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Modulation of Immune Response by Gold Nanoparticles Intramuscular Injection to Rats

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ABSTRACT
Gold nanoparticles (GNPs) are an innovative chemical compound, their size is between 1-100 nm. These metal nanoparticles (NPs) exhibit exceptional properties entirely different from their larger sizes. The huge surface area, diversity of shape and size, stability, loaded ability, ease of creation and biocompatibility, making them valuable materials used in pharmacological applications. In addition. GNPs characteristics and their surface modification can modify toxicity and biodistribution. The current study aims to understand enhanced immune response by intramuscular (IM) injection of gold nanoparticles into rats. GNPs were IM injected into rats once at different doses. On the 15th day, samples from all rats were collected. The results revealed no toxic signs. Gold nanoparticles made a significant elevation of the level of antioxidant enzymes comprising superoxide dismutase(SOD) and catalase (CAT), whereas the level of oxidative stress marker Malondialdehyde (MDA) decreased in the injected groups at dose dependent manner when compared with the non-injected control group. Moreover, GNPs exhibit a modulation effect through enhanced immune cell activation via elevation of the level of CD4 and CD8 along with elevation of the level of TNF-α in the injection groups at dose dependent manner when compared with the non-injected control group.

Keywords: Modulation Immune System, Gold Nanoparticles, Antioxidant Enzyme, Immunotherapy

INTRODUCTION

Nanotechnology represents a significant industrial innovation of the 21stcentury, which is applied in numerous medical applications and industrial products. The nanoparticles (NPs) are nanoscale materials with at least one dimension falling between 1-100 nm. The NPs can be classified into different kinds according to their composition, including inorganic (e.g., carbon, silver, gold), organic (e.g., a polymeric matrix, polysaccharide, lipid) and liposomes. The several applications of these nanoparticles are quickly growing because of their optical, electronic and chemical properties [1]. Gold nanoparticles (GNPs) have lately gained the wide attention of scientists in medical uses. They are broadly studied for their unique chemical and physical properties [2].The small size and huge surface area of GNPs compared to their larger counterparts result in large surface energies, unique electronic structures, improved binding affinity, plasmon excitation, oxidation resistance and high loading capacity, making them utilized in different biomedical applications such as treatment, imaging and detection[3, 4]. Due to their magnetic and optical properties, GNPs have been employed in the treatment of several diseases. These properties exhibit decreased cytotoxicity and the capability to interact with various functional and ligand groups that possess a substantial affinity towards the GNPs surface, thereby enhancing interactions with other biomolecules [5].High-atomic-number of GNPs enhances the radiotherapy treatment via their X-ray absorption. In addition, surface plasmon resonance of GNPs improves the radioactive properties such as scattering and absorption. Thus, they are used in photothermal therapy due to their efficient ability to absorb near-infrared and convert them into local heat energy [6, 7]. Some studies have illustrated that GNPs efficiently load and deliver different therapeutic agents such as proteins [8], glucose [9], antineoplastic medications [10], antibiotics [11], antioxidants [12] and nucleic acids [13]. The current study aims to understand the effect of rod-gold nanoparticles citrate-coated in the modulation of immune response.

Method

Gold nanoparticles

In this study rod-gold nanoparticles their dimensions are 10 nm in widthand 38 nm in length, sodium-citrate coated. Manufactured by Sigma with the batch number MKCS7674. Other properties are shown in Table (1).

Table 1: presented properties of gold nanoparticles.

NO	Test	Result
1	Color	Very faint brown to brown
2	Form	Liquid
3	Length	38 nanometers
4	Diameter	10 nanometers
5	PH	7
6	SPR peak	785 nanometers
7	Absorbance SPR OD	1.20
8	Transverse peak LSPR	510 nanometers
9	Absorbance LSPR OD	0.25
10	Concentration	42.0 µg/ml
11	Citrate	Confirmed
12	Inductively Coupled Plasma analysis Confirms gold component	Confirmed

