated dosing in rats. The *in vivo* toxicological evaluation of AuNP has been performed in Sprague dawley rats after intravenous administration for a period of 28 consecutive days. The study provides information on biological effect, biodistribution and accumulation in various organs of the animals.

2. Materials and methods

2.1. Preparation of biofunctionalized gold nanoparticles

The AuNP were synthesized using 100 mL of 0.01% chloroauric acid ($HAuCl_4$. $4H_2O$) solution and 5 mL of clove bud aqueous extract. The pH value of the biologically functionalized gold nanoparticle solution was adjusted to 7.4 using dilute NaOH buffer solution, similar to the physiological environment of mice. The AuNP solution was filtered through 1 μm filters to remove the precipitates, aggregates, fibrous matters and other unreacted water soluble impurities and the filtrate was stored at 4 °C in order to prevent aggregation. The gold nanoparticle suspension (1 mL) was centrifuged at 10,000 rpm for 30 minutes and the supernatant was removed. The size and shape of the gold nanoparticles were analyzed by field emission scanning electron microscopy using a FEI Nova nano 600, Netherlands and the images operated at 15 kV on a 0° tilt position. The optical absorption spectrum was measured in the wavelength range of 300–750 nm using a ECIL 5704SS UV–vis spectrophotometer at 1 nm resolution.

2.2. Animals and husbandry

Sprague Dawley rats (Hsd:SD) conventionally bred (In-house random bred) of 8 weeks old, males weighing 240–290 g and females weighing 160–220 g were used for the experiment. Rats were acclimatized for one week before initiation of the experiment. Rats were housed in polypropylene cages (2 rats per cage) each with stainless steel top grill which has facilities for holding pelleted food (Hindustan Lever Ltd., Bombay) and filtered drinking water in polycorbonated bottles. The rats were maintained under controlled conditions of temperature (23 ± 2 °C), humidity (55 ± 5 %), and a 12:12 h light-dark cycle. Paddy husk was used as bedding material.

2.3. Experimental design

This study was performed according to the OECD test guideline 407 (Adopted 3 October 2008) by repeated 28-day dose to find the toxicity of AuNP in rodents. The concentration dependent toxicological experiment comprising of one control group and three experimental groups allocated to different doses of biofunctionalized AuNP. For this experiment, 40 male and 40 female rats (consisting of 10 rats/sex/group) were used. One day before the start of the treatment, rats were assigned to groups stratified by weight randomization method so that they were evenly distributed with respect to the mean body weight. Three dose levels of 100 (low), 200 (mid) and 500 (high) μg/kg Bwt/day were selected based on the preliminary study conducted in our laboratory. The test material was soluble in distilled water. Hence, the same was selected as the vehicle. The test solution was prepared in distilled water for complete dissolution and was administered by intravenous route to the rats. The control animals administered an equivalent amount of distilled water through intravenous route. The test item formulations or the vehicle was administered daily once at the same time each day (varied by ±2 h) for 28 days to rats of the specific treatment or vehicle control groups. The dose volume administered was 5 mL/kg and the dose volume for the individual rat was calculated based on the body weight recorded during different intervals of the treatment period. The stability of the test solution at ambient condition has been confirmed for up to six months before the experiment.

2.4. Parameters evaluated

2.4.1. Clinical observation

All animals were observed twice daily for signs of toxicity (morbidity & mortality). Detailed clinical examination was performed once a week. During the detailed clinical examination, animals were removed from their cages and examined for skin, fur, eyes, salivation, piloerection, tremors, convulsions, gait and posture, handling etc.

2.4.2. Ophthalmic examination

Eyes were examined prior to treatment and during the week prior to sacrifice using an ophthalmoscope after inducing of the Mydriatic agent, 1% Tropicamide. During the examination the cor-

nea, lense, iris, retina, vitreous, humor and optic disc nerve were examined.

2.4.3. Body weight and food consumption

The body weights of all animals were measured before treatment and on days 8, 15, 22 and 28. Fasting body weight was recorded prior to sacrifice on Day 29. Food consumption was measured once in a week and the mean daily food consumption per animal for each weighing period was calculated.

2.4.4. Hematology and blood chemistry

Animals were fasted overnight on treatment day 28, and blood samples were collected by retro-orbital puncture on 29th day from all rats. The blood samples were collected in dipotassium EDTA tubes as an anticoagulant for hematology and with no anticoagulant tubes for clinical chemistry.

