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Bio-effects of present of AL NPs in Different Vaccines on Hematology Analysis of Rabbit's In-Vivo Study

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Abstract In present study aims to measure the effects of different types vaccines (polio, and BCG) and aluminum nanoparticles (AL NPs) at different concentrations (0.5 and 1M) on eight hematological parameters: White blood cells (WBCs), red blood cells (RBCs), hemoglobin (HGB), platelets (PLT), monocytes (MONO), granulocytes (GRANUL), hematocrit (HCT) and lymphocytes (LYMPHO). In the experimental twelve male rabbits in vivo non-randomized control trials were divided into four groups. Group control (n-6) and three groups injected with AL NPs (n-6) at different doses, BCG vaccine (n-6), and polio vaccine (n-6).

The results after injected with AL NPs, BCG, and Polio vaccines with different doses and study the CBC analysis of all parameters are significant statistical analysis except for PLT values are non-significant. It seems from the results that the AL NPs has highly affected on the blood components and also, the both polio and BCG vaccines which have some percentage of nanoparticles of aluminum. The results of CBC analysis of blood mentioned the parameters analyzed are abnormal values that affected on the immune system of the animal. It can be concluded that the nanoparticles have been affected on the blood parameters, was long-duration more harmful and effect.

Key Words AL NPs, BCG vaccine, polio vaccine, hematology analysis, rabbits blood samples

1. Introduction

One of the components of vaccines, especially those used for children, is aluminum, and it is scientifically known that the aluminum compound has toxicity in the blood. Based on the recommendations and warnings of the US Food and Drug Administration (FDA), these are the calculations for the amount of aluminum that is permissible to be injected in one go into the body [1]–[7]:

- A healthy infant weighing 3,600 kg takes 18 mcg of aluminum.
- A healthy infant weighing 6,800 kg takes 34 mcg
- A healthy baby weighing 13 kg absorbs 68 mcg
- A healthy baby weighing 22 kg absorbs 113 mcg
- An adult weighing 68 kg takes 340 mcg
- Aluminum amounts according to the dose of vaccinations
  - Hib (pedVaxHib brand) 224mcg per shot.
  - DTaP 170mcg to 625mcg depending on the manufacturer. pneumococcus 125 μg.
  - Hepatitis A 250 mcg.
  - HPV 225mcg.
  - DTaP, HIB & Plio combo 330mcg.
  - DTaP, Hep B & Polio combo 850mcg.

BCG, a live vaccine strain of Mycobacterium bovis, is commonly used to prevent the prevalence and burden of tuberculosis (TB) but is not used in the United States due to the low prevalence of TB in the general population and the fact that BCG vaccine complicates the interpretation of skin tests for TB. However, OPV and BCG vaccine recipients who are in the United States as visitors or immigrants may present healthcare providers with complex medical problems related to vaccines other than those recommended by the CDC and the American Academy of Pediatrics, Case reports and cohort studies from several countries other than the United States show the continued incidence of iVDPVs and the need for continued monitoring [8]. The study focused specifically on vaccination coverage with Bacille de Calmette et Guérin (BCG), birth dose of oral polio vaccine (OPV zero dose). This study aimed to shed light on vaccination coverage at birth doses and factors associated with timely vaccination

in the Budur health district. Timely administration of birth doses is a challenge in the Bodur Health District [9].

It is recommended that oral polio vaccine (OPV) be given at birth along with the BCG vaccine. OPV was not available during some periods and therefore some children did not receive OPV at birth, but only the Bacillus Calmette (BCG) vaccine. We investigated the effect of an oral vaccine given concomitantly with BCG at birth on the immune response to BCG. This study is the first to address the consequences of the immune response to BCG given concomitant administration with OPV. The results suggest that the common practice of administering the oral vaccine with BCG at birth may down regulate the response to BCG [10].

In this research, the aim is to achieve safety in the use of children's vaccines free from nanoparticles that cause many problems in the blood components.