Modulation of immune response

In this study, male Wister albino rats were employed, their weights were between 150 g to 250 g and aged 6 to 8 weeks. They were gained from a medical center for animal breeding. The rats were categorized into three groups according to the doses, with an additional fourth group serving as control: the first group of 10 rats received intramuscularly (IM) injection of GNPs at a dose of 5 µg/kg. The second group of 10 rats received IM injection of GNPs at a dose of 10 µg/kg and the third group of 10 rats received IM injection of GNPs at a dose of 20 µg/kg. Whereas the fourth 10 rats that employed as a control group were not injected with GNPs. Throughout the experiment days, each rat was housed in a cage alone at a temperature (18–28°C) and a 12-hour light-dark system. They were courteously provided with water and a chow meal until 24 hours before the IM injection of GNPs they prevented feeding. While the water was prevented one hour before the IM injection. The study was conducted in agreement with the ethical requirement of the Veterinary Medicine College at the University of Diyala. On the 15^{th} day post-injection with GNPs all the rats of the groups were anesthetized and the procedure was done as described by Abood, et al. [14]. Afterward, blood samples were obtained from the anesthetized rats for analysis of the study parameters by using the ELISA technique. After that, the anesthetized rats were sacrificed to collect the organs (spleen, kidney, and liver) for histo-pathological examinations.

Evaluation the level of TNF- α, CD8, CD4, MDA, CAT, and SOD

The collected blood was put in the free-anticoagulant tube. Afterwards, the tubes were allowed for 30 minutes at (25°C) to coagulate. Then, the tubes were put in a centrifuge for 10 minutes at (4000RPM). Ultimately, the obtained serum was placed in an eppendorf tube and kept at -20°C until use. The study parameters level were measured by ELISA kit from (bioassay-tech lab). The cat.No for catalase is (E0869Ra), CD4 (E0044Ra), CD8 (E0045Ra), superoxide dismutase (E0168Ra), tumor necrosis factor alpha (E0764Ra) and Malondialdehyde (E0156Ra). The tests were carried out in a manner that was completely in accordance with the guidelines that were prescribed by the manufacturer.

Statistical analysis

The collected data was analyzed statistically by utilizing SPSS software. The findings were obtained via using ANOVA and T-test, provided as mean ± SD, with significance at a p-value of ≤ 0.05.

RESULTS

Intramuscular injection of GNPs to rats with different doses exhibited, no mortality occurred, no toxic signs, and there were no neurological signs or evidence of hair loss. Additionally, the weight of rats in all study groups was significantly increased at a p-value of ≤ 0.05 as shown in the Figure 1.

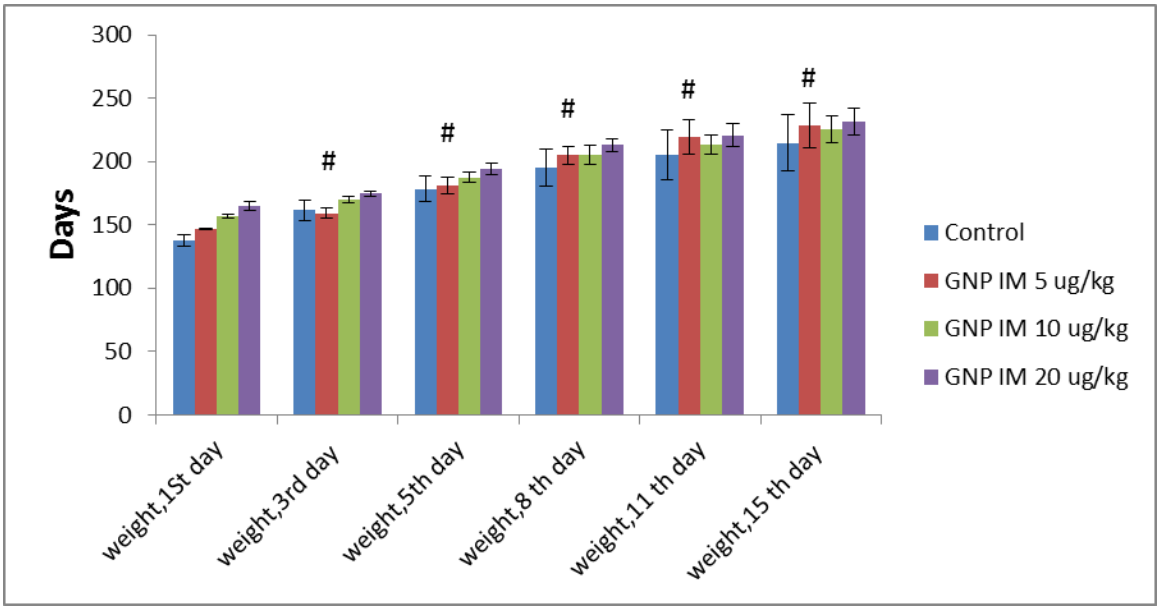

Figure 1: showed the weight of the rats association to days in the control group and IM injection of gold nanoparticles groups at doses (5, 10, and 20 µg /Kg), 10 rats/groups. #Significant at $P \leq 0.05$.