Hematological parameters such as red blood cell count (RBC), white blood cell count (WBC), haemoglobin concentration (Hb), hematocrit value (HCT), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean platelet volume (MPV), differential leucocyte count were measured using fully automated hematology analyser ARTOS Vesatis biochemical analyser.

Clinical chemistry parameters such as sodium (Na), calcium (Ca), chloride (Cl), potassium (K), creatinine, glucose, (Glu), total protein (TP), total cholesterol (TC), triglycerides (TG), albumin, blood urea nitrogen (BUN), creatinine (CRE), creatine kinase, aspartate amino transferase (AST), alanine amino transferase (ALT), γ-glutamyl transpeptidase (γ-GT), alkaline phosphatase (ALP) were measured using ARTOS Vesatis biochemical analyser.

2.4.5. Urinalysis

On day 28, urine samples were collected from rats by placing in individual metabolic cages following overnight fasting. The parameters such as pH, glucose, bilirubin, ketone, protein, urobilinogen, nitrite, specific gravity, erythrocytes and leukocytes were analyzed.

2.4.6. Necropsy and gross examination

On day 29, after the blood collection necropsy was performed in all the rats and subjected to detailed macroscopic examination. All the rats were sacrificed by anaesthetising with isoflurane, weighed, exsanguinated and subjected to detailed necropsy.

2.4.7. Organ weights

Absolute and relative organ weights were recorded based on terminal body weights. Organs such as liver, kidney, spleen, thymus, thyroid, adrenal glands, brain, heart, testes, seminal vesicles, epididymides, ovaries, oviducts and uterus were weighed and relative organ weights were calculated.

2.4.8. Histopathology

The following organs and tissues were collected for histopathological examinations; liver, kidney, adrenal gland, cecum, colon, duodenum, esophagus, ileum, jejunum, lymph node (mandibular & mesenteric), stomach, rectum, trachea, epididymis, eyes with optic nerve, pituitary, salivary glands, prostate, pancreas, seminal vesicle, spleen, thyroid, testes, thymus ovary, oviduct, brain (medulla/pons, cerebrum, cerebellum), peripheral nerve (sciatic), urinary bladder, vagina. The tissues were fixed in 10% neutral buffered formalin except testes, epididymides and eyes with optic nerve were fixed in Davidson's fixative. Microscopic examination was performed for the slides of all control (0 μg/kg Bwt/day) and high dose (500 μg/kg Bwt/day) group animals. In brief, 4–5 μm microtome sections were stained with hematoxylin and eosin (H & E) following a standard staining protocol for histopathological investigations.

3. Statistical analysis

The data was statistically analyzed using GraphPad prism 5.0 and recorded as mean ± SD. The data for mean body weight, food consumption, hematology, clinical chemistry and organ weight were analyzed using Bartlett's test for homogeneity of variance. One-way analysis of variance (ANOVA) was performed on homogenous data. Dunnett's test was used for multiple comparisons.

4. Results

The objective of the study was to determine the effect of biofunctionalized gold nanoparticles by intravenous administration on various organs in rats.

4.1. Biofunctionalized gold nanoparticles

The microwave assisted extracellular AuNP was synthesized using aqueous clove buds (*S. aromaticum*) solution by treating with aqueous $HAuCl_4$ solution. The AuNP colloidal solution was characterized using different advanced spectroscopic and microscopic techniques. AuNP were highly irregular in shape and dispersed in the range of 5–100 nm as reported (Raghunandan et al., 2010). The clear morphology was reconfirmed with TEM and AFM images (Fig. 1). The method yielded amoebic unpredictable shaped particles with an average diameter of about 5–100 nm. The actual value of the mean size might vary to some extent from each preparation compared to published procedures. We evaluated the effects of AuNP in different doses (100, 200, and 500 μg/kg/day) upon the intravenous administration in mice regularly for 28 days. All the data are statistically and graphically presented.

4.2. Mortality and clinical observation

No clinical signs of toxicity or mortalities were observed at any of the doses tested. Alopecia was observed in one male rat in the vehicle treated group and in one female rat at 200 μg/kg Bwt/day treated group. There was no apparent reason for the alopecia and was common in rats and hence considered incidental finding and not related to treatment.

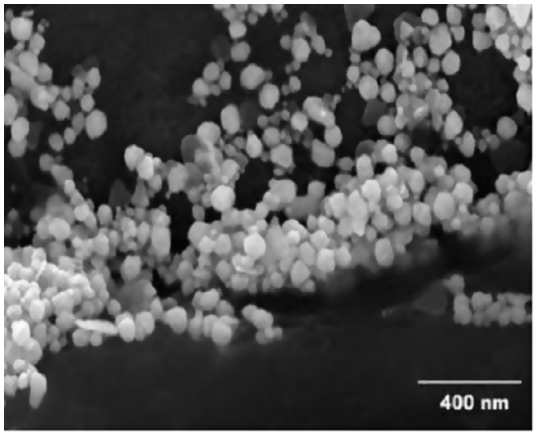

Fig. 1. FESEM images of biofunctionalized AuNP.

