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Impact of Gold Nanoparticles in Blood Parameters in Treated Mice with Mammary Adenocarcinoma

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Abstract: ***Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Nanotechnology may be able to create many new materials and devices with a vast range of applications, such as in nanomedicine, nanoelectronics, biomaterials energy production, and consumer products. In this study four groups of mice with mammary adenocarcinoma treatment intra injection with gold nanoparticles(GNP), radiation by diode laser and photothermal therapy (GNP plus radiation), so the fourth group leave as control positive. Blood samples collection from all groups and analyzed in different times for measure blood parameters which include Hb, PCV, MCV, RBCs, WBCs and MCHC. The results revealed that all the parameters measurement less than the normal rang which indicate to that GNP, radiation and photothermal therapy not have any effect on blood parameters.***

Keywords: gold nanoparticles, radiation, photothermaltherapy,blood parameters, cancer

1. Introduction

Cancer is so far a national and international health problem [1], and according to national cancer control strategy (2011-2016) that cancer together cardiovascular diseases, diabetes and chronic respiratory diseases they cause over 60% of total global mortality every year, it is estimated that cancer kill over 7.9 million people globally every year constituting close to 13% of total death worldwide[2]. Over more than 5 decades mortality due to can regardless of the discovery of several dozens of novel anticancer drug. Nanomaterial have diverse effects that draw the attention of scientists from different specialties, attributed to involved in numerous applications including biomedical uses [3]. Gold nanoparticles are subclass of nanomaterial intensively investigated for biomedical uses which is attribute tissues.

Complete blood count (CBC) is a routine test which is used to support the working diagnosis of several diseases, like anemia's, acute infection, hemorrhagic states, allergic disorders, cancers and immune disorders, for example, the low hematocrit was consider as indicator to anemia which established in the breast cancer patients, as well as, leucopoenia and thrombocytopenia [4] . This observation is corroborated by a report of Ufelle*et al*, in which significantly reduced hematocrit, total white blood cells and platelets count values in pre and post-surgery breast cancer patients were reported [5].

Complete blood count is considered another way to limits the toxilogical effect of GNPs by study hematological parameters such as Red Blood Cells (RBCs), White Blood Cells (WBCs) , Hemoglobin (Hb), Packed cell volume %(PCV), Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC) [6,7]. Different results of studies which revealed the effects of GNPs on blood parameters, some of these studies emphasized on that effect of GNPs on blood parameters were dependent on doses and concentration, size of GNPs and the injection of routes [7,8].

2. Materials and Methods

Hematology study included Complete Blood Count (CBC) analysis by measurement several parameters such as haemoglobin (Hb), haematocrit (Hct or PCV), red blood cells (RBCs), white blood cells (WBCs), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) in groups of treatment as well as control positive and negative.

Gold nanoparticles (GNPs) was purchased from Sigma Aldrich Company with 10nm, spherical shape and at concentration 6×10^{12} particles/ml.

Laboratory animals

About 100 female mice were obtained from the animal house of Iraqi Centre for Cancer and Medical Genetic Research (ICCMGR), at (3-4) weeks of age and (15-20) gm of weight. Diet and water were given to the animals.

Implantation of tumour cell in the mice

Spontaneous mammary adenocarcinoma (AM3) established by AL-Shammari, 2008, was implanted in female mice as follows:-

1) Tumour – bearing female mouse (Source) was obtained from the ICCMGR. The mouse was anesthetized by formaldehyde solution, the tumour mass sterilized was by 70% alcohol then a needle gage -18 inserted into tumour mass to aspirate 3-5 ml of tumour content that is transferred into a sterile beaker and suspended into 30-50 ml of sterile PBS which contain antibiotic.
2) The solid contents were allowed to settle down for 20 minutes while the supernatant discarded.