The modulation effect of GNPs on the function immune system was obviously noticeable in the activity of the antioxidant enzyme through raising the level of superoxide dismutase (SOD) and catalase (CAT) in the injection GNPs groups and significantly lowering the level of Malondialdehyde (MDA) when compared to the control group, as shown in Table 2. Furthermore, the impact of GNPs on lymphocytes was examined and the finding revealed a modulation effect of GNPs on the lymphocytes and raising the level of CD8 and CD4 (Figure 2&3), as well as an elevation in TNF-α levels within the study groups (Figure 4) when compared to the control group.

Table 2: showed the level of antioxidant enzyme in the control group and IM injection of gold nanoparticles groups at doses (5, 10, and 20 µg /Kg).

Groups	CAT ng/mL	SOD ng/mL	MDA nmol/mL
control	79.23 ± 8.96	1.97 ± 0.41	1.60 ± 0.67
GNPs IM 5 µg/kg	173.35 ± 152.11	2.15 ± 0.41	0.81 ± 0.27 #
GNPs IM 10 µg/kg	267.60 ± 74.15 #	2.31 ± 0.18	0.59 ± 0.089 #
GNPs IM 20 µg/kg	279.91 ± 89.99 #	3.54 ± 1.07 #	0.59 ± 0.12 #

Rat 10 / groups. #Significant at $P \leq 0.05$

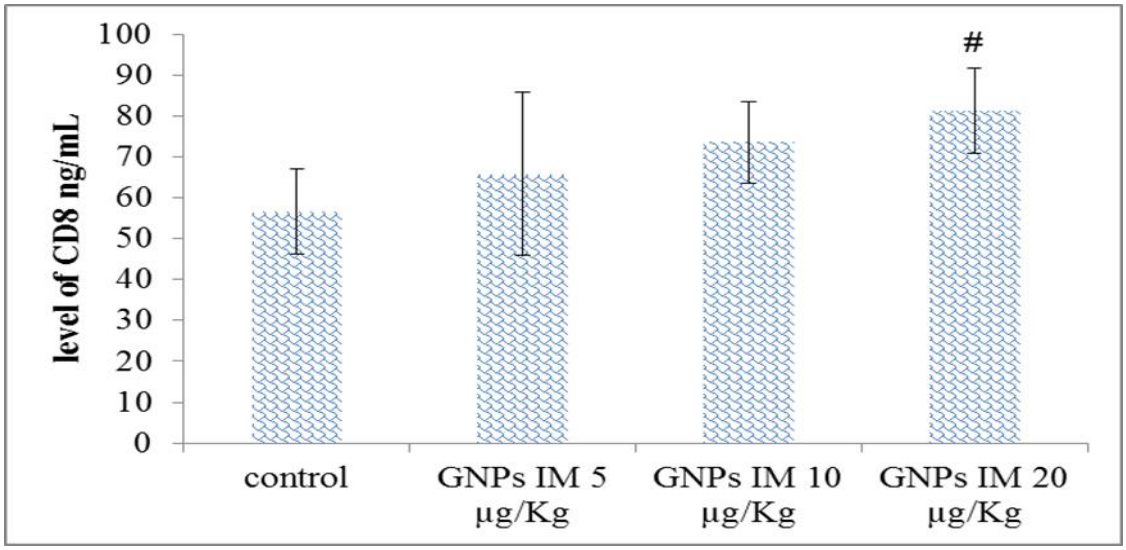

Figure 2: showed the level of CD8 in the control group and IM injection of gold nanoparticles groups at doses (5, 10, and 20 µg /Kg), 10 rats/groups. #Significant at $P \leq 0.05$.

