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Clinicopathological evaluation of some immunostimulants' effects in Barki lambs

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Abstract

Levamisole, BCG, vitamin E & Selenium are traditional immunopotentiating agents. This study aimed to monitor and compare between their effects on some clinicopathological and immunological parameters. For this purpose, sixty clinically-healthy 6-months Barki male lambs were equally divided into three groups: The first group was injected S/C with l ml of levapan®10% /50kg B.Wt (100 mg of levamisole) for 3 consecutive days, while the second group was injected S/C with 0.1 ml of BCG vaccine, and the third group was injected S/C for one time with E and Se 0.5 ml /10 kg B. Wt. Blood samples were collected at 0, 3, 7, 14, 21, and 35 days. Clinicopathological and immunological parameters were estimated and statistically analyzed. The three groups displayed a significant enhancement in the estimated immunological parameters (elevated neutrophils count, neutrophils phagocytic activity and index, globulin, and acute phase proteins), but the BCG group had the highest degree of immunopotentiating action for a longer time. The E and Se group and levamisole group were almost equal. On the other hand, the erthyrogram, total antioxidant capacity, liver and kidney functions with the BCG, and levamisole groups were negatively affected, while they were enhanced in the E and Se group for 14 days. In addition, the iron profile showed significant hypoferremia, hypotransferrinemia, and hyperferritinemia with the BCG group, and non-significant changes with both, the levamisole and E and Se groups. We concluded that the BCG has a powerful and sustainable immunomodulatory effect and it is recommended to inject it combined with E and Se to avoid its side effects.

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Introduction

Recently, Immunostimulants attracted many researchers' attention, in both human and animal medicine. They were prescribed as a part of different treatment and prophylactic programs due to their magical effects on the immune system (1-4). Levamisole, the levorotatory isomer of tetramisole, is one of these immunostimulants. Its action was approved as anthelmintic, anti-rheumatic, adjuvant, antibacterial, and antiviral for animals and humans, as well. It non-specifically improves innate and adaptive immunity resulting in the augmentation of antibodies formation, T-cell activation, proliferation, phagocytosis, and chemotaxis by monocyte and macrophage and neutrophils mobility, adherence, and chemotaxis (2). In human medicine, levamisole was helpful for patients suffering from malignant conditions, autoimmune diseases, and covid-19 (2,5). In veterinary medicine, it reduced the severity of endometritis in repeat breeder cows (6) and enhanced the body responses against FMD and PPR vaccines in sheep and goats (7,8). In fish and poultry industries, it is widely used to potentiate the innate immune response, inhibit cortisol increase in stressed fish, decrease mortality, boost productivity, and improve the vaccination action (9,10). Bacillus Calmette-Guérin (BCG) vaccine is another immunostimulant, mainly used for protection against tuberculosis in human medicine and some

researchers referred to its immunopotentiating effect against some non-mycobacterium infections as well as some neoplasms (3). In veterinary practice, the BCG vaccine was used for the immunization of small ruminants against *Corynebacterium pseudotuberculosis.* BCG also has a protective effect against some pathological conditions such as equine endometritis, equine sarcoid tumor, ocular squamous cell carcinoma in cows, and upper respiratory tract infections in horses (11). Furthermore, it maximizes the immunogenicity of sheep to the Brucella vaccine Rev.1 (12). Vitamins and trace elements are another group of immunostimulants. They were recommended by physicians and veterinaries to raise the host`s resistance to different infections. Among them, vitamin E and Selenium (E and Se) combination, as Vit E was known for its potent antioxidant, anti-sterility, and anti-inflammatory action. Selenium is an important cofactor in the synthesis of glutathione peroxidase enzyme (GPx), which is responsible for the neutralization of the lipid peroxidation products and protecting the cells from their harmful oxidative action (4,13). In veterinary medicine, using Vit E and/ or Se, parenterally or in oral supplementation before parturition increased GPx activity, neutrophils phagocytic index, and metabolic activity index in the pregnant ewe. It also reduces the stillbirth rate, retained placenta, and clinical mastitis in ewe and cattle (1,14,15). E and Se improves the reproductive performance of ewes, and lamb growth and increases fertility and metabolic rates, if given before breeding season (16,17). Vit E and/ or Se decrease the adverse effects of the high heat load and enhance the antibody titer against the *Clostridium tetani* and *Clostridium perfringens* vaccine in sheep (18,19).