3) The sediments washed 2-3 times with sterile PBS. In the final wash, an appropriate amount of PBS was kept (the amount of PBS is depending on the cell concentrations).
4) Homogenized suspension of cells made through mechanical disaggregation of cells, by using syringe for this purpose (with draw and return of contents several times).
5) Tumour cell suspension (0.1 ml) was implanted through insertion of a needle gage subcutaneously from pelvic region toward the cervical region where the injection occurred. After appearance of tumour mass (approximately 10-15 days following inoculation) and the tumour reached to suitable size (about 0.5-1cm^3), the mice became ready to be used in the therapeutic experiment.

The laboratory animals were divided to five groups, the first group as positive control and contain 20 animals, second group also contain 20 animals which injected intratumour with150µl of stock colloidal gold nanoparticles for 28day, the third group also contain 20 animals and treated by radiation (low level laser (LLL), 532wave length, 0.9 w/cm^2 power density) for 28day (4min for each day), the fourth group contain 20 mice and treated by GNPs as in second group then irradiated by LLL as in third group (this called Photo thermal therapy PTT), the animals of all groups were killed after different times 24hrs, 72hrs, 7days, 14days and 28days, the blood collected in EDTA tubes and mixed slowly for the blood parameters analysis.

3. Results and Discussions

Table (1) illustrates comparison between groups of treatment with control positive (tumour bearing mice and without treatment) and time on measurement of Hb (gm/L).There were follow up in Hb after all period of treatment except the period 7day in group of treatment GNPs alone in comparison with control positive, in radiation treatment, the decreasing occurred after 14 day, while in photothermal therapy, the lowest level of Hb occurred after 28 day of treatment when compared with control positive, there was significant variation at level (P<0.05).

The table also shows significant variation between groups of treatment in the same period when compared with control positive, all period of time showed significant variation expect the period 7day, which recorded no significant variation about other groups at level (P<0.05).

For the comparison between groups and period of treatment with control positive on PCV. All groups of treatment had lowest rate of PCV after period 28day and there is a significant variation except the treatment of PTT group with no significant variation. In comparison between time of treatment, all times of treatment had significant difference when compared with control positive at level (P<0.05). (Table 2).

Table (3) exhibits a comparison between groups and time of treatment with control positive in RBCs. There was follow up in RBCs count in treatment groups, a significant difference between them expect in PTT group, in which, no significant difference, while in treatment time, there is asignificant variation expect after 24 and 28 period of treatment in comparison with control positive (P<0.05).

Table (4) illustrates comparison between groups of treatment and time with control positive on percentage of MCV%, in group of GNPs, follow up in MCV% except the period 7day, while in radiation group, the highly decreasing only after 28 day of treatment, in photothermal therapy, slightly increasing was occurred only after 72hrs , there was significant variation at level (P<0.05) except in PTT group, but no significant difference for effect the different times of treatment on MCV% except after 28day of treatment.

Table (5) shows comparison between groups of treatment and time with control positive on WBCs. The lowest WBCs was occurred in GNPs treatment after 28day, in radiation and photothermal therapy treatment, the difference was clearing, especially after 7days, there is highly increasing in WBCs, as well as, after 28 day in radiation group, in which,a significant difference at level (P<0.05), in effect of time on WBCs, the table revealed variation in all times of treatment.

For the comparison between groups of treatment and time on MCHC, no significant variation between values of MCHC in the same group for all type of treatment except the radiation group , as well as between time, except the period 28day, in which, a significant difference at level (P<0.05). (Table 6).

