Modulation of Immune Response by Gold Nanoparticles Oral Administration to Rats

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KEYWORDS

Gold nanoparticles, Modulation immune system

ABSTRACT

Gold nanoparticles (GNPs) are a new chemical compound and have recently been used in many pharmacological applications. This study aims to understand enhanced immune response by the oral administration of gold nanoparticles to rats. Gold nanoparticles are administration once oral at different doses to rats, at 14th day collected sample from all administrated rats. The results were shown no effect on the rats' weight. Gold nanoparticles were caused elevated significant in the antioxidant enzyme CAT and SOD, while decreased in MAD in the administrated groups at dose dependent manner compared with non-administrated control group. Also, GNPs have immune response through modulation in the enhanced immune cell activation by elevated the level of CD8 and CD4 in the administrated groups at dose dependent manner compared with non-administrated control group with the keep the level of TNF-α no differ within all study groups.

1. Introduction

Gold nanoparticles(GNPs) have recently become of interest to scientists in medical use. Its chemical and physical properties like their sizes and high chemical stabilization made them the best choice in the different purposes for industry and medicine [1]. GNPs synthesis at different shapes rods ,spherical and colloid and can be blue, red or other color, besides at different bright and wavelength [2]. The unique properties of GNPs as photonic, electronic and catalytic made them have a lot of used in the different applications in technology and biomedical applications [3] as improve of PCR methods efficiency [4], transport of material into cells [5], cell motility investigation [6], and successful in the cancer therapeutically [7]. One of the cancer treatment by thermal way and can be used GNPs in this treatment through its ability to absorption light and be hot when irradiated with the light, could be inside or around the target cell so that can combine GNPs with and treatment to be more active. One of the more interest to use GNPs in the enhancement of immune response or treated immunological diseases. In 1997 they used GNPs in the treat of human rheumatoid arthritis [8]. In previous study used colloidal gold was get positive result in the decreased inflammation through decrease macrophage infiltration and enhanced antiangiogenic potential from coupling GNPs with VEGF [9, 10]. Other study was showed the effect of colloidal gold in the elevated TNF for tumor at rats [11, 12]. In this study was investigated the effect of rod gold nanoparticles coated with citrate in the modulation of immune response.

2. Methods

Gold nanoparticles

This study used rod gold nanoparticles coated with sodium citrate, their dimensions are 38 nanometers in length and 10 nanometers in width. According to manufacture Sigma batch number MKCS7674. Tables (1) was showed other properties.

Table (1): showed properties of gold nanoparticles

NO	Test	Result
1	Form	Liquid
2	Color	Very faint brown to brown
3	Inductively Coupled Plasma analysis Confirms gold component	Confirmed
4	PH	7
5	Length	38 nm
6	Diameter	10 nm
7	SPR peak	785 nm
8	Absorbance SPR OD	1.20
9	Transverse peak LSPR	510 nm

10	Absorbance LSPR OD	0.25
11	citrate	Confirmed
12	concentration	42.0 µg/ml

Modulation of Immune Response

For this study, male rats (Wister albino) were utilized, their ages ranged from 6 to 8 weeks old and their weights were between (150-250) grams. The rats were obtained from Iraqi medical Centre for Animal Breeding. All rats were housed in standard temperature (18–28°C), humidity (30–70%), and light (12–12 hour light:dark cycle) condition. Each rat was put in a cage housing alone for the length of the test. They were provided with chow meal (pellets) and water. They prevented feeding for 24 hours, while water was given continuously until one hour before giving medication. The research was carried out in accordance with the ethical guidelines established by the College of Veterinary Medicine at the University of Diyala. According to the doses, the rats were divided into three groups, with a fourth additional group as a control: A group of 10 rats received orally of GNPs at a concentration of 5 µg/kg. Other a group of 10 rats received orally of GNPs at a concentration of 10 µg/kg. And agroup of 10 rats received orally of GNPs at a concentration of 20 µg/kg. While a fourth group of 10 rats was employed as a control not administered with GNPs. After 15 day from administered with GNPs all rats were anesthetized and the method was done as described by Abood, et al. [13] . Blood sample was collected from the rats after they were anesthetized for diagnostic parameters by the ELISA method, and they were subsequently sacrificed to collect kidney, liver, and spleen samples for histopathology examinations.