Although, levamisole, BCG, and E and Se are widely used in sheep husbandry, there is only a little information about their effect on the hematological and biochemical parameters, acute phase response, and iron profile in sheep. Hence, this study aimed to study their effect on some hematological and biochemical parameters, and acute phase response of Barki lambs with special reference to their effect on iron profile.

Materials and methods

Animals' groups

After the ethical approval of the animal and poultry health department, animal and poultry health division, DRC, Egypt; sixty clinically healthy Barki lambs, aged 6 months were selected for the study. They were clinically examined and the parasitic load was determined according to Jackson (20), then were housed in closed pens in the Sustainable Development Centre of Matrouh Resources Farm, subjected to a proper nutrition system and environmental conditions. They were divided equally into 3 groups: The first group: 20 lambs were injected subcutaneously for 3 consecutive days, with l ml of levapan® 10% (Parma swede-Egypt) /50kg B.Wt (100 mg of levamisole), then, the second group: 20 lambs were injected S/C with 0.1 ml of BCG vaccine supplied by the veterinary serum and vaccine research institute, El Sekka El Beda St., Abbasia, Cairo, Egypt, and the third group: 20 lambs were injected subcutaneously for one time, with 0.5 ml /10 kg B. Wt. E and Se (ADWIA Co, Egypt). Each ml contains 150 mg vit. E and 1.67 mg Se. All doses and routes of administration are recommended by the manufacturing company.

Blood samples

5 ml blood were collected from the jugular vein of each animal using a clean sterile vacutainer tube before drug injection (0 day) and at the 3rd, 7th, 14th, 21st, 28th, and 35th days after injection, then divided into 3 parts: 1st part: 1 ml of blood was collected on anticoagulant (EDTA) and was used instantly for evaluation of different hematological parameters (red blood cells count (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total leukocytic count (TLC) and differential leukocytic count, (DLC)) (21). 2nd part: 1 ml of blood was placed in a tube containing heparin and was used immediately for the estimation of neutrophils phagocytic activity following (22). 3rd part: 3 ml of blood was placed in a clean plain tube and was left to coagulate then was centrifuged at 3000 r.p.m for 20 min and serum was separated in clean Eppendorf tubes and was used for the estimation of different biochemical parameters (total protein (TP), albumin (Alb), globulin (Glob), liver enzymes (alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP)), kidney function tests (urea, creatinine (Cr)), total antioxidant capacity (TAC), serum iron (SI), total iron binding capacity (TIBC)), spectrophotometrically using commercial kits of Biodiagnostic® Company, following the manual instructions. Plasma fibrinogen (Fb), serum amyloid A (SAA), and serum haptoglobin (Hp) were determined using ELISA kits of IBL International Crop (Canada)®. Serum ferritin was measured by the CLIA method using Abnova® (Taipei) kits. Serum caeruloplasmin (Cp) and serum transferrin (Tf) were estimated by the turbidimetric method using Elabscience USA® kits. Transferrin saturation percent (TF sat. %) = SI/TIBC*100. Unsaturated iron binding capacity (UIBC) = TIBC-SI.

Ethical approval

The research was conducted according to the ethical committee of the faculty of medicine, Alexandria University No. 0305895.

Statistical analysis

Means of different statistical parameters among the different animal groups were compared via two-way ANOVA test using SPSS version 24 at 0.05 level of probability.