2. Material and Methods

A. Hematological parameter

Blood samples was collected via cardiac puncture by using 5ml disposable syringe, 2 ml of blood collected in heparinized tube for hematological parameters which were measured as soon as possible. The hematological tests were done By using hematology autoanalyser, (huma counts 5). Germany origin with serial number of 160247. The instrument measure and calculates 22 different parameters. The hematology autoanalyser containing four solutions (HC5-BASOLYSE contain cyanide free lyse reagent, HC-LYSECF containing cyanide free lyse reagent, HC5-EOLYSE containing cyanide free lyse reagent, and HC- cleaner cleaning solution used to clean fluidic system), and the instrument have a printer mechanic inside with thermal paper. The hematological parameters estimated by this instrument were (RBC, WBC, DWBC, Hb, PCV, and platelets count) [11], [12].

B. Procedure

Blood collecting in tubes containing an anticoagulants to prevent forming clots that will clog the cell count. Then position the sample tube in the sample rotor and press start key, The sample rotor turns in moving the sample inside the instrument and the needle draw (90 μl) of blood sample from the tube. The aspirating needle retracted after a few second the rotor turn again returning the blood sample tube after that we can remove the tube from adaptor of the rotor. The ends of analysis were displayed in screen, including all the measured and calculated parameters which are stored automatically in the memory without any operator confirmation. After that we can printing the results by pressing print key.

C. Statistical analysis

All statistical analyzes were performed using SPSS version 21 software and results were described as mean ± SD using one-way ANOVA and least significant differences (LSD) test for a between-group comparison study. As a final point, the statistical significance difference (probability value) was at the 0.05 level [13].

Figure 1: Lyophylization instrument

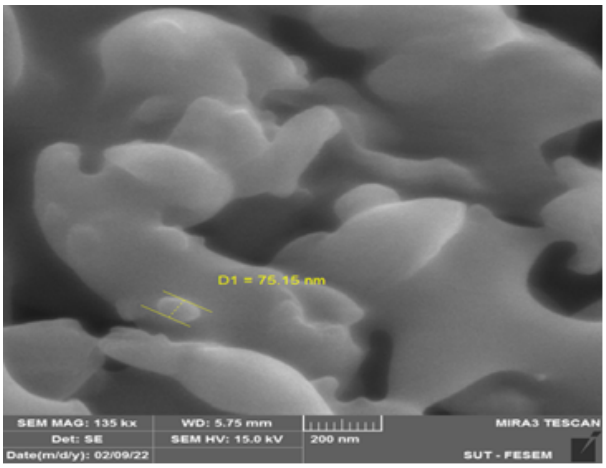

Figure 2: FESEM of Alum NPs

D. Preparation and Characterization of AL NPs

The lyophilization method was used to convert the micro alum to nanoparticles by the lyophilizer apparatus shown in Figure 1. the AL NPs NPs was characterized by spectroscopic methods using field emission scanning electron microscopy (FESEM), atomic force microscopy (AFM), and Transmission electron microscopy (TEM) to confirm its conversion to nanoparticles as shown in Figures 2- 4 respectively. It was found from FESEM analysis of the morphology and dimension of nanoparticles of Alum NPs as shown in Figure 2, about 75.15 nm the dimension of AL NPs which included with nanoparticles size. also, the spherical form of AL NPs was shown in AFM analysis as shown in in Figure 3.

3. Results and Discussion

Standard hematology markers were selected for analysis such as hemoglobin level (HGB), red blood cell (RBC), platelets (PLT), white blood cells (WBCs), lymphocyte (LYMPHO), granulocytes (GRANUL), monocyte (MONO), and hemat-

