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Acute And Chronic Hematological Study Of Gold Nanorods In Rats.

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ABSTRACT

Cancer is the second leading cause of death worldwide. Cancer is usually managed by chemotherapy, radiotherapy and immunotherapy. However, most of these approaches leads to serious adverse effects especially myelo suppression. A new trend for cancer management is the nano medicine has been discovered. Unlike other cancer therapies, nano medicine has unique properties due to its ability to target tumor cells without affecting normal cells by several pathways. This article aimed to investigate the acute and chronic hematologic effects of Gold nanorods (AuNRs). In the acute study, forty adult male albino rats weighing 180-200 gm were used. They were allocated into two equal groups. The first group received IP injection of normal saline 0.9% and the second group received 1 ml of 300 µg/kg body weight of AuNRs dissolved in normal saline. At day 1, 3, 7, and 14 post treatments, five animals from each group were sacrificed and blood was collected on EDTA test tubes for hematological study. For the chronic study, twelve adult male Albino rats were used. Rats were also allocated into two equal groups. The first group received IP injection of normal saline 0.9% and the second group received IP injection of 1 ml of 30 µg/kg body weight AuNRS dissolved in normal saline for five consecutive days in the first month (from day one to day five) and from day thirty-one to day thirty-five in the second month. At day sixty, rats of both groups were sacrificed to obtain blood on EDTA test tubes for hematological study. This study concluded that AuNRs have mild adverse effects on hematological parameters. It has been found that the intraperitoneal administration of GNRs (acute and chronic study) caused mild hematological abnormalities with no mortality.

Keywords: Male Albino rats, AuNRs, acute and chronic and hematological study

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INTRODUCTION

According to WHO's World Cancer Report 2014, nearly 25 million cancer cases will occur over the next two decades worldwide. Because of advances in cancer diagnosis and treatment, the survival rate of patients with cancer has been increasing, but the side effects of cancer treatments, like radiotherapy, chemotherapy and immunotherapy are still a major problem. Chemotherapy, as a standard regimen, has been used for treating cancers for 70 years. However, in recent years, there is a controversy as to whether it should continue to be used because it causes major side effects, especially hematotoxicity[1]. The main toxicity of most anticancer agents is hematological; it corresponds to a decrease in the production of rapidly dividing cells such as blood cell progenitors (Cytopenia) [3].

Nanomedicine is an emerging method for treatment of cancer. Unlike cancer chemotherapy, nanoparticles have unique properties that it has the ability to target tumor cells without affecting normal cells by several pathways [5]. Nanomaterials such as gold nanoparticles (AuNPs) are already being used in diagnostic imaging, [1] drug delivery, [2, 3] and cancer photothermal therapy. [4, 5]. The increasing biomedical applications of AuNPs have raised major concerns regarding their *in vivo* fate and potential toxic effects in living organisms, including humans. [6]. Although AuNPs are recognized as being as nontoxic, (Connor et al., 2005; Shukla et al., 2005) there have been still some reports on their toxicity (Chithrani and Chan, 2007; Pan et al., 2007), which has been shown to depend on the physical dimension, surface chemistry, and shape of the AuNPs. Gold nanorods has on adverse effect on blood profile in human (He et al., 2018), dogs and cats (Abdoon et al., 2015, 2016), rats (Lee et al., 2018). However, other studies found that after the administration of 25 nm AuNP, the concentration of white blood cells was increased in rabbit (Glazer et al., 2012). Several studies have reported no significant short-term toxicity of AuNPs (1 day to 3 months) [14] ***Connor, et al., (2005).*** The dose-dependent effects of gold nanoparticles on biological systems have been widely recognized [15]. The available literature on in vitro and in vivo toxicity of AuNPs showed contradicting findings, primarily because in vivo behaviors of AuNPs are dependent on their physicochemical characteristics [26]. Since the blood is the first point of contact in any therapy, and it is necessary to have a thorough in vivo investigation of gold nanoparticles on blood profile to avoid any adverse effects. This study aimed to investigate the possibility of hematotoxic effect of acute and chronic administration of gold nanorods in male rats.