Results

Levamisole administration caused a significant decrease in RBCs, Hb, and PCV on the 3rd day with non-significant changes in MCV, MCH, and MCHC. While, TLC, neutrophils, Phagocytic index of neutrophils, TP, Glob, liver function tests (ALT, AST, ALP), kidney function tests (urea, creatinine), and acute phase proteins (Fb, Hp, SAA, Cp) significantly increased till reaching their peaks at the 14th day, then started decreasing returning to their 0-day values at the 35th day. Contrariwise, Alb, A/G, and TAC significantly declined, achieving their lowest values on the 14th day and then raised approaching their baseline values on the 35th day for Alb but A/G and TAC didn`t achieve theirs. Non-significant (P≥0.05) changes were determined in the iron profile. BCG group results displayed a significant reduction in RBCs, and Hb (peaks at 14th), PCV (lowest values were at 3rd, 7th), MCV (lowest values at 3rd day), MCH (lowest values at 7th day), then they started increasing towards 0-day values till the end of the experiment, but they didn`t score it. MCHC significantly increased on the 3rd day then significantly decreased till the 35th day and didn`t return to its original values. On the other hand, TLC, neutrophils, phagocytic activity and index of neutrophils, Tp, Glob, liver enzymatic activity, kidney function tests (urea, creatinine (Cr)) and APPs (Fb, Hp, SAA, Cp), TIBC, UIBC, ferritin significantly elevated till reaching their peaks at the 14th day, then downregulating towards their primary values, but they didn`t reach them. While, Alb, A/G, TAC, SI, Tf, and Tf sat significantly decreased till the 14th day, then began to elevate approaching their 0-day value, but didn`t reach them. E and Se group showed a significant raise in RBCs, Hb, MCH, and MCHC on the 3rd and 7th days, PCV demonstrated a significant increase on the 3rd day then decreased on the 7th day but still higher than the 0-day values. All of them returned to their initial values on the 14th day. E and Se group presented a significant increase in TLC, neutrophils, phagocytic activity and index of neutrophils, TP, Glob, and a significant decrease in A/G but they reached their peaks at the 14th day, then achieving their primary values at the end of the experiment. TAC significantly increased in the 3rd, and 7th days and reached normal values on the 14th day. Liver enzymatic activities significantly decreased on the 3rd and 7th days, then returned to their initial values on the 14th day. Non-significant changes were detected in Alb, kidney function tests, AAPs, and iron profile throughout the study (Table 1-6).

Table 1: Red blood cell parameters in LG, BCG, and E+Se groups

Day	Group	RBCs ($\times10^6$/µl)^D	Hb (g/dl)^D	PCV (%)^D	MCV (fl)^D	MCH (pg)^D	MCHC (%)^D
0	LG	11.88±0.77^d	13.62±0.88 ^{d}	33.01±0.85 ^{d}	27.88±1.80	11.47±0.30	41.29±2.76
	BG	11.82±0.75^d	13.71±0.84 ^{d}	33.07±0.96 ^{d}	28.07±1.70^d	11.61±0.29^d	41.49±2.68^d
	EG	11.82±0.75^d	13.62±0.89 ^{d}	33.01±0.85^d	28.01±1.73^d	11.52±0.32^d	41.28±2.80^d
3	LG	10.72±0.52^c	12.62±0.85^c	31.01±0.85^c	28.99±2.15	11.78±0.40	40.75±2.87
	BG	9.28±0.38^c	9.98±0.34^c	23.53±0.64^c	25.39±1.30^c	10.76±0.35^c	42.41±1.39^c
	EG	13.15±0.86^c	15.80±0.86^c	36.53±0.92^c	27.70±2.37	12.00±0.18^c	43.34±3.15^c
7	LG	11.32±0.83	13.12±1.01	32.01±0.85	28.40±2.01	11.60±0.55	40.99±3.04
	BG	9.22±0.40^c	9.69±0.45^c	23.53±1.46^c	25.60±2.15^c	10.52±0.46^c	41.32±3.09^c
	EG	13.20±0.80^c	15.96±0.91^c	34.60±0.94^c	27.97±1.65	12.12±0.21^c	46.15±2.90^c
14	LG	11.35±0.85	13.12±1.01	32.01±0.85	28.33±2.17	11.57±0.33	41.02±2.87
	BG	9.15±0.46^c	9.66±0.52^c	25.20±0.77^c	27.61±1.68^c	10.56±0.45^c	38.35±2.83^c
	EG	12.08±0.74	13.83±0.90	33.60±0.83	27.91±1.87	11.44±0.35	41.16±2.71
21	LG	11.65±0.85	13.12±0.88	32.01±0.85	27.60±2.06	11.27±0.31	41.02±2.87
	BG	9.72±0.49^c	10.73±0.50^c	27.01±0.85^c	27.62±1.86^c	11.04±0.05^c	39.75±2.05^c
	EG	12.05±0.86	13.83±0.77	33.33±0.82	27.78±1.85	11.49±0.27	41.51±2.19
28	LG	11.74±0.81	13.59±0.91	32.01±0.85	27.38±1.91	11.60±0.30	42.47±2.89
	BG	10.62±0.49^c	11.33±0.50^c	29.33±0.82^c	27.69±1.68^c	10.67±0.03^c	38.65±2.24^c
	EG	11.96±0.77	13.71±0.93	33.33±0.82	27.97±1.65	11.47±0.15	41.13±2.47
35	LG	11.82±0.83	13.59±0.91	32.01±0.85	27.19±1.93	11.50±0.36	42.47±2.89
	BG	10.72±0.49^c	11.53±0.50^c	29.93±0.88^c	27.99±1.69^c	10.76±0.03^c	38.55±2.25^c
	EG	11.96±0.7	13.71±0.93	33.33±0.82	27.97±1.65	11.47±0.15	41.13±2.47