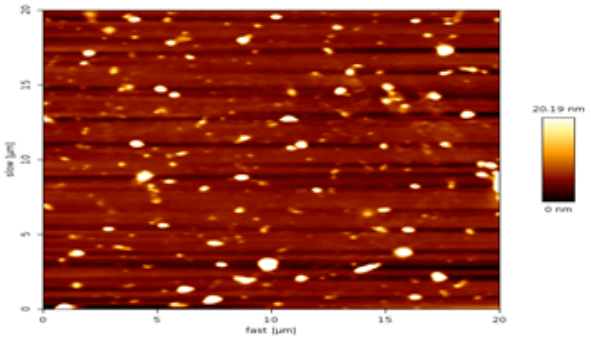

Figure 3: AFM of Alum NPs

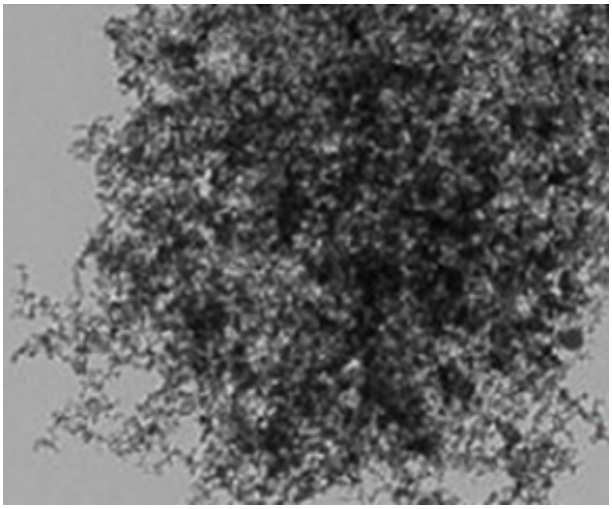

Figure 4: TEM of Alum NPs

ocrit (HCT). The blood samples were received from rabbits that injected with AL NPs, polio vaccine, and BCG vaccine at different doses.

A. Effects of AL NPs, BCG, and Polio vaccine at different doses on hematology analysis of rabbits blood

Each of the vaccines (polio and BCG) and different doses of AL NPs (0.5 and 1 ml of 0.1M) were studied in experiments using rabbits in order to identify their effect on the blood composition through the necessary analyses of the blood of rabbits dosed with different vaccines and at different concentrations of AL NPs., by CBC analysis can be examined the rabbits blood at different parameters as in the following studies:

B. Effect of AL NPs, Polio vaccine, and BCG vaccine on hematology White blood cells (WBCs)

The percentage of WBC in the blood of rabbits was analysed in different cases, where the value of the statistical mean was identified for the blood of rabbits that did not receive any of the vaccines or AL NPs (control group), and then compared the results with blood of rabbits dosed with polio and BCG vaccines and with AL NPs (1 and 2), positive results were found with the high significant for all cases as shown in Table 1, these mean that the vaccines (polio and BCG) and AL NPs at different concentrations were effected on the WBC values and in turn, it effects on the immunity of the animal [14].

As a result, a significant in the account of WBC in the vaccinated of animals was an indicator of stress-related consequences, and further research into the immunomodulatory effect on vaccinated rabbits was indicated. Since the development of leukocyte count showed tissue injury, and white blood cell disorders (leukemia), the effect of AL NPs or the nanoparticles contained in vaccines led to leukocytosis, which was then followed by lymphocytosis [15].

C. Effect of AL NPs, Polio vaccine, and BCG vaccine on hematology red blood cells (RBCs)

Table 2 illustrated the RBC values of the control group that comparing with other values of RBC of groups doses with vaccines and with AL NPs, it was observed the decreasing of RBC in these groups as shown in Table 2., so the effect of polio vaccine has less with significant than in other cases with high significant. The decrease of account of RBC that causes anemia disease [16].

RBCs was a target as the main and most affected by the nanoparticles. The interaction between RBC and the vaccines and AL NPs is very complex and involves several oxidative stress [17].

D. Effect of AL NPs, Polio vaccine, and BCG vaccine on hematology hemoglobin HGB

There has been a change in the value of hemoglobin (HGB) in the blood of rabbits in each of the AL NPs at different concentrations, as they are increased in HGB values compared with their in the vaccines value when compared with the control group as shown in Table 3. So, it was found a high significant statistical study [18].

The changes in blood parameters of CBC analysis especially in HGB values may be caused by oxidative stress, leading to uncontrolled hemoglobin test, which can lead to anemia and bone marrow suppression, as well as an increase in the hematopoietic function of the bone marrow. This theory can also be applied to other conditions of erythrocyte malformation [19].