MATERIAL AND METHODS

Ethical approval: ZU/IACUC number: *ZU-IACUC/2/F/49/2018* from IACUC committee of Zagazig University, Egypt

Chloroauric acid ($HAuCl_4.\ H_2O$), Cetyltrimethylammonium bromide (CTAB), ascorbic acid and sodium borohydride ($NaBH_4$) were purchased from Sigma-Aldrich Co. All solutions were prepared using deionized water (Milli-Q water).

Synthesis of gold nanorods (GNRs)

GNRs solution was prepared using the seed-growth approach according to the method adopted by **Nikoobakht and El-Sayed [30]**. Briefly, the reaction was carried out through the following steps: 1) **Seeding solution,** 5 mL of (5×10^{-4} M) $HAuCl_4$ is added to CTAB solution (5 mL, 2 $\times10^{-1}$ M) with gentle shaking and an orange solution was obtained. 600 µL of (10^{-2} M) ice-cold $NaBH_4$ is injected at once to the above mixture. The color of the mixture is instantly turned from orange to reddish brown. 2) **Growth solution,** 300 µL of (4×10^{-3} M) $AgNO_3$ is added to a mixture of CTAB (5 mL, 2 $\times10^{-1}$ M). To this aqueous mixture, 70 µL of (7.8×10^{-2} M) ascorbic acid is added which results in changing the growth solution from orange to colorless. Finally, 12 µL of the seed solution is injected at once to the growth solution. The color of the growth solution changes slowly within 30-45 min to the reddish purple. 3) **Gold Nanorods PEGylation:** Thiol-terminated methoxypoly-(ethylene glycol) (mPEG-SH) (MW=5000) was purchased from Nanocs Co. The raw nanorods solution was centrifuged at 15000 rpm for 20 min to pellet the nanorods, decanted, and then re-suspended to 10 ml of deionized water to remove excess CTAB. 0.05 g of mPEG-SH were added to the nanorods solution. The mixture was kept overnight at room temperature, then was centrifuged, decanted, and re-suspended in deionized water twice to remove excess CTAB and mPEG-SH.

Characterization of GNRs

The absorption spectra of GNRs solutions were recorded by using V-630 UV-VIS Spectrophotometer (Jasco, Japan). A strong absorption band with a maximum at~700 nm resulting from the electronic oscillation of the electrons of the nanorod along its long axis and a weak band at ~500nm polarized along the short axis resulting from nanorod electrons oscillations along the short axis of the gold nanorod. Transmission electron microscopic (TEM) images were obtained using JEOL JEM 2010 TEM operated at 100 kV accelerating voltage. From the TEM results, it is very important to make sure that most of the nanoparticles made have rod shapes with the long to the short axis length ratio of ~ 4 and thus absorb the light at ~700 nm (near infrared light) and not spheres or other shapes that do not absorb the near infrared light used and thus will not get hot to kill the cancer cells.

Animals and experimental design

Experiment I: Effect of acute i.p. administration of 50nm gold nanorods on blood profile in male rats.

Forty eight adult male Albino rats Wister strain weighing 180-200 gm were used in the present work. They were obtained from the laboratory animal house of the Faculty of Veterinary Medicine, Zagazig University. They were left for acclimatization for one week. Feed of balanced ration and water were added *ad libitum*. Rats were divided into two equal groups. The first group was received i.p. injection of 1 ml normal saline 0.9% and kept as a control group, While the second group was received 1 ml of 75 µg AuNRs/kg body weight as one shot **[8]**. On Day-1, Day-3, Day-7 and Day-14 post treatment, five animals from each group were sacrificed to obtain blood on EDTA vacutainer tube for hematological study.

Experiment II: Effect of chronic i.p administration of 50nm gold nanorods on blood profile in male rats.