^{D} on the parameters (significant between the three drugs effect along the study), d on the 0day value (the effect of the drug significant along the study in the same group), c (significant with the control group), significant when P<0.05.

Table 2: TLC, neutrophils, lymphocytes, Monocytes, Eosinophils and Basophils counts in LG, BCG, and E+Se groups

Day	Group	TLC ($\times10^3/\mu l$)^D	Neutrophils ($\times10^3/\mu l$)^D	Lymphocytes ($\times10^3/\mu l$)	Monocytes ($\times10^3/\mu l$)	Eosinophils ($\times10^3/\mu l$)	Basophils ($\times10^3/\mu l$)
0	LG	7.78±0.34^d	4.20±0.29^d	2.59±0.23	0.53±0.07	0.43± 0.06	0.04±.005
	BG	7.82±0.34^d	4.18±0.23^d	2.65±0.26	0.53±0.07	0.43±0.06	0.04±.005
	EG	7.78±0.33^d	4.22±0.23^d	2.60±0.22	0.53±0.07	0.43±0.06	0.04±.005
3	LG	7.93±0.38^c	4.38±0.24^c	2.56±0.28	0.53±0.08	0.43± 0.06	0.04±.005
	BG	9.83±040^c	6.27±0.23^c	2.56±0.27	0.53±0.08	0.42±0.06	0.04±.005
	EG	8.28±0.37^c	4.67±0.22^c	2.59±0.21	0.53±0.08	0.44±0.06	0.04±.005
7	LG	8.32±0.30 ^{c}	4.77±0.16^c	2.55±0.28	0.53±0.06	0.43± 0.06	0.04±.005
	BG	10.42±0.32^c	6.87±0.08^c	2.56±0.21	0.53±0.05	0.43±0.06	0.04±.005
	EG	8.48±0.39^c	4.89±0.19^c	2.59±0.20	0.53±0.04	0.43±0.06	0.04±.005
14	LG	8.49±0.31^c	4.94±0.03^c	2.53±0.27	0.53±0.04	0.43± 0.06	0.04±.005
	BG	10.49±0.29^c	6.94±0.05^c	2.50±0.27	0.53±0.06	0.43±0.06	0.04±.005
	EG	8.61±0.33^c	5.03±0.12c	2.52±0.21	0.53±0.05	0.43±0.06	0.04±.005
21	LG	8.38±0.30^c	4.84±0.06^c	2.54±0.21	0.53±0.08	0.43± 0.06	0.04±.005
	BG	9.80±0.30^c	6.25±0.02^c	2.56±0.28	0.53±0.05	0.43±0.06	0.04±.005
	EG	8.41±0.40^c	4.82±0.20	2.59±0.24	0.53±0.08	0.43±0.06	0.04±.005
28	LG	8.19±0.31^c	4.63±0.04^c	2.53±0.27	0.53±0.07	0.43± 0.06	0.04±.005
	BG	9.61±0.31^c	6.06±0.04^c	2.54±0.22	0.53±0.07	0.43±0.06	0.04±.005
	EG	8.16±0.37^c	4.57±0.16^c	2.58±0.20	0.53±0.06	0.43±0.06	0.04±.005
35	LG	8.00±0.31	4.45±0.02	2.59±0.27	0.53±0.07	0.43± 0.06	0.04±.005
	BG	9.61±0.31^c	6.06±0.04^c	2.56±0.27	0.53±0.07	0.43±0.06	0.04±.005
	EG	8.06±0.29	4.47±0.08	2.59±0.24	0.53±0.07	0.43±0.06	0.04±.005