E. Effect of AL NPs, Polio vaccine, and BCG vaccine on hematology platelets (PLT)

A platelet (PLT) count is a type of test that measures the number of platelets in the blood. Platelets are used by the body in the process of blood clotting. A decrease in the number of platelets in the blood can be a sign of cancer, infection, or various health problems. It may be suffer from blood clots or a stroke if their number of PLT in blood decreases [20]. In this study Table 3 illustrated the effect of the account of platelets with different vaccines and AL NPs at different concentrations, the control group indicted with lower account of PLT comparing with high values of the PLT of the blood samples doses with different vaccines and with

		N	Mean	Std.	Std.	Range		ANOVA test
						Mini.	Maxi.	
WBC	Control			Deviation	Error			(P-value)
	1	6	13.183	1.2576	.5134	11	14.5	
	2	6	13.667	1.8886	.7710	11	16.0	
	3	6	14.167	1.5055	.6146	12	16.0	
	4	6	13.00	1.414	.577	11	15	
	Total	24						
WBC	Polio							0.001 High Sign
	1	6	.4167	.11325	.04624	.30	.60	
	2	6	5.4083	.58345	.23819	4.50	6.00	
	3	6	2.8850	.76115	.31074	2.00	.25	
	4	6	.4033	.12910	.05270	4.00	.60	
	Total	24						
WBC	BCG							0.000 High Sign
	1	6	7.617	. 5419	.2212	7.0	8.5	
	2	6	2.333	. 3502	.1430	1.9	2.9	
	3	6	2.2683	.17440	.07120	2.00	2.50	
	4	6	.4067	.14236	.05812	.20	.60	
	Total	24						
WBC	ALNP1							0.000 High Sign
	1	6	1.4650	.19532	.07974	1.20	1.70	
	2	6	1.3950	.14195	.05795	1.20	1.60	
	3	6	2.4517	. 30069	.12276	2.00	2.90	
	4	6	.4050	.14195	.05795	.20	.60	
	Total	24						
WBC	ALNP2							0.000 High Sign
	1	6	.7650	.16538	.06752	.59	1.00	
	2	6	2.3533	.18403	.07513	2.10	2.60	
	3	6	1.3333	.21602	.08819	1.00	1.60	
	4	6	.7583	.18552	.07574	.50	1.00	
	Total	24						

Table 1: Effect of AL NPs, Polio vaccine, and BCG vaccine on hematology white blood cells (WBCs)

		N	Mean	Std.	Std.	Range		ANOVA test
						Mini.	Maxi.	
RBC	Control			Deviation	Error			(P-value)
	1	6	5.06	0.75	0.309	4	6	
	2	6	5.18	0.74	0.305	4	6	
	3	6	5.41	0.58	0.238	4.5	6	
	4	6	5.33	0.87	0.358	4	6.5	
	Total	24						
RBC	Polio							0.021 Sign
	1	6	2.83	0.68	0.278	2	4	
	2	6	5.01	0.70	0.288	4	6	
	3	6	4.65	1.02	0.419	3	6	
	4	6	5.6	0.89	0.366	4.5	6.9	
	Total	24						
RBC	BCG							0.000 High Sign
	1	6	1.25	0.68	0.281	0.50	2	
	2	6	4.21	0.88	0.360	3	5.3	
	3	6	2.68	0.75	0.308	2	4	
	4	6	4.56	0.97	0.397	3	6	
	Total	24						
RBC	ALNP1							0.002 High sign
	1	6	3.03	0.68	0.278	2	4	
	2	6	5.1	1.43	0.585	3	7	
	3	6	7.11	1.42	0.583	5	9	
	4	6	3.55	1.007	0.411	2	5	
	Total	24						
RBC	ALNP2							0.002 High Sign
	1	6	1.6	0.58	0.238	1	2.5	
	2	6	5.11	1.44	0.589	3	7	
	3	6	4.63	1.08	0.444	3	6	
	4	6	6.76	1.14	0.466	5	8	
	Total	24						