Twelve adult male Albino rats weighing 180-200 gm were used. They were obtained from the laboratory animal house of the faculty of veterinary medicine, Zagazig University. They were left to acclimatize in the laboratory of pharmacology for one week. They were given clean tap water *ad libitum* and balanced ration. Rats were allocated into two equal groups. The first group was received i.p. injection of normal saline 0.9% for five consecutive days in the first month (from day one to day five) and from day thirty-one to day thirty-five in the second month. In the second group , rats were i.p. injected with 1 ml of 7.5 µg of 50nm AuNRs/kg body weight for five consecutive days in the first month (from day one to day five), and from day thirty-one to day thirty-five in the second month. At day sixty, rats of both groups were sacrificed to obtain blood on EDTA vacutainer tube for hematological study **[9]**.

Hematological studies

Hematological autoanalyzer (Automatic cell counter: Sysmex KX 21 N, Sysmex Co., Japan) was used to determine hematological parameters such as red blood cells (RBC), white blood cells (WBC), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets (PLT) **[10]**.

Statistical analysis:

In order to assess the influence of intraperitoneal administration of gold nanorods on hematological parameters in experiment 1 and 2, Independent T- test followed by Levene's Test Significant Difference (Levene's HSD) test as post hoc test were used**. [11].** Analysis was done using Statistical Package for Social Sciences version 22.0 (IBM Corp., Armonk, NY, USA). Results were presented as means ± SEM (Standard Error of Mean). The value of $P < 0.05$ was used to indicate statistical significance

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RESULTS

AuNRs preparation and characterization

TEM image of the prepared AuNRs is presented in Fig. 1A. It was noticed that a number of non-agglomerated AuNRs were formed. These AuNRs were to a large extent homogenous in shape and size. The obtained AuNRs averaged 50.0± 5.0 nm in length and 12.0 nm width. The optical absorption spectrum of AuNRs solution in the visible near IR region is displayed in Fig. 1B. The spectrum exhibits two distinct peaks at 530 and 808 nm. The higher energy (808 nm peak) is attributed to the longitudinal Plasmon mode while the lower (530 nm peak) is due to the transverse Plasmon mode.

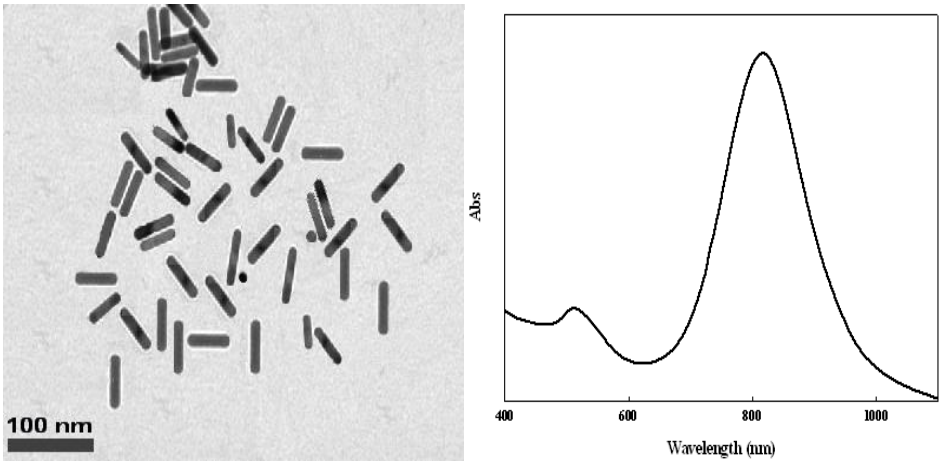

Fig 1: TEM images (A), and absorption spectra of aqueous of 50nm AuNRs solution (B).