^{D} on the parameters (significant between the three drugs effect along the study), d on the 0day value (the effect of the drug significant along the study in the same group), c (significant with the control group), significant when P<0.05.

Table 3: Phagocytic activity, phagocytic index, TP, Alb, Blob, and A/G in LG, BCG, and E+Se groups

Day	Group	Phagocytic activity (%)^D	Phagocytic index^D	TP (g/dl)^D	Alb (g/dl)^D	Glob (g/dl)^D	A/G^D
0	LG	61.00±0.85	2.02±0.01^d	6.45±0.16^d	4.51±0.20^d	1.94±0.18^d	2.35±0.28^d
	BG	61.00±0.85^d	2.02±0.01^d	6.45±0.16^d	4.53±0.19^d	1.91±0.16^d	2.39±0.25^d
	EG	61.00±0.85^d	2.02±0.01 ^{d}	6.47±0.17^d	4.53±0.26	1.94±0.24^d	2.38±0.34^d
3	LG	61.47±0.64	2.12±0.01^c	6.84±0.16^c	3.57±0.20^c	3.27±0.27^c	1.10±0.14^c
	BG	71.00±0.85^c	3.03±0.04^c	7.42±0.17^c	3.05±0.02^c	4.37±0.17^c	0.70±0.03^c
	EG	61.93±0.80^c	2.22±0.03^c	7.05±0.02^c	4.69±0.15	2.36±0.29^c	1.99±0.19^c
7	LG	61.47±0.64	2.12±0.03 ^{c}	7.07±0.03^c	3.05±0.02^c	4.02±0.04^c	0.76±0.01^c
	BG	71.00±0.85^c	3.03±0.04^c	8.06±0.02^c	2.51±0.08^c	5.54±008^c	0.45±0.02^c
	EG	61.93±0.80^c	2.22±0.03^c	7.44±0.21^c	4.69±0.15	2.75±0.29^c	1.72±0.22^c
14	LG	61.46±0.63	22.22±0.03 ^{c}	7.48±0.04^c	2.96±0.02^c	4.52±0.04^c	0.65±0.01^c
	BG	71.40±0.74^c	3.16±0.10^c	8.46±0.02^c	1.95±0.02^c	6.51±0.03^c	0.30±0.01^c
	EG	62.13±1.13^c	2.33±0.01^c	7.45±0.21^c	4.69±0.15	2.76±0.29^c	1.71±0.22^c
21	LG	61.07±0.80	2.12±0.03^c	7.16±0.02^c	3.06±0.02^c	4.10±0.04^c	0.75±0.01^c
	BG	71.01±0.85^c	3.14±0.10^c	8.15±0.03^c	2.15±0.02^c	6.00±0.04^c	0.36±0.01^c
	EG	61.53±0.64^c	2.23±0.01^c	7.15±0.02^c	4.69±0.15	2.47±0.16^c	1.90±0.18^c
28	LG	61.07±0.80	2.10±0.03 ^{c}	6.82±0.06^c	3.58±0.04^c	3.23±0.06^c	1.11±0.51^c
	BG	66.01±1.70^c	3.12±0.08^c	7.85±0.03^c	2.64±0.05^c	5.21±0.07^c	0.51±0.02^c
	EG	61.53±0.64^c	2.13±0.02^c	6.83±0.04^c	4.69±0.15	2.15±0.17^c	2.20±0.24^c
35	LG	61.07±0.80	2.02±0.03	6.46±0.02	4.06±002	2.06±0.03	1.70±0.03^c
	BG	64.01±1.70^c	3.00±0.05^c	7.26±0.02^c	2.64±0.05^c	4.61±0.06^c	0.57±0.02^c
	EG	61.03±0.64	2.03±0.02	6.46±0.02	4.69±0.15	2.05±0.17	2.30±0.24