Table 2: Effect of AL NPs, Polio vaccine, and BCG vaccine on hematology red blood cells (RBCs)

		N	Mean	Std.	Std.	Range		ANOVA test
						Mini.	Maxi.	
HGB	Control			Deviation	Error			(P-value)
	1	6	10.300	0.5692	0.2324	9.5	11.0	
	2	6	9.467	1.1928	0.4869	7.4	10.9	
	3	6	9.600	0.7874	0.3215	9.0	11.0	
	4	6	9.900	0.6693	0.2733	9.0	11.0	
	Total	24						
HGB	Polio							0.000 High sign
	1	6	9.917	0.7910	0.3229	9.0	11.0	
	2	6	10.583	1.0206	0.4167	9.0	12.0	
	3	6	10.917	0.9704	0.3962	9.5	12.0	
	4	6	10.200	0.8343	0.3406	9.0	11.1	
	Total	24						
HGB	BCG							0.003 sign
	1	6	10.1	0.8	0.170	9.0	11.0	
	2	6	10.75	0.935	0.187	9.50	12.0	
	3	6	7.983	1.897	0.335	5.40	10.5	
	4	6	10.516	0.884	0.205	9.50	12.0	
	Total	24						
HGB	ALNP1							0.000 High Sign
	1	6	13.05	0.71	0.292	12	14	
	2	6	11.98	0.70	0.289	11	13	
	3	6	13.48	0.88	0.214	12	14.5	
	4	6	8.25	0.52	0.407	7.5	9	
	Total	24						
HGB	ALNP2							0.000 High Sign
	1	6	11.96	0.711	0.290	11	13	
	2	6	12.43	0.86	0.352	11	13.5	
	3	6	10.43	0.72	0.296	9.5	11.5	
	4	6	17.30	0.59	0.243	16.5	18	
	Total	24						

Table 3: Effect of AL NPs, Polio vaccine, and BCG vaccine on hematology hemoglobin (HGB)

AL NPs, so the results in Table 4 show non-significant in statistical analysis for all groups. It has been shown from these results that there are direct positive effects on blood composition when using different vaccines and AL NPs [21].

F. Effect of AL NPs, Polio vaccine, and BCG vaccine on hematology lymphocyte (LYMPHO)

Lymphocytes (LYMPHO) are one of the types of white blood cell. It helps the immune system to defend against to different diseases such as cancer, viruses and various bacteria by measuring the number of LYMPHO. Lymphocyte levels vary with age, race, sex, height, and lifestyle [22]. Table 5 shows the values of LYMPHO in the control group and other groups doses with different vaccines and AL NPs at different concentrations, which shows the high significant values of all groups.

G. Effect of AL NPs, Polio vaccine, and BCG vaccine on hematology monocyte (MONO)

A type of white blood cell in the immune system is monocyte (MONO), these cells may be kill the invader cell or alert other blood cells to help destroy it and prevent infection [23]. Table 6 illustrated the MONO analysis decreased values were observed when administering vaccines to rabbits

H. Effect of AL NPs, Polio vaccine, and BCG vaccine on hematology granulocytes (GRANUL)

Granule cells or granulocytes (GRANUL) are one of the most well-known types of white blood cells. They are small granules that secrete enzymes when the immune system is exposed to disease. These GRANUL cells happens during the occurrence of disease infection. Granulocytes are produced from stem cells in the bone marrow and only live for a few days [24].

Such an analysis of GRANUL is important to know the effect of vaccines on the immune system through these test, in Table 7 shows a dropping of the GRANUL values than in normal group (control) this decreasing of GRANUL after doses the rabbits with vaccines and AL NPs effected on the immune system, the all cases have high significant as shown in Table 7.

I. Effect of AL NPs, Polio vaccine, and BCG vaccine on hematology of hematocrit (HCT)

Hematocrit (HCT) or the volume of stacked cells, scientifically meaning hematocrit, is the process of separation of blood, as well as it is measured by calculating the percentage of the volume of red blood cells from the total volume of blood. As for the normal values in human, it is 47% for men and 42% for women and for children, it is about 36% to 44% [25].