Table 1: Effect of treatment groups and time in Hb (g/L)

Time	Groups				LSD value
	Control positive	GNPs only	Radiation	Photothermal	
24 hr.	127.5 ±5.7	123.0 ±6.1	93.6 ±4.1	90.25 ±4.3	15.49 *
72 hr.	144.0 ±8.4	86.0 ±4.8	100 ±6.4	112 ±6.2	28.75 *
7 day	86.6 ±3.5	114.6 ±7.3	116 ±6.1	109.75 ±6.9	30.36 NS
14 day	43.0 ±1.9	84 ±3.8	56.5 ±2.6	130 ±5.3	22.52 *
28 day	62.5 ±2.7	54 ±2.8	61 ±2.9	91.5 ±5.0	24.90 *
LSD value	22.54 *	26.70 *	26.25 *	24.81 *	---

*** (P<0.05), NS: Non-significant.**

Table 2: Effect of treatment groups and time on PCV (%)

Time	Groups				LSD value
	Control positive	GNPs only	Radiation	Photothermal	
24 hr.	42.45 ±2.6	40.5 ± 2.2	30.2 ± 1.2	29.75 ± 1.3	7.15 *
72 hr.	45.0 ± 2.7	25.4 ±1.9	22.6 ± 0.85	29.45 ±1.1	6.67 *
7 day	21.0 ± 0.85	37.9 ±2.5	36.2 ± 1.5	30.00 ± 1.03	7.91 *
14 day	12.7 ± 0.52	27.2 ± 1.9	19.6 ± 0.73	32.00 ±1.5	7.25 *

28 day	20.8 ± 0.74	19.0 ± 0.84	10.24 ± 0.46	27.15 ± 0.79	6.92 *
LSD value	7.72 *	8.39 *	6.51 *	6.34 NS	---

***(P<0.05),NS:Non-significant**

Table 3: Effect of treatment groups and time on RBCs (10^{12}/L)

Time	Groups				LSD value
	Control positive	GNPs only	Radiation	Photothermal	
24 hr.	8.42 ± 0.50	8.0 ± 0.43	6.7 ± 0.26	6.5 ±0.30	2.29 NS
72 hr.	8.7 ± 0.38	5.1 ± 0.16	5.5 ± 0.09	5.86 ± 0.11	2.18 *
7 day	5.36 ±0.36	7.6 ± 0.25	8.24 ± 0.11	6.4 ± 0.24	2.26 *
14 day	2.83 ± 0.07	6.4 ±0.34	4.31 ± 0.09	7.0 ± 0.39	2.45 *
28 day	4.5 ± 0.12	4.25 ± 0.19	4.0 ± 0.12	6.0 ± 0.07	2.49 NS
LSD value	3.24 *	3.17 *	2.69 *	2.58 NS	---

***(P<0.05),NS:Non-significant**

Table 4: Effect of treatment groups and time on MCV (%)

Time	Groups				LSD value
	Control positive	GNPs only	Radiation	Photothermal	
24 hr.	49 ±2.9	50.9 ± 2.7	45 ±1.8	45.5 ±2.7	7.092 NS
72 hr.	52.0 ± 2.7	45.0 ±2.6	46.5 ± 2.0	50.4 ± 2.3	7.659 NS
7 day	40.1 ± 1.4	49.9 ± 1.9	44.0 ± 1.8	46.0 ± 2.3	6.368 NS
14 day	45.1 ± 2.8	44.5 ± 1.6	45.3 ± 2.3	45.5 ± 1.9	4.61 NS
28 day	48.6 ± 2.1	44.25 ± 1.2	26.8 ± 2.2	45.15 ± 2.4	7.54 *
LSD value	7.32 NS	5.82 *	6.42 *	6.85 NS	---

***(P<0.05),NS:Non-significant.**

Table 5: Effect of treatment groups and time on WBCs (10^{9}/L)

Time	Groups				LSD value
	Control positive	GNPs only	Radiation	Photothermal	
24 hr.	15.0 ±0.44	11.3 ± 0.57	8.0 ±0.31	11.25 ±0.62	3.72 *
72 hr.	31 ± 1.2	10.1 ±0.41	8.0 ±0.34	8.0 ± 0.16	7.61 *
7 day	12.5 ± 0.63	12.3 ± 0.51	21.1 ±0.29	38 ± 0.1.4	7.59 *
14 day	6.6 ± 0.24	14.5 ±0.70	8.4 ± 0.28	12.35 ±0.66	4.15 *
28 day	8.8 ±0.46	8.0 ± 0.26	39.8 ±2.98	10.5 ± 0.39	3.86 *
LSD value	5.60 *	4.03 *	3.88 *	5.85 *	---