Measured The Level Of CD8, CD4, TNF- A, MDA, Catalase, SOD

Blood was collected and then put in a glass tube free of anticoagulant, with each group separately marked. After that, the collected blood was left for 30 minutes at room temperature (25°C) to coagulate. The samples were then centrifuged at (4000 RPM) for 10 minutes. After that, the serum that was got was put in an eppendorf tube and stored in -20^{o} C until it was used. The CD8 level was measured by ELISA Kit (Bioassay technology laboratory. Cat.No: E0045Ra). The CD4 level was measured by ELISA Kit (Bioassay technology laboratory. Cat.No: E0044Ra). The catalase level was measured by ELISA Kit (Bioassay technology laboratory. Cat.No: E0869Ra). The Malondialdehyde (MDA) level was measured by ELISA Kit (Bioassay technology laboratory. Cat.No: E0156Ra). The superoxide dismutase (SOD) level was measured by ELISA Kit (Bioassay technology laboratory. Cat.No: E0168Ra). The tumor necrosis factor alpha (TNF- α) level was measured by ELISA Kit (Bioassay technology laboratory. Cat.No: E0764Ra). The tests had been carried out entirely according to the instructions provided by the manufacturer.

Statistical analysis

The data was collected and statistically analyzed by using SPSS software. The results were got through applying ANOVA and T test and expressed by Mean ± SD with significant at P value ≤ 0.05.

3. Results and Discussion

Oral administration of GNPs to rats at different doses was appeared no toxic effect, no death was happened and there is no any sign of hair losing or neurological sign. The weight of rats was increased significant at P value ≤ 0.05 in all study groups as shown in the figure 1.

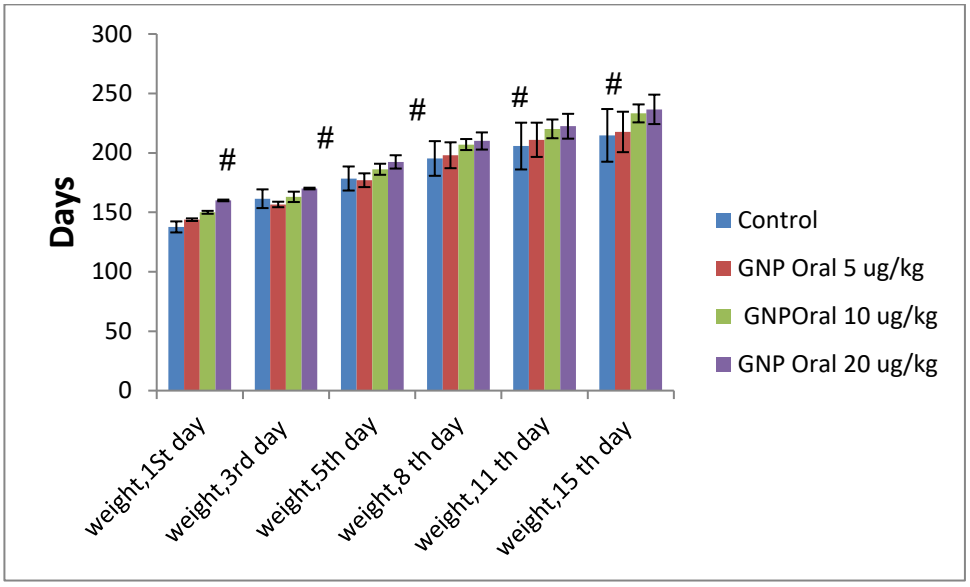

Figure 1: Presented weight of rats related to days among groups; control and gold nanoparticle (GNP) groups at dose (5 µg /Kg, 10 µg /Kg and 20 µg /Kg) orally administrated to rat, 10 rats / groups. #Significant at P ≤ 0.05. The modulation effect on the immune system function was clearly notice in the level of antioxidant enzyme activity by elevated the level of CAT and SOD in the administrated GNPs groups and decreased in MDA significant compared with control group (Table 2). On the other hands the effect of GNPs on the level of lymphocytes was investigated and the results was showed the modulated effect of GNPs on the elevated the number of CD4 and CD8 lymphocytes compared with control group (Figure 2&3), while no effect on the level of TNF-α among all study groups (Figure 4).