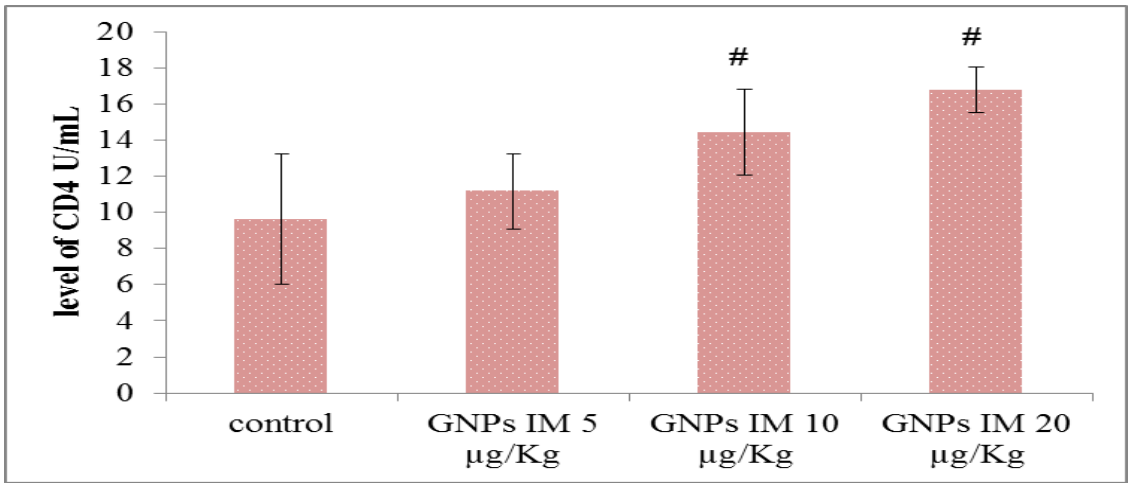

Figure 3: showed level of CD4 in the control group and IM injection of gold nanoparticles groups at doses (5, 10, and 20 µg /Kg), 10 rats/groups. #Significant at $P \leq 0.05$.

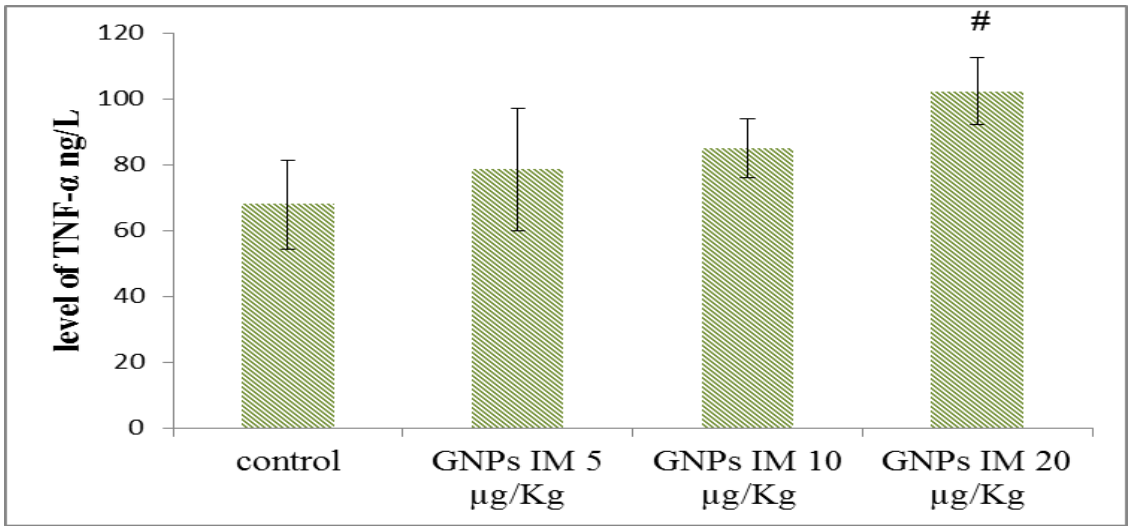

Figure 4: showed the level of TNF-α in the control group and IM injection of gold nanoparticles groups at doses (5, 10, and 20 µg /Kg), 10 rats/groups. #Significant at $P \leq 0.05$.

Histopathological investigation revealed no toxic signs for the spleen, kidney, or liver in all the study groups injected GNPs compared to the control group (Figure 5).