4.3. Ophthalmic examination

Examination of eyes did not reveal any toxic effect.

4.4. Body weight and food consumption

Weekly evaluation showed increase in body weight of the animals of both sexes (Figs. 2 and 3). No biologically significant variations were observed in the food consumption between control and treated group of male and female animals (Figs. 4 and 5).

4.5. Hematology and blood chemistry

No significant changes were observed for the hematology values (Table 1) and blood biochemical measurements (Table 2). The statistically significant decrease in the PLT and MCH values at 200 μg/kg Bwt in males were considered incidental as the changes were minimal and lack of dose relation.

4.6. Organ weight and organ to body weight ratios

There was an insignificant usual variation in the weight of all the organs tested in both male and female rats. The analysis has not affected their biological functions (Table 3 and 4).

5. Discussion

Surface chemistry is important to know the biocompatibility and stability of gold nanoparticle before implementing into the clinical trials. Surface coating had more impact on toxicity rather than on biodistribution of the AuNP (Fraga et al., 2014). Our results suggest that the surface coating of nanoparticles helps in improving the drug design, biodistribution and nontoxicity. Biofunctionalized AuNP have the potential biomedical application as compared to the chemically designed surface coated nanoparticles. The biosynthesized AuNP coated with biological moieties from clove revealed no toxicity in the rats at selected doses. Hence rats remained healthy during the experimental analysis. The potentiality of gold nanoparticles in biological application has not yet fully determined due to its toxicity *in vitro* and *in vivo* (Mi-Rae et al., 2015). There are multiple techniques to apply AuNP in the diagnostics, treatments including uptake and distribution throughout the cell (Botchway and Coulter, 2015). Successful biodistribution of gold nanoparticles (AuNPs) with surface coatings like citrate, 11-MUA and 3 pentapeptides, CALNN, CALND and CALNS were evaluated in rats. Liver showed the maximum Au level, followed by spleen and blood after 24 h. Liver slices showed AuNP in Kupffer cells and hepatocyte by TEM analysis (Morais et al., 2012). The gold nanoparticles were time dependent in intravenous dose and have distributed in various organs within short period (Cho et al., 2009). The irradiation of 11.2 nm nanoparticles has increased the survival of mice by 50% and appropriate dose of Au nanoparticles could be less toxic which improves the lifespan (Hainfeld et al., 2013). The accumulation and spreading of AuNP in mesenteric lymph nodes was analyzed with respect to size and duration. The 15 and 50 nm gold nanoparticles were detected in the cluster form in the cytoplasm of macrophages and lymphocyte (Zlobina et al., 2013). No toxicity was observed in liver and kidney and concluded its potentiality in obesity related diseases (Chen et al., 2013). The biokinetic changes was observed between pregnant and non-

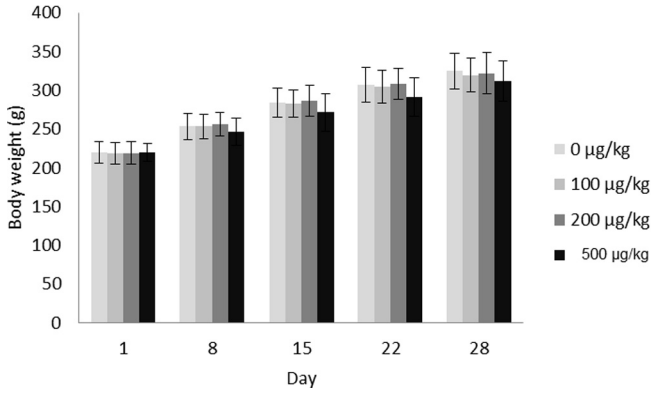

Fig. 2. Mean (±SD) body weights (g) of male rats treated with AuNP for 28-days.