The effect of acute intraperitoneal injection of 75 µg 50 AuNRs/kg body weight on hematological values of male rats is presented in Table 1. Data revealed that on Day-1 post to i.p AuNRs injection, the values TLC, MCV and MCH were significantly ($P<0.05$) higher in AuNRs injected group when compared with the control one. Meanwhile, the values of RBCs and PLT counts were higher ($P<0.05$) in control than AuNRs injected rats. In addition, on Day-3 post to AuNRs injection showed a significant ($P<0.05$) increase in TLC, HGB, MCV and PLT values in AuNRs injected rats when compared with the control group. On Day-7, RBCs count was higher in AuNRs injected rats than in control one, while, PLT values were higher ($P<0.05$) in control than the AuNRs injected rats. Hematological analysis of samples on Day-14 post to AuNRs injection revealed that TLC, RBCs count, MCV and PLT count were significantly ($P<0.05$) higher in AuNRs injected animals when compared with the control one. At the same time, MCH and MCHM values were higher ($P<0.05$) for control group than AuNRs injected rats.

The effect of chronic administration of 7.5 µg 50nm AuNRs/kg bwt for five consecutive days over a period of two months in male rats is illustrated in Table 2. On day 60 post treatment, TLC and PLT values were significantly ($P<0.05$) decreased in AuNRs injected animals corresponding to the control group, while the values of HGB were significantly ($P<0.05$) higher for AuNRs injected group when compared to control one. The values of RBCs, MCV, MCH, and MCHC were not significantly affected by the chronic administration of AuNRs in male rats (Table 2).

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Table 1: Changes in hematological values on Days-1, 3, 7 and 14 after acute administration of 75 µg 50nm AuNRs/kg bwt in male rats.

Blood parameters	Day 1		Day 3		Day 7		Day 14	
	Control	AuNRs	Control	AuNRs	Control	AuNRs	Control	AuNRs
TLC	7.58 ±0.31	13.76±0.24*	7.21 ±0.13	12.18±0.39*	7.3±0.43	7.86±0.23	7.78±0.47	14.58±0.25*
HGB	12.36±0.44	12.76±0.38	12.71± 0.14	13.82± 0.29*	12.22±0.19	13.08± 0.44	12.08± 0.17	12.55± 0.39
RBCs	5.67± 0.39	5.12± 0.19*	5.58± 0.10	5.68± 0.35	5.22± 0.04	6.20± .19*	5.55± 0.08	5.98± 0.12*
MCV	61.71± 1.20	66.86±1.47*	61.73± 0.59	63.61± 0.59*	59.28± 0.57	58.81± 0.39	59.35± 0.17	60.53± 0.21*
MCH	21.75± 0.76	23.91±0.27*	24.43± 0.25	23.15± 0.22	23.46± 0.24	23.0± 0.19	22.41± 0.21	21.26± 0.37*
MCHC	35.51± 0.64	37.66± 0.89	36.88± 0.13	36.21±0.36	40.18± 0.53	39.48± 0.87	37.80± 0.28	36.61± 0.25*
PLT	827.5± 1.05	617.2±0.60*	729.7±1.17	741.83±0.88*	755.5± 1.06	708.67±0.88*	612.17±2.40	690.33± 0.76*

Superscript within the same column differ carrying (*) significantly at $P < 0.05$.

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Table 2: Changes in hematological values on Day-60 after chronic administration of 7.5 µg 50nm AuNRs/kg bwt in male rats. (Mean ± SE)

	Day-60	
	Control	AuNRs
TLC	13.56± 0.97	6.58± 0.42*
HGB	12.43±0.34	13.86± 0.39*
RBCs count	5.48± 0.17	6.19± 0.25
MCV	58.23± 1.61	57.08± 0.83
MCH	22.53± 0.39	21.55± 0.39
MCHC	39.46± 0.69	38.03± 0.73
PLT	594± 2.12	539± 1.15*

Number of animals=6
Superscript within the same column differ carrying (*) significantly at $P < 0.05$.