Table 2: Presented the level of antioxidant enzyme in the rats among groups; control and gold nanoparticle (GNP) groups at dose (5 µg /Kg, 10 µg /Kg and 20 µg /Kg) orally administrated

Groups	**CAT ng/mL**	**SOD ng/mL**	**MDA nmol/mL**
Control	79.23 ± 8.96	1.97 ± 0.41	1.60 ± 0.67
GNP Oral 5 µg /Kg	127.04 ± 23.29	1.92 ± 0.56	1.21 ± 0.16
GNP Oral 10 µg /Kg	158.01 ± 22.35	2.33 ± 0.86	0.77 ± 0.17 #
GNP Oral 20 µg /Kg	474.64 ± 149.88 #	2.89 ± 0.88	0.72 ± 0.21 #

Rat 10 / groups. #Significant at P ≤ 0.05

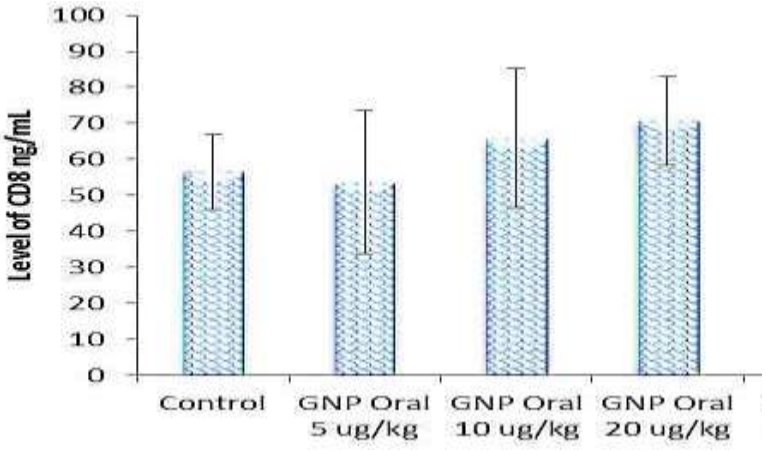

Figure 2: Presented level of CD8 in the rats among groups; control and gold nanoparticle (GNP) groups at dose (5 µg /Kg, 10 µg /Kg and 20 µg /Kg) orally administrated, 10 rats / groups. #Significant at P ≤ 0.05.

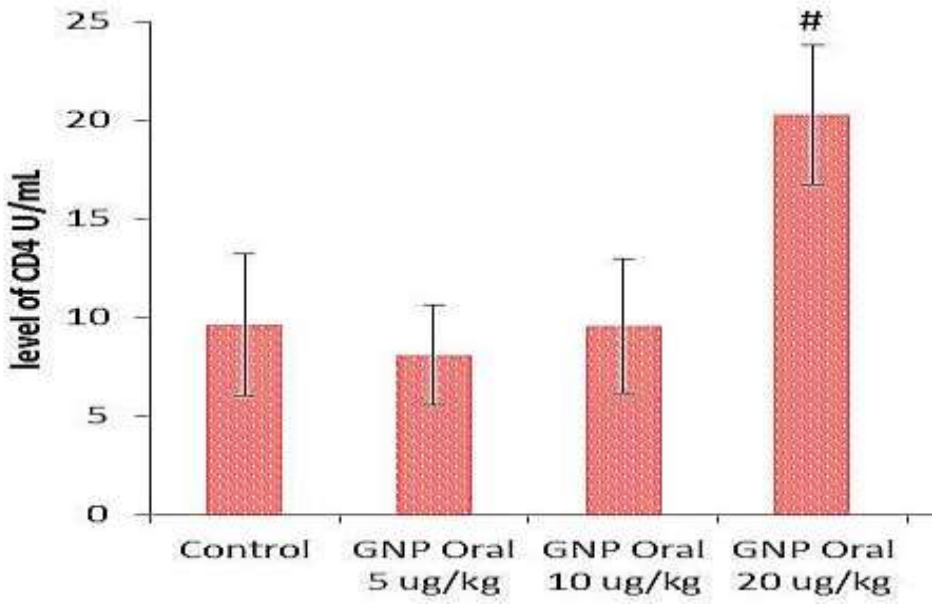

Figure 3: Presented the level of CD4 in the rats among groups; control and gold nanoparticle (GNP) groups at dose (5 µg /Kg, 10 µg /Kg and 20 µg /Kg) orally administrated, 10 rats / groups.
[#]Significant at $P \leq 0.05$