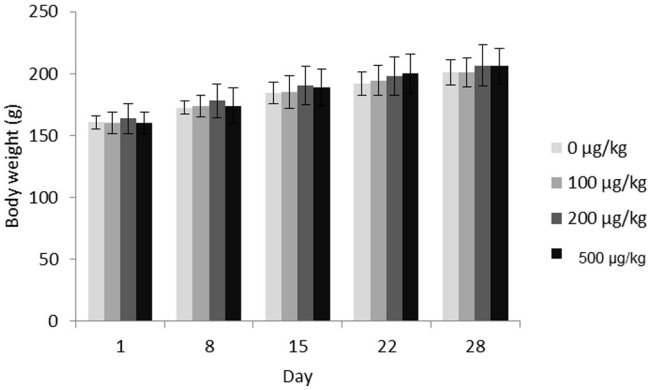

Fig. 3. Mean (±SD) body weights (g) of female rats treated with AuNP for 28-days.

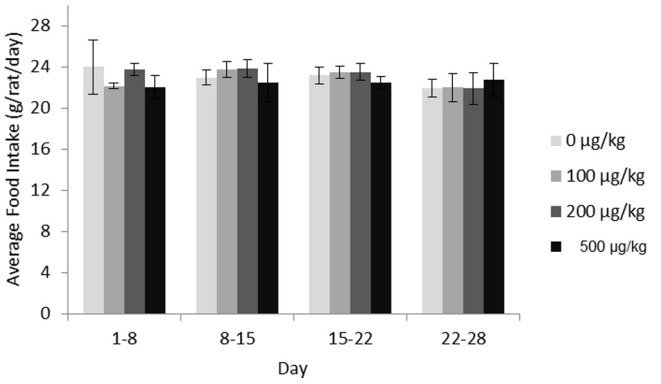

Fig. 4. Average (±SD) food consumption (g/rat/day) of male rats treated with AuNP for 28-days.

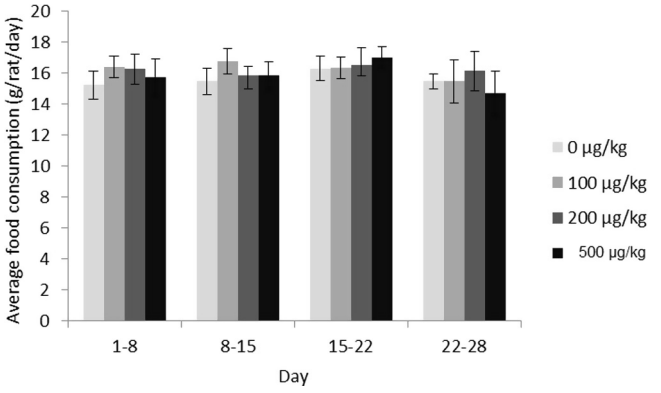

Fig. 5. Average (±SD) food consumption (g/rat/day) of female rats treated with AuNP for 28-days.

Table 1
Hematological values of male and female rats after 28-day intravenous administration of AuNP (mean ± SD).