^{D} on the parameters (significant between the three drugs effect along the study), d on the 0day value (the effect of the drug significant along the study in the same group), c (significant with the control group), significant when P<0.05.

Table 4: Concentration of AST, ALT, ALP, Urea, Cr, and TAC in LG, BCG, and E+Se groups

Day	Group	AST (U/L)^{D}	ALT (U/L)^{D}	ALP (U/L)^{D}	Urea (mg/dl)^{D}	Cr (mg/dl)^{D}	TAC (Mm/L)^{D}
	LG	30.60±1.88^{d}	28.00±1.46^{d}	24.40±3.48^{d}	22.01±1.46^{d}	1.40±0.23^{d}	1.34±0.05^{d}
0	BG	30.60±1.88^{d}	28.00±1.46^{d}	24.40±3.48^{d}	22.00±1.46^{d}	1.42±0.23^{d}	1.34±0.05^{d}
	EG	30.60±1.88^{d}	27.60±1.46^{d}	24.16±3.66^{d}	22.00±1.46	1.41±0.23	1.34±0.05^{d}
	LG	32.00±2.06^{c}	30.87±1.51^{c}	27.40±1.64^{c}	25.20±1.65^{c}	2.54±0.25^{c}	0.87±0.01^{c}
3	BG	39.67±2.38^{c}	35.20±2.14^{c}	36.35±2.33^{c}	30.00±2.93^{c}	3.01±0.15^{c}	0.47±0.04^{c}
	EG	25.60±1.46^{c}	24.27±0.85^{c}	22.80±1.66^{c}	21.07±1.16	1.48±0.22	2.05±0.02^{c}
	LG	34.03±0.88^{c}	32.13±1.60^{c}	29.87±1.41^{c}	26.33±1.47^{c}	3.32±0.21^{c}	0.79±0.01^{c}
7	BG	45.20±1.78^{c}	40.01±1.69^{c}	43.00±0.89^{c}	38.80±2.24^{c}	3.60±0.15^{c}	0.39±0.02^{c}
	EG	27.40±1.46^{c}	24.01±1.69^{c}	22.40±1.69^{c}	21.60±1.45	1.20±0.15	1.64±0.01^{c}
	LG	36.00±1.25^{c}	34.01±1.69^{c}	35.20±1.21^{c}	30.13±1.76^{c}	3.80±0.15 ^{c}	0.69±0.01^{c}
14	BG	47.00±1.46^{c}	42.00±1.69^{c}	44.00±1.46^{c}	42.00±1.69^{c}	4.01±0.17^{c}	0.38±0.02^{c}
	EG	30.26±1.78	27.60±1.69	23.40±1.69	23.20±2.95	1.58±0.16	1.51±0.07
	LG	32.53±1.06 ^{c}	31.60±1.72^{c}	27.67±1.70 ^{c}	26.20±2.67 ^{c}	3.49±0.24^{c}	0.78±0.01^{c}
21	BG	43.01±1.46^{c}	38.00±1.69^{c}	42.00±1.69^{c}	38.00±1.69^{c}	3.69±0.24^{c}	0.65±0.02^{c}
	EG	30.26±085	27.60±1.69	23.86±0.89	23.20±2.99	1.20±0.17	1.49±0.07
	LG	32.66±1.35^{c}	30.13±1.55^{c}	26.01±1.69^{c}	23.40±0.82 ^{c}	2.60±1.69^{c}	0.95±0.04^{c}
28	BG	40.01±0.85^{c}	35.00±0.85^{c}	38.00±1.69^{c}	32.00±1.69^{c}	2.90±0.08^{c}	0.78±0.02^{c}
	EG	30.60±0.85	27.66±1.70	23.86±1.69	23.20±2.37	1.40±0.17	1.49±0.07
	LG	31.46±083	29.01±0.85	24.80±1.97	22.00±1.46	1.90±0.08	1.07±0.01^{c}
35	BG	31.60±1.55^{c}	32.13±1.30^{c}	28.00±1.46^{c}	25.60±2.41^{c}	2.20±0.15^{c}	0.97±0.01^{c}
	EG	30.60±0.85	27.60±0.85	23.86±0.85	23.20±1.69	1.70±0.08	1.49±0.07