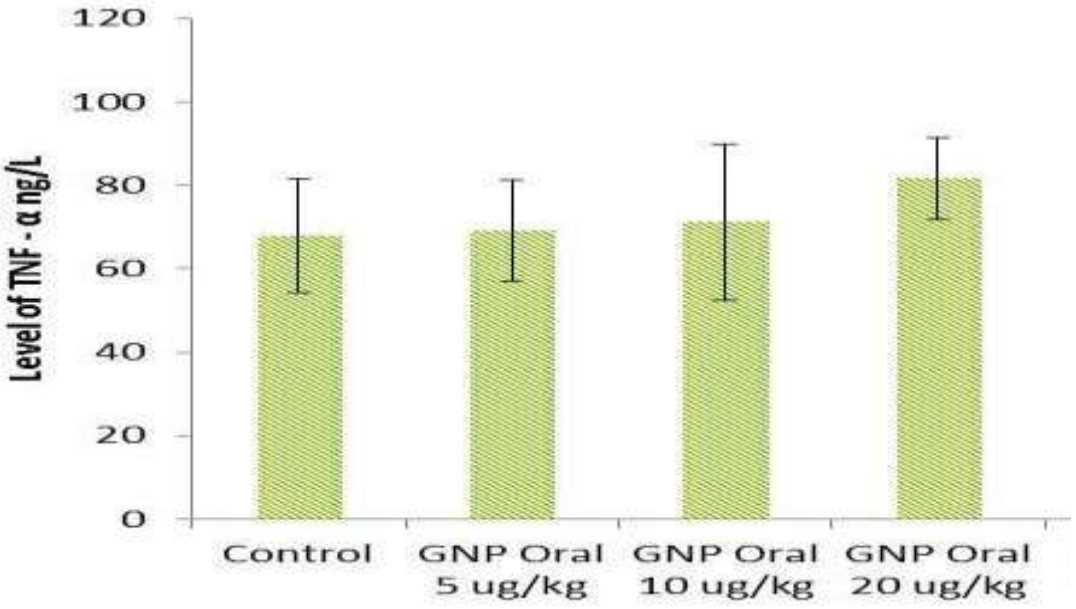

Figure 4: Presented the level of TNF-α in the rats among groups; control and gold nanoparticle (GNP) groups at dose (5 µg /Kg, 10 µg /Kg and 20 µg /Kg) orally administrated, 10 rats / groups.
[#]Significant at $P \leq 0.05$

Histopathlogical examination was indicated no toxic sign for the liver, kidney or spleen in all groups administrated GNPs compared with control group (Figure 5).