Parameters	Unit	Doses (μg/kg Bwt/day)			
		0 (control)	100 (low dose)	200 (mid dose)	500 (high dose)
Male					
RBC	T/L	9.17 ± 0.48	9.48 ± 0.93	8.98 ± 0.66	9.10 ± 0.84
WBC	G/L	7.01 ± 0.92	7.00 ± 0.58	7.84 ± 1.10	7.26 ± 0.73
Hb	g/L	159.43 ± 3.21	164.22 ± 3.06	157.60 ± 4.81	160.04 ± 3.94
HCT	L/L	0.48 ± 0.04	0.47 ± 0.75	0.44 ± 0.07	0.44 ± 0.61
PLT	G/L	968.30 ± 103.21	966.84 ± 274.82	944.86 ± 186.09*	1016.49 ± 210.75
MCV	fL	48.21 ± 1.10	49.30 ± 1.48	51.37 ± 1.77	48.38 ± 0.99
MCH	Pg	18.14 ± 0.98	18.29 ± 0.85	16.64 ± 0.85*	17.93 ± 1.26
MCHC	G/L	364.22 ± 4.13	359.22 ± 3.21	361.66 ± 3.47	352.06 ± 3.79
MPV	fL	9.63 ± 0.84	9.26 ± 0.75	10.21 ± 0.58	9.86 ± 0.83
Differential leucocyte counting					
Neut	%	22.33 ± 0.64	21.97 ± 1.22	24.37 ± 0.84	24.90 ± 0.24
Ly	%	71.31 ± 6.73	77.53 ± 5.82	73.64 ± 5.11	79.63 ± 4.63
Mono	%	2.78 ± 0.41	2.25 ± 0.75	2.37 ± 0.92	1.86 ± 0.67
Eo	%	1.31 ± 0.6	1.36 ± 0.56	1.54 ± 0.36	1.37 ± 0.61
Ba	%	0.28 ± 0.06	0.31 ± 0.04	0.26 ± 0.22	0.30 ± 0.07
Female					
RBC	T/L	7.21 ± 0.85	7.82 ± 0.74	8.05 ± 0.07	7.42 ± 0.17
WBC	G/L	4.32 ± 1.03	3.95 ± 0.79	3.81 ± 0.42	4.00 ± 0.63
Hb	g/L	147.23 ± 2.93	151.74 ± 3.22	142.00 ± 2.47	150.01 ± 3.59
HCT	L/L	0.37 ± 0.14	0.33 ± 0.06	0.31 ± 0.13	0.40 ± 0.09
PLT	G/L	1206.0 ± 210.07	1241.71 ± 195.28	1184.62 ± 261.33	1230.22 ± 301.64
MCV	fL	56.97 ± 2.31	58.96 ± 1.64	58.25 ± 1.74	57.33 ± 1.38
MCH	Pg	18.44 ± 0.67	18.69 ± 0.81	18.55 ± 0.37	19.0 ± 0.09
MCHC	G/L	337.95 ± 6.93	321.06 ± 4.10	328.19 ± 3.42	331.05 ± 3.95
MPV	fL	9.06 ± 0.48	10.53 ± 0.83	9.32 ± 0.46	9.59 ± 0.84
Differential leucocyte counting					
Neut	%	21.64 ± 0.37	22.8 ± 0.92	21.48 ± 0.39	23.34 ± 0.68
Ly	%	74.26 ± 3.68	72.5 ± 5.38	72.29 ± 4.19	73.22 ± 4.52
Mono	%	2.32 ± 0.36	2.44 ± 0.09	2.78 ± 0.72	2.69 ± 0.39
Eo	%	1.84 ± 0.71	2.01 ± 0.45	1.97 ± 0.41	1.81 ± 0.37
Ba	%	0.26 ± 0.12	0.21 ± 0.31	0.28 ± 0.05	0.3 ± 0.74

pregnant rats and found that AuNP translocate from maternal blood or placental tissues to the fetus but not from the amniotic fluids. It was also concluded the translocation of AuNP was size dependent involving transcellular mechanisms (Semmler-Behnke et al., 2014). The short (30 min) and long-term (28 days) biodistribution and toxicity of 20 nm acitrate- and pentapeptide CALNN-coated AuNPs was evaluated after a single intravenous injection in rats. It was found that AuNPs were quickly removed from the bloodstream and accumulated in the liver. However Spleenatrophy and hematological findings showed mild anemia (Fraga et al., 2014). AuNPs prominently deposited in the liver whereas AgNPs was accumulated in more organs including heart, kidney, lung etc. AgNPs have induced greater alterations in gene expression causing ion transport, oxidative stress and apoptosis. The importance of chemical composition of NPs played a critical role in their *in vivo* biodistribution and toxicity (Yang et al., 2017).

The toxicity of biometal nanoparticles were reported in rats. The increasing levels of GNP have injured the hepatocytes and caused metabolic and structural disturbances (Abdelhalim and Jarrar, 2011). The highest toxicity was caused by smaller GNP and time exposure which accumulated in organs like liver, lung followed by kidney and heart (Abdelhalim, 2012). AuNP has proliferated 8-hydroxydeoxyguanosine, caspase-3 and heat shock protein70 which has damaged the DNA leading cell death (Siddiqi et al., 2012). AuNPs and AgNPs were directly translocated to secondary organs like central nervous system (CNS) when administrated systemic or subcutaneous, or through the olfactory system. AuNP and AgNP have increased the numbers of proliferating and apoptotic HNPCs affecting the growth profile (Soderstjerna et al., 2013). GNPs of different shape (spherical and hexagonal) and size 10, 20 nm and 50 nm were used to study the *in vivo* accumulation in rats. The fluorescence peak of particle size, shape, surface area and exposure time plays an important role in toxicity and accumulation in different rat organs (Abdelhalim, 2013). 21 nm spherical AuNPs was found accumulated and decreased the mass of abdominal fat tissue without effecting the daily energy and body weight of rats. GNPs has injured renal tubules by accumulating and resulted in metabolic and structural disturbances (Doudi and Setorki, 2014). The toxicity of chemically synthesized nanoparticles in various organs of rats was reported. The effect of 12- and 22-nm chitosan-capped gold nanoparticles on rats suggested that larger size NP has damaged the brain and liver without significantly affecting the body weights of the rat. The agglomeration of nanoparticles was observed in cytoplasmic cellular regions causing damage (Stefan et al., 2013). The components of the Au core and citrate surface coating showed altered biodistribution in the organs and tissues hence could be utilized as drug delivery vehicles (Rambanapasi et al., 2015). AuNP have caused oxidative stress and a reduction of antioxidant enzyme like glutathione peroxidase activity in rat brain. The 5 mg kg^{-1} AuNP have caused hypoglycemic effect and increases HDL cholesterol in normal rats. The increased concentration of AuNP has damaged the lungs causing inflammation which suggested the lung as major target organ (Aravinthan et al., 2016). The intravenous and oral doses study shows the bioavailability of 1, 2 and 5 kDa PEG-coated 5 nm gold nanoparticles (AuNPs) in male rats. The oral dose revealed the highest concentrations of NP in kidneys and similar concentrations in different organs on intravenous doses (Alalaiwe et al., 2017). Our study revealed AuNP intravenous administration has no toxicity in the organs of rats, which supports the practice of biofunctionalized AuNP with further detail molecular study for the treatment and drug delivery in diseases. The surface and size of nanoparticles plays very important role in toxicity. A particular dose contains different size of nanoparticles, when this dose administered in rats

