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Effects of administration of 10 nm or 50 nm gold nanoparticles (AuNPs) on blood profile, liver and kidney functions in male albino rats

Sahar H Orabi[1]*, Doaa A Mansour[1], Said I Fathalla[2], Shaaban M Gadallah[3], Amina A Gamal ElDin[4] & Ahmed Sabry S Abdoon[5]

[1]Department of Biochemistry and Chemistry of Nutrition; [2]Department of Physiology, Faculty of Veterinary Medicine; [3]Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, University of Sadat City, Egypt
[4]Depatment of Pathology, Medical Research Division; [5]Depatment of Animal Reproduction, Veterinary Research Division, National Research Center, Dokki, Cairo-12622, Egypt

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This work aimed to investigate the effect of acute and chronic administration of gold nanoparticles (GNPs) on liver and kidney functions, blood glucose concentration, lipid profile, and haematological parameters in male albino rats. Two experiments were conducted. In acute study: Fifty-four adult mature male rats were randomly assigned into three equal groups (18 per group). Group 1 (control group): in which rats were received intramuscular (i.m) injection of 1 ml normal saline 0.9%. Group 2 (50 nm GNPs group): rats were i.m. injected with a single dose of 75 µg 50 nm GNPs/kg body weight (bwt). In Group 3 (10 nm GNPs group): rats were i.m. injected with a single dose of 75 µg 10 nm GNPs/kg bwt. In chronic study: Eighteen adult male rats were randomly divided into three equal groups (6 per group). Group I (control): rats were intramuscular (i.m) repeatedly injected with 1 ml normal saline 0.9% once/week 5 for weeks. Group 2 (50 nm GNPs): rats were i.m. injected with once/week with a dose of 75 µg 50 nm GNPs/kg bwt) for 5 weeks. In Group 3 (10 nm GNPs): male rats were i.m. injected with once/week with a dose of 75 µg 50 nm GNPs/kg bwt for 5 weeks, followed by 3 weeks washout period for all groups. Blood was collected at 3, 7, and 60 days in acute experiment, while, they were collected only before and after 2 months in chronic experiment. Acute and chronic administration of GNPs (10 or 50 nm size) in male albino rats induced no significant alterations for liver and kidney functions, lipid profile parameters and different haematological parameters at days 3 and 60 of the study. However, on day-7 post-treatment, GNPs-treated rats showed significantly (P <0.05) higher serum ALT, AST, ALP, urea, creatinine, glucose, and different lipid profile and decreased HDL level. Chronic administration of 10 nm or 50 nm GNPs significantly (P <0.05) decreased serum glucose levels. In conclusion acute or chronic administration of 10 nm or 50 nm GNPs could alter the liver, kidney functions and blood profile on day 7 post-treatment, however, these values returned to the normal levels on day 60 post- injection. Also, the chronic administration of GNPs induced a hypoglycemic effect in male albino rats.

Keywords: Blood profile, GNPs, Liver and kidney function, Male rats

Nanotechnology is a new technological field that benefits from the unique characteristics of particles in nano dimensions. It is widely used in biomedical and many industries applications[1]. Gold nanoparticles (AuNPs) which are easily modified in size, shape, and functionalization, are among the most prominent inorganic nanoparticles[2]. Nanoparticles have anticancer activity against neuroblastoma cells[3]. The combination of biotechnology and nanotechnology has encouraged the rapid development of fungal extracellular enzyme cocktail through nanoencapsulation[4].

Recently, AuNPs have a wide range of applications in medicine, catalysis, diagnostics, sensors[5], and different industries such as beverages, toothpaste, vehicles, air handling units[6], as well as nanomedicine, drug delivery, diagnosis, gene therapy, photothermal therapy, radiotherapy, biosensing, as well as cancer diagnosis and therapy[7-9].

The extensive use of AuNPs in biomedical and industrial fields has raised the significant concerns about their capacity to impair human health. Some reports have recorded that exposure to AuNPS is toxic[6,10]. On the other hand, other studies have reported that AuNPs are not toxic[11,12]. The toxicity and distribution of AuNPs are based on their physicochemical properties such as size, shape, surface composition and chemistry (such as coating)[13], techniques for synthesis, exposure duration, and a dose of gold nanoparticle and route of administration[14].