Table 8 shows the statistical calculation of the means values of HCT between the control group and mean values

		N	Mean	Std.	Std.	Range		ANOVA test
						Mini.	Maxi.	
	Control			Deviation	Error			(P-value)
PLT	1	6	342.5	13.32	5.439	325	360	
	2	6	344.16	12.00	4.901	325	360	
	3	6	342.5	13.32	5.439	325	360	
	4	6	342.5	13.32	5.439	325	360	
	Total	24						
	Polio							
PLT	1	6	1758.33	115.83	47.28	1600	1900	0.986
	2	6	1001.16	69.65	28.43	900	1100	
	3	6	138.83	16.005	6.53	120	160	Non Sign
	4	6	27	15.93	6.50	10	50	
	Total	24						
	BCG							
PLT	1	6	1357	33.43	13.65	1300	1400	0.797
	2	6	186.33	12.11	4.94	170	200	
	3	6	296.33	18.56	7.57	270	320	Non Sign
	4	6	28.5	14.61	5.96	10	50	
	Total	24						
	ALNP1							
PLT	1	6	2488.33	266.86	108.94	2000	2700	0.507
	2	6	46.83	16.12	6.58	30	70	
	3	6	293	17.37	7.09	270	320	Non Sign
	4	6	95.16	10.40	4.24	80	110	
	Total	24						
	ALNP2							
PLT	1	6	1008.83	80.05	32.68	900	1100	0.906
	2	6	928.66	20.46	8.35	900	950	
	3	6	36.5	16.53	6.75	19	60	Non Sign
	4	6	67.16	11.10	4.53	50	80	
	Total	24						

Table 4: Effect of AL NPs, Polio vaccine, and BCG vaccine on hematology platelets (PLT)

		N	Mean	Std.	Std.	Range		ANOVA test
						Mini.	Maxi.	
	Control			Deviation	Error			(P-value)
LYMPHO	1	6	3.81	0.604	0.270	3	4.6	
	2	6	3.23	0.394	0.176	2.5	3.6	
	3	6	2.5	0.294	0.131	2	3	
	4	6	2.75	0.170	0.076	2.5	3	
	Total	24						
	Polio							
LYMPHO	1	6	2.08	0.134	0.060	1.9	2.3	0.000
	2	6	2.25	0.170	0.763	2	2.5	
	3	6	1.33	0.195	0.087	1	1.6	High Sign
	4	6	1.11	0.13	0.060	0.9	1.3	
	Total	24						
	BCG							
LYMPHO	1	6	5.55	0.18	0.076	5.3	5.8	0.000
	2	6	1.83	1.16	0.066	1.6	2	
	3	6	0.83	0.14	0.060	0.6	1	High Sign
	4	6	0.8	0.14	0.057	0.6	1	
	Total	24						
	ALNP1							
LYMPHO	1	6	1.26	0.169	0.075	1	1.5	0.000
	2	6	1.27	0.158	0.070	1	1.5	
	3	6	1.39	0.131	0.058	1.2	1.6	High Sig
	4	6	1.4	0.129	0.057	1.2	1.6	
	Total	24						
	ALNP2							
LYMPHO	1	6	0.26	0.149	0.066	0.10	0.50	0.000
	2	6	1.66	0.177	0.079	1.40	1.90	
	3	6	0.69	0.129	0.058	0.50	0.90	High Sign
	4	6	0.1	0	0.000	0.10	0.10	
	Total	24						

Table 5: Effect of AL NPs, Polio vaccine, and BCG vaccine on hematology lymphocyte (LYMPHO)