Table 2
Clinical chemistry values of male and female rats after 28-day intravenous administration of AuNP (mean ± SD).

Parameters	Unit	Doses (μg/kg Bwt/day)			
		0 (control)	100 (low dose)	200 (mid dose)	500 (high dose)
Male					
Na	mmol/L	144.16 ± 1.3	144.67 ± 1.78	143.67 ± 0.98	143.48 ± 1.63
Ca	mmol/L	2.41 ± 0.11	2.52 ± 0.06	2.40 ± 0.09	2.44 ± 0.05
Cl	mmol/L	107.8 ± 0.78	107.75 ± 0.59	108.72 ± 1.24	107.63 ± 0.68
K	mmol/L	4.02 ± 0.4	3.93 ± 0.24	4.00 ± 0.61	3.88 ± 0.25
Glu	mmol/L	6.84 ± 0.26	6.39 ± 0.13	6.5 ± 0.42	6.61 ± 0.41
TP	g/L	59.81 ± 1.97	60.82 ± 2.42	59.72 ± 2.61	61.52 ± 1.85
TC	mmol/L	2.11 ± 0.41	1.98 ± 0.13	2.01 ± 0.42	2.00 ± 0.20
TG	mmol/L	0.71 ± 0.17	0.74 ± 0.44	0.82 ± 0.28	0.78 ± 0.45
ALB	g/L	39.83 ± 1.6	38.27 ± 1.72	41.62 ± 1.25	40.59 ± 1.04
BUN	mmol/L	5.67 ± 1.04	5.59 ± 0.64	6.02 ± 0.83	6.02 ± 1.11
CRE	μmol/L	32.33 ± 4.21	34.05 ± 3.65	32.05 ± 3.16	31.62 ± 2.84
CK	IU/L	244.52 ± 98.07	251.01 ± 84.28	260.61 ± 107.5	257.50 ± 97.22
AST	IU/L	127.05 ± 42.6	121.94 ± 27.90	117.43 ± 33.77	124.39 ± 31.7
ALT	IU/L	34.27 ± 6.39	33.54 ± 5.33	36.46 ± 6.01	34.7 ± 4.88
γ-GT	IU/L	0.54 ± 0.7	0.51 ± 0.52	0.61 ± 0.08	0.57 ± 0.45
ALP	IU/L	81.26 ± 10.6	78.8 ± 7.92	85.75 ± 12.84	80.6 ± 8.99
Female					
Na	mmol/L	142.68 ± 1.44	143.72 ± 2.06	142.4 ± 2.12	144.68 ± 1.27
Ca	mmol/L	2.63 ± 0.05	2.6 ± 0.17	2.56 ± 0.08	2.71 ± 0.21
Cl	mmol/L	106.41 ± 1.2	107.53 ± 0.69	107.28 ± 1.02	106.97 ± 0.74
K	mmol/L	4.06 ± 0.42	4.14 ± 0.56	4.02 ± 0.44	4.28 ± 0.24
Glu	mmol/L	6.28 ± 0.48	6.71 ± 0.25	6.39 ± 0.64	7.05 ± 0.63
TP	g/L	62.7 ± 2.53	60.86 ± 1.94	62.66 ± 2.21	64.76 ± 81
TC	mmol/L	1.89 ± 0.56	1.92 ± 0.61	1.88 ± 0.87	1.90 ± 0.64
TG	mmol/L	0.53 ± 0.43	0.56 ± 0.27	0.61 ± 0.53	0.56 ± 0.36
ALB	g/L	42.84 ± 2.1	40.82 ± 1.64	40.26 ± 1.38	39.88 ± 1.62
BUN	mmol/L	6.24 ± 1.22	6.17 ± 0.97	6.0 ± 1.31	6.86 ± 1.48
CRE	μmol/L	31.75 ± 2.58	33.25 ± 4.52	33.7 ± 3.28	30.6 ± 3.46
CK	IU/L	186.43 ± 123.6	197.6 ± 87.63	182.52 ± 105.7	193.94 ± 75.83
AST	IU/L	121.85 ± 52.31	124.26 ± 44.4	119.41 ± 38.61	127.4 ± 46.17
ALT	IU/L	27.49 ± 4.36	29.42 ± 3.94	30.26 ± 4.74	27.8 ± 5.37
γ-GT	IU/L	0.64 ± 0.43	0.81 ± 0.72	0.61 ± 0.39	0.64 ± 0.25
ALP	IU/L	44.26 ± 6.57	47.7 ± 8.62	50.52 ± 7.71	46.11 ± 4.64