It has been proven that smaller gold particles are more toxic than larger particles. This may be because the

*Correspondence:
E-mail: saher977@yahoo.com

interactions which are often linked to their physiochemical feature between smaller AuNPs and biological systems that allow them to be internalized within cells than larger particles[15]. Some researchers indicated that gold nanorods (AuNRs) are more toxic than gold nanospheres (AuNSs)[16]. Also, the frequency of administration affected the toxicity of AuNPs, chronic administration of GNPs at size 10 and 30 nm resulted in higher DNA damage than their acute administration[17].

It has been shown that the main mechanism of the toxicity of AuNPs is through oxidative stress and *via* inflammatory response[18].On the other hand, other experimenters demonstrated no significant induction of oxidative stress or inflammatory response since GNPs are potential antioxidants, GNPs influenced catalase enzyme to reduce the oxidative stress in tissues[19] and efficient for quenching ROS, including hydrogen peroxide (H2O2) and superoxide anion radical (O.2) in a dose-dependent manner[20,21].

Here we focus our attention on studying the acute and chronic effects of GNPs on the liver, and kidney functions, blood glucose concentration, lipid profile, and hematological parameters.

Materials and Methods

Gold nanoparticles (GNPs) at size 10 nm were purchased from Sigma-Aldrich Com., Germany (PCode: 4100798324). While, GNPs solution at size 50 nm was prepared according to the method adopted by Turkevich[22].

Experimental design

Seventy-two mature male albino Wister rats (110-150 g/bwt) were obtained from AL-Zyade Experimental Animals Production Center, Giza, Egypt. They were kept in well-ventilated metal cages at 23 ± 2°C, 40–60% relative humidity, and light/dark cycle of 12 h. The composition of the diet was as follows for 1 kg: corn starch, 620.7 g; mineral mixture (AIN-93M-MX), 35 g; casein (85%protein),140 g; mineral mixture(AIN-93M-VX), 10 g; sucrose, 100 g; L-cysteine, 1.8 g; soybean oil, 40 g; choline chloride, 2.5 g; fiber, 50 g; tetra-butyl-hydroquinone, 0.008 g. Water was supplied ad-libitum all over the study and the rats were left 2 weeks for adaptation.

Rats were random divided into two experiments for evaluation of the effect of acute and chronic effects of gold nanoparticles (GNPs) (10 and 50 nm size).

Experiment 1: To evaluate the acute effect of 10 or 50 nm GNPs on liver, kidney functions, and blood profile in male rats. Animals were assigned into 3 groups:

Group 1 (control group): Eighteen rats were received IM injection of 1 mL normal saline 0.9%.

Group 2 (10 nm GNPs): Eighteen rats in each group were received a single dose of 75 µg 10 nm GNPs/kg/bwt, i.m. according to Abdoon *et al*.[7].

Group 3 (50 nm GNPs): Eighteen male rats in each group were received a single i.m. dose of 75 µg 10 nm GNPs/kg/bwt. Rats were weighed weekly and 6 rats from each group were scarificed under anesthesia by diethyl ether after 3, 7 and 60 days.

Experiment II: To evaluate the chronic effect of 10 or 50 nm GNPS on the liver, kidney functions and blood profile in male rats. Animals were allocated into 3 groups:

Group 1 (control group): Six rats were fed on a basal diet, normal water, and i.m. injected with 1 mL saline solution every week and for 5 weeks, followed by 3 weeks of observation.

Group 2 (GNPs at size 10 nm size): Six rats in each group were administered 75 µg 10 nm GNPs/kg/bwt once weekly for successive five weeks followed by a 21-day washout period.

Group 3 (GNPs at size 50 nm size): Six rats in each group were administered 75 µg 10 nm GNPs/kg/bwt once weekly for successive five weeks, followed by a 21 day washout period. Rats were weighed weekly and at the end of the experiment; they were scarificed under anesthesia by diethyl ether.

Blood sample collection, biochemical and hematological analyses

Three blood samples were collected. The first blood was collected on EDTA vacutainer tube for analysis of blood profile. Second blood samples were collected on plan vacutainer tube (without EDTA) for biochemical analysis. Blood was centrifuged at 1000 g for 15 min and clear serum samples were separated and stored at –20°C until biochemical analysis. The third part of the blood was collected in a tube containing sodium florid for measuring blood glucose concentration.