*** (P<0.05), NS: Non-significant.**

Table 6: Effect of treatment groups and time on MCHC (g/L)

Time	Groups				LSD value
	Control positive	GNPs only	Radiation	Photothermal	
24 hr.	303.0 ±16.7	303.3 ±13.3	307.0 ±15.8	304.3 ±13.9	38.06 NS
72 hr.	312.0 ±14.3	312.0 ±19.4	340.0 ±22.5	355.0 ±16.4	49.58 NS
7 day	310.0 ±14.0	301.6 ±14.2	300.0 ±15.3	317.0 ±13.6	39.41 NS
14 day	336.6 ±21.6	305.0 ±14.9	290.0 ±13.9	337.0 ±23.5	53.92 NS
28 day	298.0 ±15.9	279.5 ±12.5	251.5 ±15.7	336.0 ±18.5	55.64 *
LSD value	53.67 NS	46.92 NS	58.75 *	61.50 NS	---

***(P<0.05),NS:Non-significant**

Number of studies were carried out demonstrate the effect of GNPs on blood parameters, like studies of [7, 9]in which, all hematological values did not significantly change when compared to untreated control after 14 day of oral route in rats. But when the GNPs injected as a highly exposure at 2mg/kg for multiple days, it leads to a significant dose dependent increase in RBC, Hb and WBC count [10].

The decreasing in RBC, PCV and Hb because those RBCs were derived from hemopoietic stem cells in bone marrow. Following a series of maturation steps, directed mainly by erythropoietin, red cells enucleate and enter the circulatory system, therefore, the variation in RBCs can be related to the hematopoietic system, in addition, the high doses of GNPs, long term of treatment and the administration route have effect in this system, then, the GNPs can interaction with blood components and induce the inflammatory response, which lead to increase or decrease the activity of immune system and altered hematologic factors such as blood cell count [8,11]. Study of Axial-Bechtel and his team [12], in which prostatic cancer bearing dog and treatment with GNPs plus imaging for 4 weeks revealed no statistically significant differences in total WBCs and RBCs count.

The results of the hematological study showed that control positive group (tumour bearing female mice) manifested decrease in Hb value, PCV (%), RBCs count.The most common hematological cancer effect is anemia (decrease in Hb value, PCV and RBCs count), whilein some research which revealed that higher WBCs, Hb and PCV in women with breast cancer than other healthy. Hence, the team of this research suggested that C.B.C may be used as an independent predictor to breast cancer[13].

Anemia can develop in cancer patients through a number of mechanisms, including malnutrition, blood loss due to neoplastic invasion of tissues, autoimmune hemolysis and decrease red blood cell production [14].Decrease vitamin B_{12} absorption, coupled with anemia, is a common finding in cancer cases; the blood losing may be occult and leads to an iron-deficiency anemia. An immune-type hemolytic anemia resulting from production of antibodies directed against the patient's own erythrocytes is seen in certain types of cancer [15].

According to some reports,the normal range of Hb about (140-150), HCT (PCV) (45-50), RBC(9-10), WBC(5-14.8), MCV(46-51) and MCHC(29-33)[16, 17].

All table revealed that Hb, PCV, MCV, RBCs and WBCs in all the treatment groups and positive group less than the normal rang, especially, in the last period of treatment, expect in MCHC, in which, the value is nearby to the normal rang, this may be to the effect of cancer reaction.

From this study we conclude that GNPs, radiation and photothermal therapy can not to return the blood parameters to the it's normal rang due to the effect of cancer on this parameters.

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