Table 3
Organ weights and organ to body weight ratios (% of body weight) of male rats after 28-day intravenous administration of AuNP (mean ± SD).

Organs	Unit	Doses (μg/kg Bwt/day)			
		0 (control)	100 (low dose)	200 (mid dose)	500 (high dose)
Number of rats	10	10	10	10	
Terminal body weight					
Liver	g	8.28 ± 0.78	8.64 ± 1.22	8.20 ± 1.11	7.44 ± 1.41
	%	3.64 ± 1.31	3.29 ± 0.39	3.75 ± 0.94	2.96 ± 1.05
Kidney	g	1.94 ± 0.63	2.01 ± 0.96	1.96 ± 0.63	1.823 ± 0.85
	%	0.650 ± 0.53	0.67 ± 0.74	0.64 ± 0.48	0.62 ± 0.09
Spleen	g	0.811 ± 0.121	0.785 ± 0.064	0.772 ± 0.174	0.8 ± 0.053
	%	0.24 ± 0.024	0.283 ± 0.063	0.251 ± 0.035	0.224 ± 0.056
Thymus	g	0.651 ± 0.362	0.740 ± 0.274	0.622 ± 0.34	0.704 ± 0.274
	%	0.239 ± 0.024	0.281 ± 0.055	0.264 ± 0.062	0.274 ± 0.026
Adrenals	g	0.051 ± 0.005	0.053 ± 0.003	0.050 ± 0.003	0.063 ± 0.006
	%	0.015 ± 0.002	0.012 ± 0.001	0.014 ± 0.001	0.012 ± 0.002
Brain	g	2.01 ± 0.14	1.98 ± 0.056	1.86 ± 0.143	2.0 ± 0.13
	%	0.58 ± 0.054	0.52 ± 0.13	0.60 ± 0.16	0.56 ± 0.073
Heart	g	0.064	0.96 ± 0.068	0.095 ± 0.137	0.99 ± 0.082
	%	0.424 ± 0.052	0.376 ± 0.011	0.401 ± 0.083	0.4 ± 0.026
Testes	g	2.96 ± 0.185	3.0 ± 0.12	2.84 ± 0.201	2.93 ± 0.163
	%	0.97 ± 0.085	1.11 ± 0.073	1.05 ± 0.14	0.961 ± 0.095
Seminal vesicles	g	1.218 ± 0.318	1.164 ± 0.194	1.064 ± 0.179	1.217 ± 0.133
	%	0.362 ± 0.071	0.384 ± 0.083	0.411 ± 0.068	0.375 ± 0.086
Epididymides	g	0.901 ± 0.064	0.922 ± 0.072	1.053 ± 0.088	0.961 ± 0.764
	%	0.273 ± 0.023	0.269 ± 0.031	0.248 ± 0.017	0.262 ± 0.042
Pituitary gland	g	0.014 ± 0.002	0.013 ± 0.001	0.014 ± 0.001	0.012 ± 0.002
	%	0.002 ± 0.001	0.002 ± 0.001	0.003 ± 0.001	0.002 ± 0.00

starts translocating with the blood in the body and gets accumulated in different organs. Size dependent AuNP (15, 50, 100 and 200 nm) have distributed in tissue or organ in mice. Sonavane et al., 2008 reported smaller size NP has major distribution in tissues than the larger nanoparticles which accumulated in different organs. They also found that 15 and 50 nm gold NP have passed blood–brain barrier in brain. The study shows traces of AuNP in the brain could be used in central nervous system (CNS) drug

Table 4
Organ weights and organ to body weight ratios (% of body weight) of female rats after 28-day intravenous administration of AuNP (mean ± SD).