^{D} on the parameters (significant between the three drugs effect along the study), d on the 0day value (the effect of the drug significant along the study in the same group), c (significant with the control group), significant when P<0.05.

Table 5: Concentration of acute phase proteins concentrations in LG, BCG, and E+Se groups

Day	Group	Fb (mg/dl)^{D}	Cp (mg/dl)^{D}	Hp (g/dl)^{D}	SAA (mg/L)^{D}
	LG	121.01±8.70^{d}	2.32±1.19^{d}	0.15±0.02^{d}	2.30±0.15^{d}
0	BG	121.67±4.08^{d}	2.32±1.19^{d}	0.15±0.02^{d}	2.30±0.15^{d}
	EG	121.00±8.70	2.32±1.19	0.15±0.02	2.30±0.15
	LG	150.01±7.32^{c}	5.75±0.25^{c}	0.40±0.15^{c}	2.80±0.15^{c}
3	BG	166.01±10.72^{c}	6.93±0.33^{c}	1.01±0.15^{c}	3.71±0.35^{c}
	EG	122.01±7.32	2.43±0.25	0.25±0.15	2.40±0.17
	LG	169.33±5.69^{c}	6.73±0.24^{c}	1.01±0.15^{c}	3.48±0.21^{c}
7	BG	192.33±5.94^{c}	8.01±0.15^{c}	1.60±0.15^{c}	4.86±0.25^{c}
	EG	123.01±7.32	2.55±0.25	0.35±0.15	2.68±0.21
	LG	186.01±2.93^{c}	7.60±0.15^{c}	1.60±0.19^{c}	4.40±0.29^{c}
14	BG	221.33±11.25^{c}	8.60±0.15^{c}	2.52±0.21^{c}	5.80±0.29^{c}
	EG	124.01±1.69	2.68±0.15	0.38±0.15	2.80±0.29
	LG	163.40±2.47^{c}	5.91±0.20^{c}	1.23±0.14^{c}	3.66±0.25^{c}
21	BG	192.00±5.61^{c}	7.10±0.08^{c}	1.80±0.15^{c}	4.79±0.33^{c}
	EG	125.20±3.28	2.44±0.25	0.36±0.15	2.74±0.25
	LG	143.40±247^{c}	4.09±0.20^{c}	0.80±0.15^{c}	2.76±0.16^{c}
28	BG	171.80±5.54^{c}	4.68±0.21^{c}	1.08±0.21^{c}	3.66±0.25^{c}
	EG	124.40±3.48	2.40±0.15	0.28±015	2.60±0.15
	LG	127.98±2.47	2.44±0.25	0.33±0.15	2.29±0.23
35	BG	132.53±9.05^{c}	3.07±0.63^{c}	0.47±0.21^{c}	3.19±0.33^{c}
	EG	125.80±2.37	2.30±0.16	0.20±0.08	2.30±0.17

^{D} on the parameters (significant between the three drugs effect along the study), d on the 0day value (the effect of the drug significant along the study in the same group), c (significant with the control group), significant when P<0.05.