		N	Mean	Std.	Std.	Range		ANOVA test
						Mini.	Maxi.	
	Control			Deviation	Error			(P-value)
MONO	1	6	5.49	0.344	0.153	5	6	
	2	6	5.033	0.926	0.414	3.5	5.9	
	3	6	4.25	0.403	0.180	3.4	4.6	
	4	6	4.76	0.179	0.080	4.5	5	
	Total	24						
	Polio							
MONO	1	6	1.66	0.149	0.066	1.5	1.9	0.000
	2	6	1.48	0.226	0.101	1.2	1.9	
	3	6	0.42	0.140	0.062	0.2	0.6	High Sign
	4	6	0.59	0.146	0.065	0.4	0.8	
	Total	24						
	BCG							
MONO	1	6	0.60	0.14	0.057	0.4	0.8	0.000
	2	6	0.56	0.30	0.124	0.07	0.9	
	3	6	0.75	0.18	0.076	0.5	1	High Sing
	4	6	0.75	0.18	0.076	0.5	1	
	Total	24						
	ALNP1							
MONO	1	6	0.041	0.013	0.006	0.02	0.06	0.000
	2	6	0.07	0.013	0.006	0.05	0.09	
	3	6	0.55	0.171	0.076	0.3	0.8	High Sing
	4	6	0.71	0.154	0.068	0.5	0.99	
	Total	24						
	ALNP2							
MONO	1	6	0.1	0	0.000	0.10	0.10	0.000
	2	6	0.21	0.106	0.047	0.10	0.40	
	3	6	0.25	0.094	0.042	0.10	0.40	High Sing
	4	6	0.1	0	0.000	0.10	0.10	
	Total	24						

Table 6: Effect of AL NPs, Polio vaccine, and BCG vaccine on hematology monocyte (MONO)

		N	Mean	Std.	Std.	Range		ANOVA test
						Mini.	Maxi.	
	Control			Deviation	Error			(P-value)
GRANUL	1	6	4.71	0.13	0.060	4.5	4.9	
	2	6	5.33	0.19	0.088	5	5.6	
	3	6	3.75	0.17	0.076	3.5	4	
	4	6	4.38	0.24	0.107	4	4.7	
	Total	24						
	Polio							
GRANUL	1	6	1.41	0.13	0.060	1.2	1.6	0.000
	2	6	1.65	0.17	0.076	1.4	1.9	
	3	6	0.42	0.13	0.062	0.2	0.6	High Sign
	4	6	0.75	0.17	0.076	0.5	1	
	Total	24						
	BCG							
GRANUL	1	6	2.33	0.163	0.066	2.10	2.50	0.000
	2	6	0.22	0.113	0.046	0.10	0.40	
	3	6	0.68	0.146	0.059	0.50	0.90	High Sign
	4	6	0.68	0.147	0.060	0.50	0.90	
	Total	24						
	ALNP1							
GRANUL	1	6	0.22	0.107	0.048	0.10	0.4	0.000
	2	6	0.06	0.017	0.007	0.04	0.09	
	3	6	0.39	0.129	0.058	0.20	0.6	High Sign
	4	6	0.38	0.134	0.060	0.20	0.6	
	Total	24						
	ALNP2							0.000
GRANUL	1	6	0.1	0	0.000	0.10	0.10	
	2	6	0.20	0.08	0.037	0.10	0.30	High
	3	6	0.16	0.04	0.018	0.10	0.33	
	4	6	0.01	0	0.000	0.01	0.01	Sign
	Total	24						

Table 7: Effect of AL NPs, Polio vaccine, and BCG vaccine on hematology granulocytes (GRANUL)

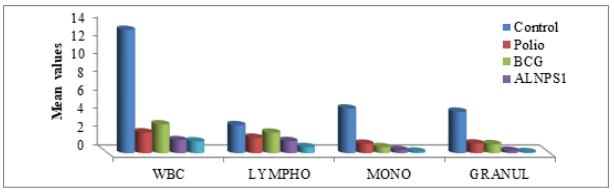

Figure 5: Relationship between the mean values of each control, polio vaccine, BCG vaccine, and AL NPs at 0.5, and 1 M, for CBC analysis of WBC, LYMPHO, MONO, and GRANUL