Organs	Unit	Doses (μg/kg Bwt/day)			
		0 (control)	100 (low dose)	200 (mid dose)	500 (high dose)
Number of rats	10	10	10	10	
Terminal body weight					
Liver	g	7.39 ± 1.27	7.26 ± 0.96	6.93 ± 1.13	7.18 ± 0.86
	%	3.84 ± 0.19	3.27 ± 0.61	3.39 ± 0.38	3.24 ± 0.40
Kidneys	g	1.178 ± 0.053	1.201 ± 0.022	1.46 ± 0.063	1.285 ± 0.063
	%	0.724 ± 0.063	0.707 ± 0.028	0.638 ± 0.058	0.679 ± 0.048
Spleen	g	0.521 ± 0.083	0.562 ± 0.047	0.632 ± 0.041	0.548 ± 0.073
	%	0.326 ± 0.017	0.349 ± 0.026	0.310 ± 0.021	0.372 ± 0.032
Thymus	g	0.461 ± 0.057	0.439 ± 0.039	0.457 ± 0.042	0.427 ± 0.056
	%	0.261 ± 0.029	0.248 ± 0.037	0.283 ± 0.062	0.270 ± 0.042
Thyroid	g	0.031 ± 0.12	0.037 ± 0.09	0.029 ± 0.11	0.034 ± 0.081
	%	0.013 ± 0.001	0.010 ± 0.001	0.014 ± 0.001	0.013 ± 0.001
Adrenals	g	0.048 ± 0.004	0.051 ± 0.004	0.043 ± 0.006	0.042 ± 0.003
	%	0.021 ± 0.002	0.026 ± 0.004	0.030 ± 0.002	0.029 ± 0.004
Brain	g	1.92 ± 0.06	1.93 ± 0.05	1.97 ± 0.08	1.95 ± 0.06
	%	0.64 ± 0.063	0.62 ± 0.038	0.67 ± 0.048	0.62 ± 0.073
Heart	g	0.867 ± 0.071	0.921 ± 0.069	0.907 ± 0.053	0.882 ± 0.084
	%	0.393 ± 0.037	0.407 ± 0.048	0.384 ± 0.031	0.402 ± 0.042
Ovaries	g	0.081 ± 0.011	0.084 ± 0.006	0.081 ± 0.017	0.089 ± 0.014
	%	0.036 ± 0.005	0.031 ± 0.008	0.037 ± 0.013	0.034 ± 0.006
Uterus	g	0.402 ± 0.061	0.384 ± 0.073	0.392 ± 0.049	0.401 ± 0.057
	%	0.021 ± 0.042	0.026 ± 0.031	0.020 ± 0.061	0.027 ± 0.038
Pituitary gland	g	0.028 ± 0.002	0.024 ± 0.001	0.025 ± 0.002	0.027 ± 0.001
	%	0.006 ± 0.001	0.005 ± 0.001	0.006 ± 0.001	0.005 ± 0.001

development. The research efforts have shown that 98% of the potential CNS drugs have failed to cross the blood brain barrier which protects central nervous system from neurotoxins (Nkansah, 2013). The concentration of 2.5 mg/L of GNP showed therapeutic benefits without effecting the liver and brain (Muller et al., 2017).

6. Conclusion

The increasing nanomaterial practice in research and clinical setting is very essential to find out the safety and toxicity profile in a fast and efficient manner. The intention to find out the effect of biofunctionalized AuNP synthesized from clove bud extract by intravenous administration in the rats. We carried out all the experiments in details with respect to each organ. The AuNP administration shows no toxicity in daily activity of male and female rats. The nontoxicity of AuNP might have produced immunity in the rats hence remained healthy even after getting daily doses of AuNP. The study shows no harmful effect on the organs tested. The biofunctionalized nanoparticles have diagnostic and therapeutic applications. The biodistribution and accumulation of nanoparticles could be used in drug delivery to the different organs including brain. The research in development of drug delivery system to control the sensory nerves of the brain is needed. However the biomedical applications of inorganic metal nanoparticles *in vivo* are yet unresolved and needs extensive molecular study. The future study extends to find out the concentration, size based biodistribution and accumulation of nanoparticles in the various organs of the rats.

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Conflict of interest

No conflict of interest declared.

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