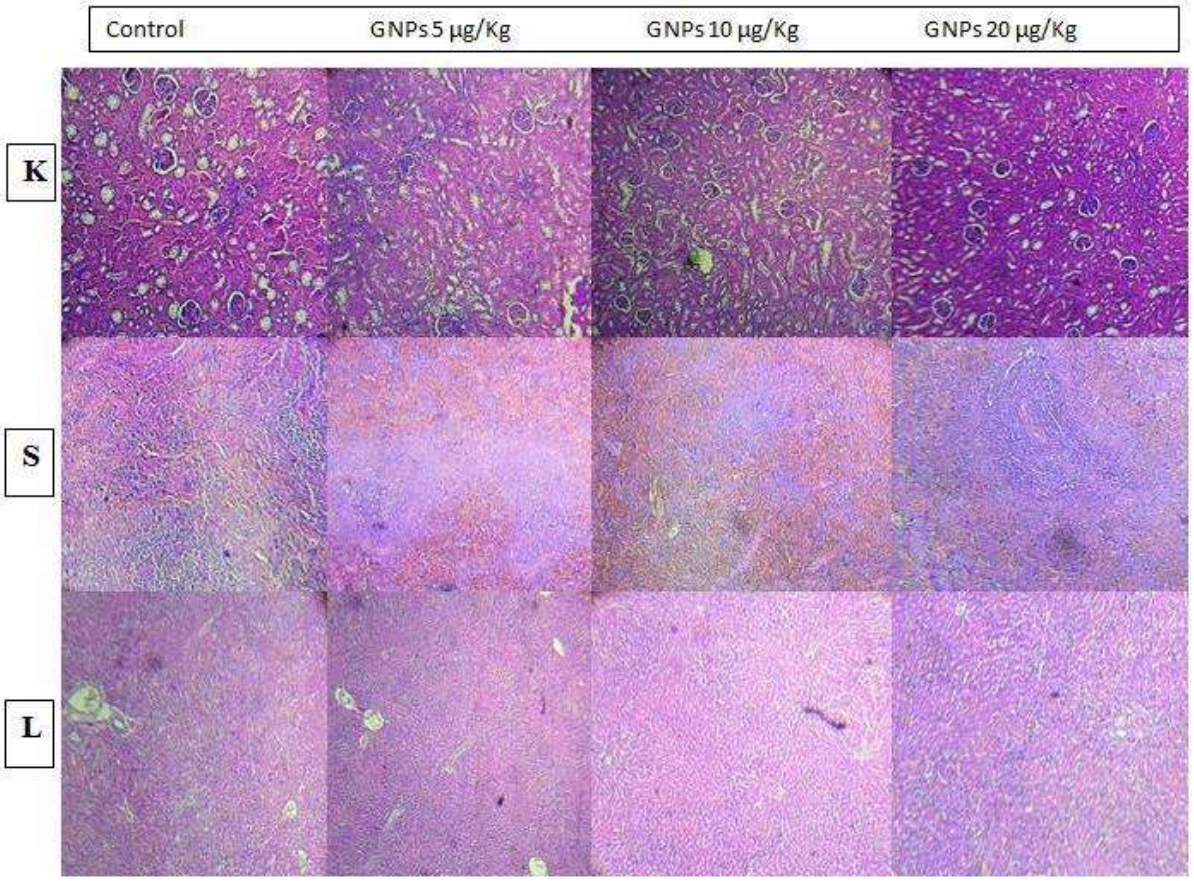

Figure 5: Histopathology H&E stain , magnification 100X , results for Kidney, spleen and liver for rats in study groups. The result was clearly appeared no toxic appearance for the organs investigated in this study at all groups were orally administrated gold nanoparticle (GNP) at dose (5 µg /Kg, 10 µg /Kg and 20 µg /Kg) no significant change in the texture compared with control group. K letter, refers to kidney tissue was shown normal glomeruli with adequate quantities blood vessels. S letter, refers to spleen tissue was shown clearly the normal spleen architecture; white pulp separated from red pulp through marginal zone. The white pulp appeared with pale germinal center (follicle), peripheral located central arteriole surrounded with peripheral lymphatic sheath. L letter, refers to liver tissue was revealed normal central vein, with normal cell texture (kupffer cell, sinusoidal capillaries and normal hepatocytes at a round nuclei.

Immunological activity of gold nanoparticles was attracting many researchers mainly at immunogenicity action of immune response. In previous study the researcher was successful to get antisera for colloidal gold. After that, many authors were investigated to couple antigen with colloid metal for enhanced antigen production [14]. Other previous study proven that haptens when adsorbed to colloid metal lead to over antibody production. A lot of data showed the effect of colloid gold on the non-specific immunity when intravenous injected to rabbits caused increasing in leukocytes [15]. Nemours researchers used the colloid gold to develop technique for production antibody for different materials as amino acid, biotin, platelet activating factors, lysophosphatide acid, quinolinic acid, and the capsid peptides for hepatitis, Yersinia surface antigen [16-18].

The gold particles were utilized in the different immunological applications as prepared an antiviral vaccine, as therapeutic for the treatment medulispinal traumas in rats [19]. And used GNPs for activating phagocytes of macrophage and enhanced lymphocytes function [20] and activation of T cells [21]. In this study was found that oral administration of GNps to rats lead to modulation of immune response through increase the number of CD8 and CD8 cells besides elevated the level of TNF-α , and antioxidant enzyme SOD and CAT. These effect of GNPs in the modulation of immune

response through interact with TLR-4 receptor for macrophages and GNPs penetrate inside the cells, this related with secretion of proinflammatory cytokines as IL-6 and TNF by suppressed of macrophages proliferation [22].Moreover, the non-inflammatory potential for GNPs is when penetration to macrophages by interacted with scavenger receptors [23].The effect of GNPs in the inhibition effect of PEG coated with GNPs on Nitric oxide production in the mechanism of stimulated macrophages by lipopolysaccharide [24].njected non-conjugated GNPs to mice could enhancement the lymphocytes proliferation and NK cells with increasing IL-2 production [17]. These previous investigated showed the importance of sued GNPs in the medical application for its safety and affectivity in the enhanced immune response.

Conclusion: The result of this study was concluded the importance used GNPs in the therapeutic application in discover a new treatment for different diseases.

Conflict of interest: Authors were declared no conflict of interest

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