DISCUSSION

Nanomedicine, the application of nanotechnology to health and medicine, is a relatively new branch of science. While nanotechnology has many applications in medicine, a key application has been the development of nanoparticle-based therapeutics. The incorporation of nanomaterials to drug formulation (nanoformulation) imparts physical advantages such as improved solubility, decreased degradation or physiologic clearance rates, decreased systemic toxicity, and improved clinical efficacy. [12]

Nanoparticles have many different shapes and forms e.g. carbon, silver and gold nanoparticles. The chemical reactivity of even very small gold nanoparticles is important because it can cause oxidative damage to cells. Certainly, it can be considered that gold nanoparticles themselves might be “drugs” in sufficiently high doses. Photothermal therapy with functionalized gold nanorods has been performed on cancerous and bacterial cells using *in vitro* and *in vivo* models. Gold nanorods have been also used as “therapeutic” agents in photothermal therapy; this is one of the evident goals of gold nanorod applications in biomedical field. [13]

The safety profile of GNRs remains unclear. Gold is a chemically inert material. Therefore, it is generally considered biocompatible and has been used in some routine clinical practices for many years (e.g., in treating rheumatoid arthritis). Several studies have reported no significant short-term toxicity of AuNPs (1 day to 3 months) [14] ***Connor, et al., (2005).*** The dose-dependent effects of gold nanoparticles on biological systems have been widely recognized [15]. This study aimed to investigate the hematological effect of intraperitoneal administration of acute and chronic dose of GNRs.

Complete blood count is the gate that can indicate many diseases especially blood disorders. The hematological parameters including TLC, HGB, RBCs, MCV, MCH, MCHC and platelets were measured. The dose of intraperitoneal administration of 300 µg/kg b.wt GNRs caused a significant decrease in RBCs count of GNRs treated rats at day one post treatment, but a significant increase in RBCs at day seven and fourteen post treatment. Of course, this fluctuation of RBCs count has a certain physiological mechanism.

Normally, RBCs have a function that it delivers oxygen to all parts of the body. When a mitochondrial dysfunction occurred, this resulted in hypoxia, so the oxygen delivery should be balanced. The major adaptive mechanism is increased production GH that led to erythropoiesis as well as increased MCV, MCH and MCHC [16]. Our results coordinated with [17] as they illustrated that I.P administration of GNRs showed a significant decrease in RBCs count of golden hamsters associated with increased MCV, MCH and MCHC.

White blood cells (WBCs) also called leukocytes are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. All white blood cells are produced and derived from multipotent cells in the bone marrow known as hematopoietic stem cells. Leukocytes are found throughout the body, including the blood and lymphatic system [18]**.** Due to the oxidant and the irritant effect of GNRs, it induced an increase in the total leucocytic count at day one and three post treatments; that’s

exactly explained by Bashandy, et al., [19]. Concerning the chronic administration of gold nanorods (30 µg/kg b.wt), a significant decrease in the total leukocytic count occurred, this may have a theory. A recent study investigated that the over production of free radicals damage bone marrow leading to decreased number of leukocytes [20]**.** No significant effect occurred at day seven post treatment, because prolonged increase of leukocytes makes the leukocytes return to its marginal pool. [21]

The main physiological role of platelets is to secure primary hemostasis and maintain the integrity of the vascular wall. However, they have also other functions, and are implicated in immunity and inflammation, vascular permeability and cancer metastasis. Under physiological conditions when blood flow is laminar, due to shear stress platelets flow close to endothelial cells (ECs) along the vessel wall and promote vascular integrity. Several studies have shown that shear stress may influence platelet aggregation through different ligands such as the vWF, GPIb/IX, GPIbα and GPIIb/IIIa [22]. Our results agreed with [23], because they found that the smaller the size of nanoparticles, the greater possibility of platelet aggregation. *In- vitro* study showed that the nanoparticle- induced platelet aggregation may be due to activation of GPIIa/GPIIIb. [24]

Regarding the decrease in platelets count in rats injected with 300µg/kg.bwt and 30 µg/kg b.wt GNRs, this result coordinated with el sayed, et al., [25], where they noted that the chronic dose of PEG- coated GNRs caused a significant decrease in the platelets. Furthermore, the *in-vivo* effects of gold nanorods on the platelets are still unclear, so this study is in need to prospective and upcoming investigations

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