For biochemical analysis, serum levels for liver function were measured spectrophotometrically using Human company kits for ALT and AST[23]and alkaline phosphatase (ALP) by using Spectrum kits[24]. Kidney functions were measured spectrophotometrically using Diamond kits for urea concentration[25] and creatinine[26] by using Spin react kits. Blood glucose concentration was measured by using Spin react

kits[27]. Determination of serum lipid profile including cholesterol (TC) concentration was measured by using Spin react kits[28], triglycerides (TAG) level was determined colorimetrically by using Spin react kits[26]. High-density lipoprotein-cholesterol concentration was determined by using Cholesterol HDL precipitating reagent kits, BioSystem[29], Low-density lipoprotein and very-low density lipoprotein were calculated as described by Friedewald *et al*.[30] as follows:

$$LDL\ cholestrol(mg/dl)=Total\ cholesterol-(\frac{triglyceride}{5}+HDL\ cholesterol)$$

$$\text{VLDL-cholesterol (mg/dL)} = \frac{Triglycerides}{5}$$

For hematological analysis: Hematological autoanalyzer (BeneSphera™ H32 VET 3-Part Differential Hematology Analyzer User's Manual, 3477 Corporate Parkway, Suite 3 200 Center Valley, PA18034 (USA) was used to determine hematological parameters.

Statistical analysis

All data were presented as mean ± standard errors (SE). Statistical significances of the different sizes of gold nanoparticles were determined by one way ANOVA[31].All statistical analysis was performed using SPSS (Statistical Package for Social Sciences) Version 22.

Results

Effects of GNPs on biochemical parameters analyses

Data illustrated in (Fig. 1), represent the effect of acute and chronic i.m. injection of 75 µg GNPs/kg/bwt on different serum biochemical parameters. Results indicated that i.m. injection of a single dose of 75 µg 50 nm GNPs/kg/bwt to male rats had no significant effect on serum ALT, AST, ALP activities, urea, creatinine levels, and blood glucose concentration