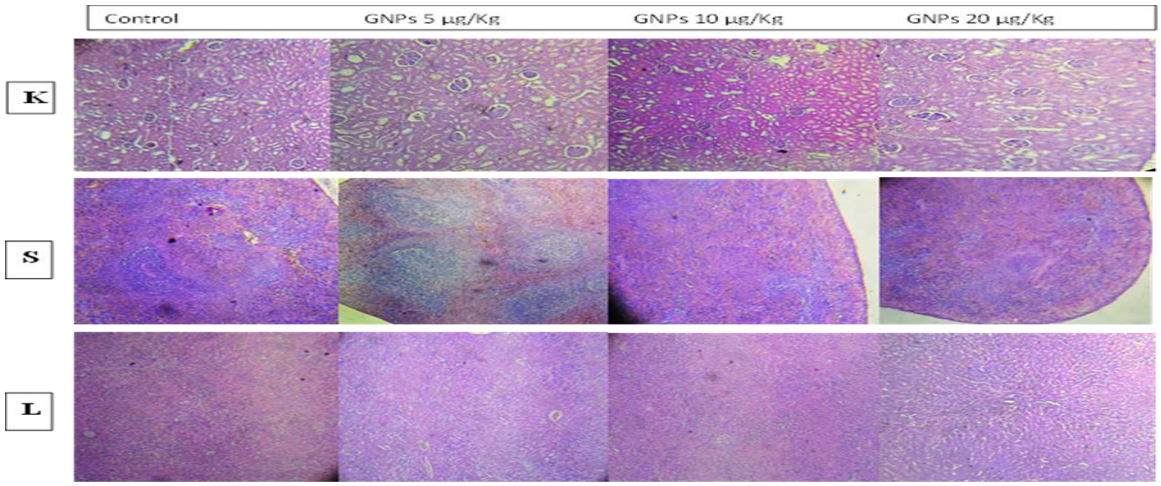

Figure 5: Histopathology H&E stain, at 100X magnification, for spleen, kidney and liver in the study group. The finding was obvious that there was no toxic appearance for the organs that were examined in the present

study in all groups were IM injected of GNPs at doses (5, 10, and 20 µg/kg), did not result in significant alteration to the texture as compared to the control group. The K letter, denotes kidney tissue, exhibiting normal glomeruli with sufficient blood vessel quantities. The S letter, denotes spleen tissue, which clearly exhibits normal spleen architecture; the white pulp is distinctly separated from the red pulp through a marginal zone. Moreover, the white pulp exhibited a pale germinal center (follicle), with a central arteriole encircled via a peripheral lymphatic sheath. The L letter, denotes liver tissue, which exhibited a normal central vein and an intact cell texture (sinusoidal capillaries, kupffer cell, and normal hepatocytes with round nuclei).

DISCUSSION

The immunological function of gold nanoparticles has garnered significant interest from researchers, particularly regarding their immunogenicity effect on the immune response. In the past study, the researcher successfully obtained antisera against colloidal gold. Subsequently, other authors were studied to conjugate antigens with colloidal metals to boost antibody production [15]. Several other studies demonstrated that when haptens adsorbed to colloidal metal result in high production of antibody. Many data have displayed that intravenous injection of colloidal gold in rabbits affect the non-specific immunity, hence result in raising the level of leukocytes [16]. Several researchers utilized the colloidal gold to improve procedure for production of antibody against diverse substances such as biotin, platelet activating factors, hepatitis capsid-peptide, amino acid, surface Yersinia antigen, quinolinic acid and lysophosphatide acid [17-19].

The gold nanoparticles were employed in the diverse immunological applications such as antiviral vaccine as well as used as therapeutic treatment in rats with medulispinal traumas [20].The GNPs also utilized for activating phagocytes within macrophages and improving lymphocyte function [21] and stimulation of T cells [22]. In the current study, the intramuscular injection of GNPs into rats results in the modulation response of immune system through raising the level of both CD4 and CD8 as well as elevation of the level of TNF-α, along with elevation of the level of antioxidant enzyme CAT and SOD. These effects of GNPs on the modulation of immune response involve interaction with the TLR-4 receptor in macrophages and internalization of GNPs within the cells. This is associated with the release of proinflammatory cytokines such as IL-6 and TNF, resulting from the suppression of macrophage proliferation [23].

Furthermore, the non-inflammatory role of GNPs is demonstrated when they penetrate macrophages through interaction to scavenger receptors [24]. Inhibitory effect investigation of GNPs coated with polyethylene glycol on the generation of nitric oxide, in macrophages stimulated-lipopolysaccharide, it was demonstrated that successfully inhibit nitric oxide generation [25].

In addition, non-conjugated GNPs that were injected into mice had the potential to enhance the proliferation of lymphocytes and NK cells with raising the IL-2 production [18]. These prior investigations presented the importance of GNPs utilization in medical applications due to their safety and efficacy in enhancing immune response.

CONCLUSION

The outcome of this study concluded that the important utilization of GNPs in therapeutic applications is discovering a novel treatment for several diseases.

Conflict of interest

Authors were declared no conflict of interest

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