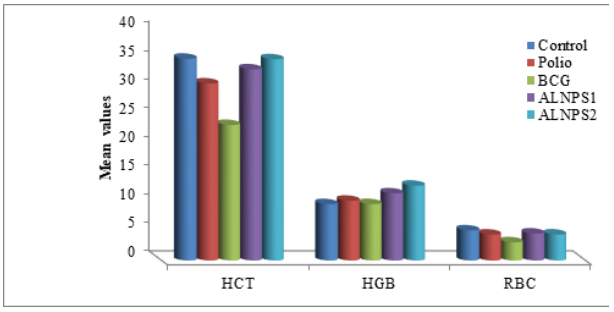

Figure 6: Relationship between the mean values of each control, polio vaccine, BCG vaccine, and AL NPs at 0.5, and 1 M, for CBC analysis of HCT, HGB, and RBC

of different blood samples of the rabbits doses with polio, BCG vaccines and AL NPs, it was found the P-value for all cases have high significant, so the effect of vaccine and AL NPs on the rabbits blood is clear by increasing the values of HCT.

Figure 5 illustrated the relationship between the mean values of each control, polio vaccine, BCG vaccine, and AL NPs at 0.5, and 1 M which injected the rabbits to examine their blood of CBC analysis of each WBC, LYMPHO, MONO, and GRANUL, Fig.6 for each HCT, HGB, and RBC. The results of these study observed that all CBC analysis of blood samples have abnormal results with significant statistical analysis except the PLT analysis has non-significant, so the results in the Fig. 5, and 6 has a homogenous in consequence, and have the effected on the blood components [26].

4. Conclusion

It is possible to conclude from the this research several important matters of concern to the health and life of children in particular by identifying the effects resulting from the use of some chemicals, especially AL NPs. The results were monitored in this research through a complete blood analysis (CBC) and obtained the following:

1) each of polio, BCG vaccines, and AL NPs were effected on the blood components of rabbits doses with these vaccines and nanoparticles by the CBC analysis.
2) all of the CBC analysis of blood samples tested of White blood cells (WBCs), red blood cells (RBCs), hemoglobin (HGB), platelets (PLT), monocytes (MONO), granule (GRANUL), hematocrit (HCT) and lymphocytes (LYMPHO) which effected with high significant statistical analysis except of PLT value that has non-significant.
3) all factors in CBC analysis mentioned to the effect the immune system with the vaccines and AL NPs.
4) the safest method that can be achieved to obtain healthy vaccines and not affect them in the future on the health of children is to be avoided the use of nanomaterial in vaccines in order to prevent harm

Conflict of interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

Authors Contribution

All authors contributed equally in this paper.

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		N	Mean	Std.	Std.	Range		ANOVA test
						Mini.	Maxi.	
HCT	Control			Deviation	Error			(P-value)
	1	6	35.033	0.86	0.352	34	36	
	2	6	35.183	0.74	0.305	34	36	
	3	6	35.666	1.75	0.714	33	38	
	4	6	34.66	1.84	0.75	33	37	
	Total	24						
HCT	Polio							0.000 High Sign
	1	6	13.98	1.281	0.523	12	15.4	
	2	6	36.55	1.065	0.434	35	37.8	
	3	6	36.85	1.137	0.464	35	38	
	4	6	35.93	0.816	0.333	35	37	
	Total	24						
HCT	BCG							0.000 High Sign
	1	6	4.083	1.744	0.712	2	6.5	
	2	6	41.2	1.104	0.45	40	43	
	3	6	16.1	4.913	2.00	10	22	
	4	6	33.016	4.737	1.03	25	38	
	Total	24						
HCT	ALNP1							0.000 High Sign
	1	6	13.85	0.99	0.407	12	15	
	2	6	41.26	1.08	0.442	40	43	
	3	6	50.41	7.14	2.91	40	60	
	4	6	27.58	4.52	1.845	20	33	
	Total	24						
HCT	ALNP2							0.000 High Sign
	1	6	7.66	0.57	0.234	7	8.5	
	2	6	43.36	0.94	0.384	42	44.5	
	3	6	30.95	3.52	1.440	25	35	
	4	6	58.2	9.28	3.791	45	70	
	Total	24						

Table 8: Effect of AL NPs, Polio vaccine, and BCG vaccine on hematocrit (HCT)

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