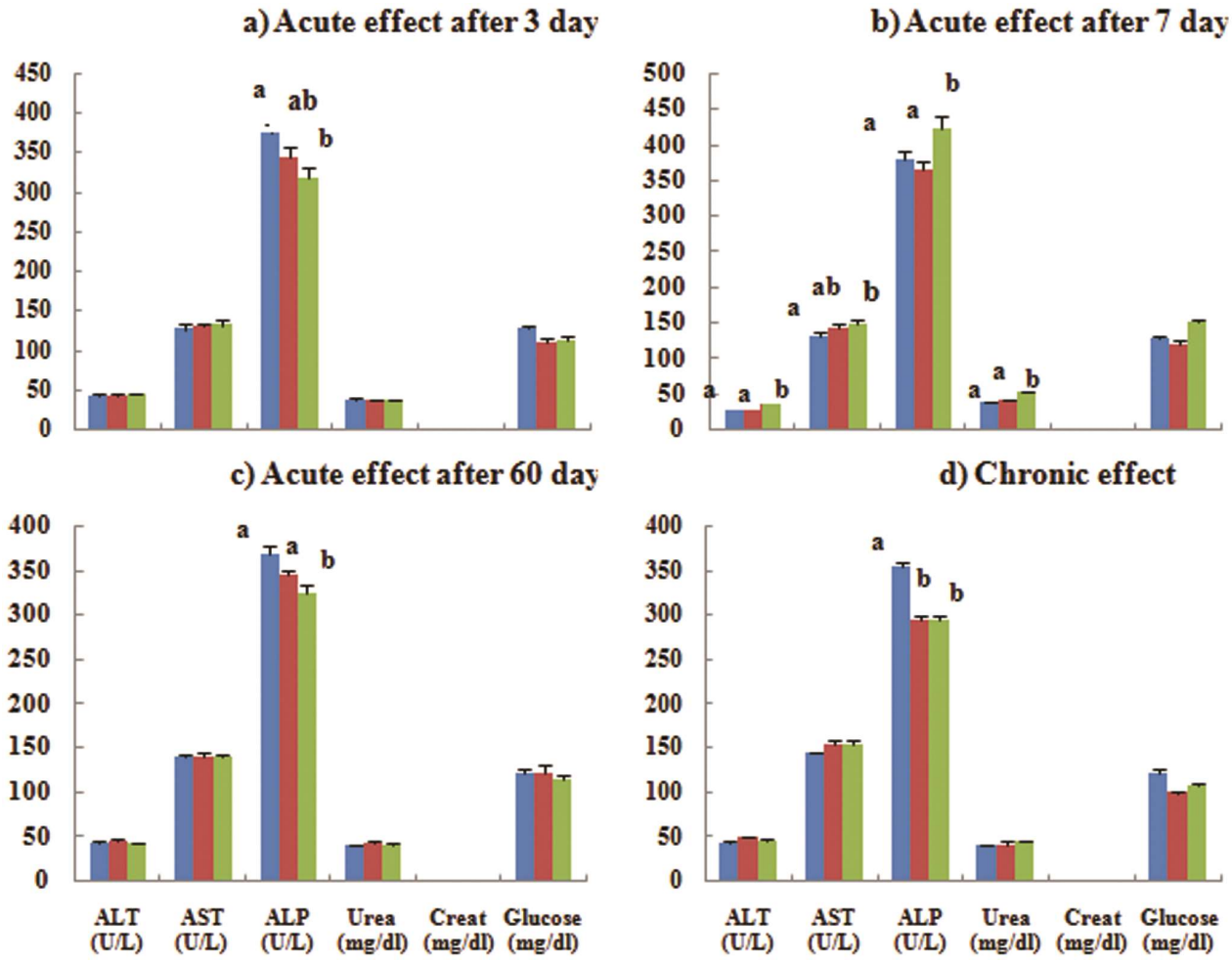

Fig. 1 — Acute and chronic effects of GNPs on different biochemical parameters after 3, 7 and 60 days in different groups of rats after (A) 3; (B) 7; (C) 60 days; and (D) chronic effect

compared to control group after 3, 7 and 60 days. However, i.m. injection of a single dose of 75 µg 10 nm GNPs/kg/bwt significantly (P <0.05) increased of serum ALT, AST, ALP activities and urea level compared to the control group on day 7 post-treatment.

Also, results in (Fig. 1) indicated that repeated administration of 75 µg 10 nm or 50 nm GNPs/kg/bwt has no significant effect on serum ALT, AST activities, urea, creatinine, levels. While they significantly reduced serum ALP activity and significantly (P <0.05) decreased blood glucose concentration on Day-60 post-treatment compared to the control group.

Effects of GNPs on serum lipid profile

Data illustrated in (Fig. 2), represent the effect of acute and chronic administration of 75 µg GNPs/kg/bwt on serum lipid profile. Results reported that i.m. injection of a single dose of 75 µg 50 nm GNPs/kg/bwt to male rats had no significant effect on serum TC, TAG, HDL, non-HDL cholesterol (LDL-VLDL) levels compared to the control group after 3, 7 and 60 days. However, the acute treatment with 10 nm GNPs significantly (P <0.05) increased serum TC, TAG, LDL, and VLDL levels but they significantly decreased serum HDL level compared to the control group on Day-7 post-injection.

As well as, results in (Fig. 2) reported that chronic administration of 10 nm or 50 nm GNPs has no significant effect on serum TC, TAG, HDL, LDL, and VLDL levels on Day-60 post-treatment compared to the control group.

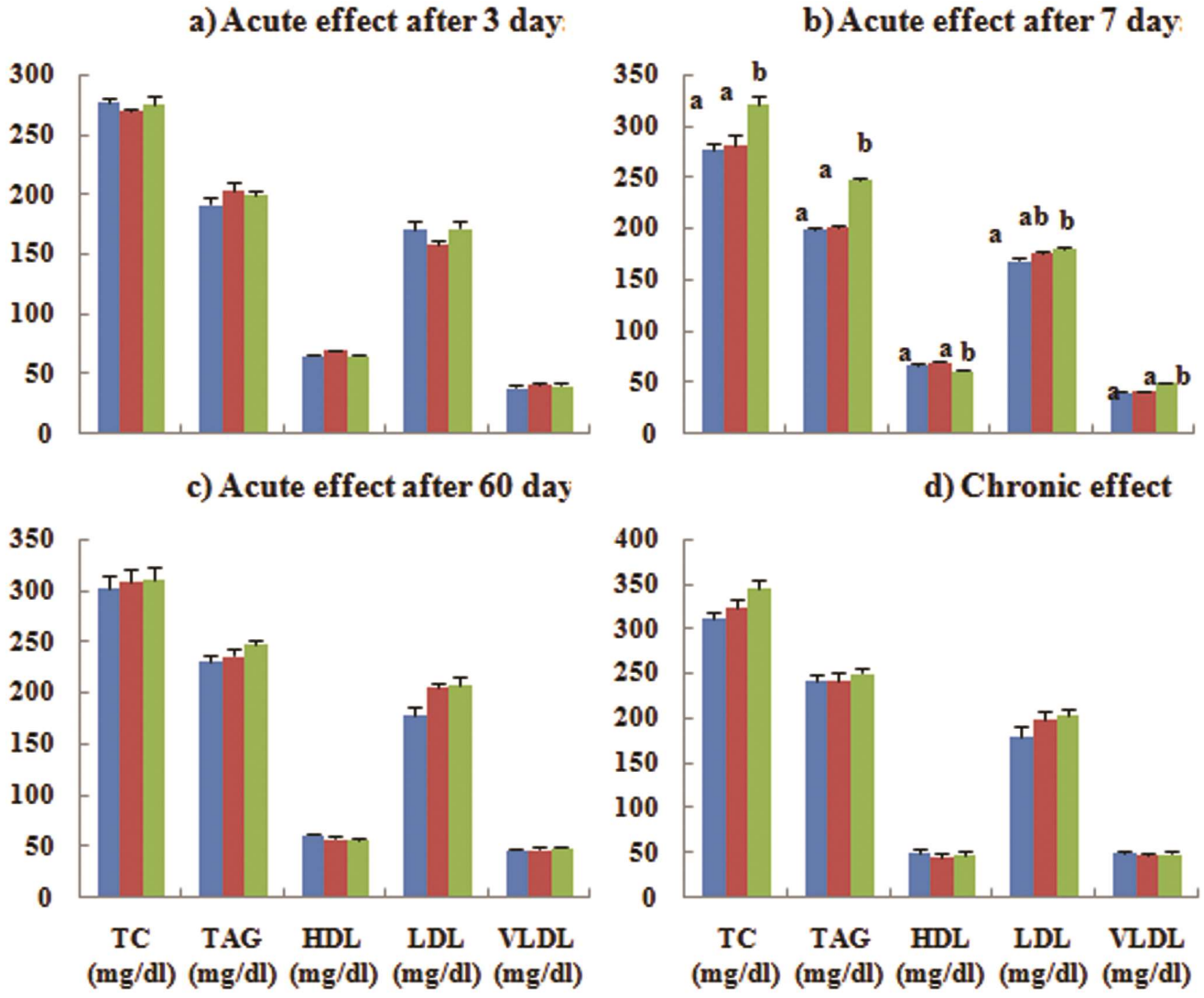

Fig. 2 — Effect of acute and chronic injection of 10 nm or 50 nm GNPs on serum lipid profile after (A) 3; (B) 7; (C) 60 days; and (D) chronic effect in different groups of rats

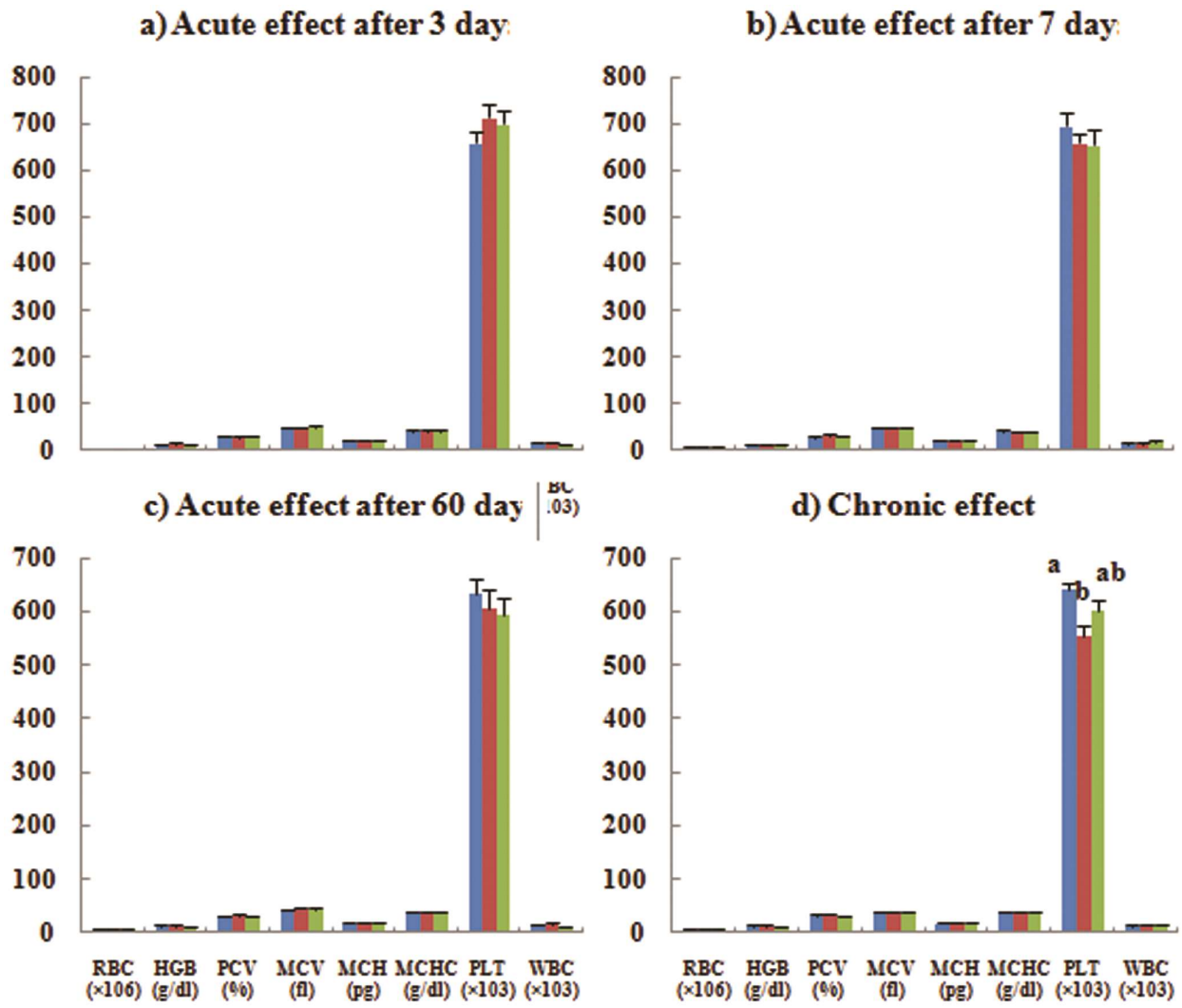

Fig. 3 — Effect of acute and chronic injection of 10 nm or 50 nm GNPs on haematology after (A) 3; (B) 7; (C) 60 days; and (D) chronic effect in male rats

Effects of GNPs on hematological analysis

Data illustrated in (Fig. 3), represent the acute and chronic effects of i.m. injection of 75 µg 10 nm or 50 nm GNPs/kg/bwt on various hematological parameters. For hematological analysis, i.m. injection of single or repeated doses of 10 nm or 50 nm GNPs has no significant effect on any of the tested hematological parameters such as RBC, HGB, PCV, MCV, MCH, MCHC, WBC, and PLT compared to the control group at any time point of the experiment. However, repeated i.m. injection of 50 nm GNPs significantly (P <0.05) decreased platelets count on Day-60 post-treatment when compared to the control group.

Discussion

GNPs have been extensively used in many medical applications. The health impact of exposure to GNPs on various biochemical and hematological parameters in rats must be assessed.

The possible toxic effects of GNPs in the present work were confirmed through the analysis of different biochemical and hematological parameters. With regard to the estimation of liver and kidney functions, on Day-3 after i.m. injection of single doses of 75 µg of 50 nm or 10 nm GNPs/kg bwt in male rats, no adverse effects on activities of ALT, AST, urea and creatinine levels were detected in 10 nm and 50 nm GNPs treated groups when compared with control one, however, Day-7 post-injection which showed significant increase of serum activities of ALT, AST and urea level, however, these values were returned to normal levels on Day- 60 post-injection. Also, in this work, repeated injection of 75 µg of 10 or 50 nm GNPS/kg bwt in male

rats for 5 weeks did not elicit any changes in liver or kidney functions 60 days after injection.

In similar, some studies reported that repeated injection of 0.9, 9 and 90 µg of 14 nm AuNPs 0.9, 9 and 90 µg does not effect on ALP, ALT, total bilirubin (BIL T), creatinine and urea levels in male rats weekly for 7 weeks, followed by a 14-day washout period indicating that there was no acute and/or subchronic damage[32]. In contrast, other studies showed that there was a significant increase in serum ALT, AST activities and creatinine levels in rats *i.p.* injected with PEG-coated and uncoated GNPs at a dose of 12.5, 25, 50, and 100 µg/kg body weight [33]. This difference could be attributed to the higher dose used, the route of administration, or species difference.

Furthermore, in the current study, i.m. injection of single or repeated injection of 75 µg of 10 or 50 nm GNPs/kg bwt has no significant effect on blood glucose level on Day-3 and 60 when compared to the control group. While in 10 nm GNPs treated male rats, blood glucose level was significantly higher on Day-7 post-injection when compared with the control group. Also, the present work showed that repeated injection of 10 nm or 50 nm GNPs significantly decreased blood glucose level on Day-60 post-injection when compared to the control group. AuNPs have the anti-oxidative and anti-hyperglycemic activities of AuNPs in diabetic mice[34]. Conversely, an oral treatment of rats with 650 µg AuNPs/Kg daily for 14 days significantly elevated serum glucose concentration[35]. This difference could be attributed to the higher dose used particle size.

For estimation of serum lipid profile i.m. injection of a single dose of GNPs at size 50 nm and 10 nm had no significant effect on serum TC, TAG, HDL, and non-HDL cholesterol (LDL-VLDL) levels compared to the control group after 3 and 60 days, also, chronic administration of GNPs at both sizes had no significant effect on these parameters. However, a single dose of 10 nm GNPs significantly elevated serum TC, TAG, and non-HDL cholesterol (LDL-VLDL) and significantly decreased serum HDL levels on Day-7 post-injection when compared to 50 nm GNPs or control groups. Similarly, administration of 2.5 mg of 20 nm GNPs/kg *i.p.* every 48 h or daily for 21 days has no changes in cholesterol and triglyceride levels in the liver and serum of GNPs treated rats compared to control group[36]. Also, there were no variations of serum levels of TC, TAG, LDL, VLDL, and HDL between AuNPs-treated mice and control group[37]. On the other hand, an oral dose of 10 mg AuNPs/kg b.w given daily for 60 days induced a significant decrease of TC, TAG, and LDL-c levels and elevation of HDL-c levels compared with diabetic group[38]. This discrepancy could be due to difference in the protocol used for preparation, size, and dose of GNPs.

For hematological analysis, i.m. injection of single or repeated doses of 10 nm and 50 nm GNPs has no significant effect on any of the tested hematological parameters such as RBC, HGB, PCV, MCV, MCH, MCHC, WBC, and PLT compared to the control group at each time point of the experiments. However, repeated doses of 50 nm GNPs significantly decreased blood platelets count on Day-60 post- injection when compared to the control group. These results are matched with other studies reported that the administration of AuNPs in rats daily for 14 consecutive days had no significant effect on all hematological values as compared to control rats[35]. Also, the exposure to 4-5 nm AuNPs at different doses for 6 h/day, 5 days/week, for 90-days in a whole-body inhalation chamber had no significant changes in the hematology value compared to the control group[6]. In contrast, chronic administration of 0.45 mg or 90 mg PEG-AuNRs/kg bwt for 6 months significantly induced hematological changes in female and male rats. This reduction in platelet count might be due to platelet aggregation (clumping), which will reduce reported counts because clumps of platelets will not be counted with any automated hematology analyzers[39].

Conclusion

Single or repeated i.m injection of 10 nm or 50 nm GNPs induced transit changes in liver and kidney functions, which retained to the normal value after 60 days of injection. Also, repeated injection of 10 nm or 50 nm GNPs over 5 weeks showed a hypoglycemic effect in experimental animals.

Conflict of interest

All authors declare no conflict of interest.

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