Table 6: Concentration of SI, TIBC, UIBC, Transferrin, Tf sat. %, and ferritin in LG, BCG, and E+Se groups

Day	Group	SI (µg/dl)^{D}	TIBC (µg/dl)^{D}	UIBC (µg/dl)^{D}	Transferrin (mg/dl)^{D}	Tf sat. %^{D}	Ferritin (ng/ml)^{D}
0	LG	107.56±2.66	327.50±212	219.93±2.40	124.40±2.47	33.00±1.00	13.60±1.06
	BG	106.76±2.62^{d}	327.43±2.25^{d}	220.67±2.58^{d}	125.40±3.38^{d}	33.00±1.00^{d}	13.67±1.11^{d}
	EG	107.68±2.08	327.41±1.89	219.74±1.16	124.40±2.47	33.00±1.00	13.60±1.06
3	LG	107.83±2.66	327.50±2.37	219.67±2.79	124.47±2.39	33.00±1.00	13.73±1.01
	BG	96.90±1.67^{c}	334.76±2.50^{c}	237.87±2.95^{c}	112.00±1.69^{c}	29.00±1.00^{c}	17.01±0.85^{c}
	EG	107.28±2.64	327.41±1.89	220.27±2.89	124.60±2.47	33.00±1.00	13.80±1.01
7	LG	107.00±2.80	328.03±2.29	220.13±2.42	124.53±2.17	33.00±1.00	13.87±0.99
	BG	93.03±1.63^{c}	341.90±2.30^{c}	248.87±2.72^{c}	106.33±1.29^{c}	27.00±1.40^{c}	20.01±0.85^{c}
	EG	107.90±2.71	327.55±2.16	220.13±3.13	124.93±2.15	33.00±1.00	13.93±1.28
14	LG	107.5±2.65	327.96±2.14	220.13±2.42	124.20±1.61	33.00±1.00	13.73±0.88
	BG	87.83±1.74^{c}	349.70±2.58^{c}	261.87±2.95^{c}	100.00±0.85^{c}	25.00±1.00^{c}	24.00±1.69^{c}
	EG	107.83±2.71	328.08±2.19	220.13±3.13	125.33±2.50	33.00±1.00	14.33±1.23
21	LG	107.05±2.44	327.83±2.04	219.80±2.46	124.53±1.68	33.00±1.00	13.53±1.06
	BG	93.83±1.74^{c}	344.25±1.77^{c}	250.42±1.24^{c}	105.00±0.85^{c}	27.00±1.00^{c}	21.87±1.06^{c}
	EG	108.03±2.71	328.08±2.19	220.13±3.32	124.93±2.31	33.00±1.00	14.20±1.42
28	LG	107.00±2.44	327.96±2.08	219.87±2.56	124.80±182	33.00±1.00	14.07±1.16
	BG	98.23±1.10^{c}	339.23±1.10^{c}	241.01±1.51^{c}	112.13±1.60^{c}	29.00±1.00^{c}	19.07±0.88^{c}
	EG	108.01±2.32	328.22±2.33	220.21±2.91	125.20±2.18	33.00±1.00	14.73±1.22
35	LG	106.00±2.06	328.03±1.91	220.07±1.98	124.00±2.01	33.00±1.00	13.93±0.96
	BG	102.68±1.60^{c}	335.58±0.92^{c}	232.90±1.85^{c}	118.60±1.99^{c}	31.00±1.00^{c}	16.93±0.96^{c}
	EG	108.00±1.89	328.22±2.11	219.68±1.85	125.20±2.18	33.00±1.00	14.73±1.22

^{D} on the parameters (significant between the three drugs effect along the study), d on the 0 day value (the effect of the drug significant along the study in the same group), c (significant with the control group), significant when $P<0.05$.

The comparison among the studied immunostimulants showed that, BCG has the most powerful immunostimulant action, although the three groups displayed a significant elevation in TLC, neutrophils, neutrophils phagocytic activity and index, TP, Glob, and APPs, but these changes were superior and persistent in BCG group. While, levamisole and E and Se were almost equal, as these immunological parameters were slight better in the E and Se group than the levamisole group for 14 days, except globulin values were better in the levamisole group than the E and Se group and APPs significantly increased in Levamisole group and non-significantly changed in E and Se group. On the other hand, the erthyrogram, TAC, liver, and kidney functions of BCG and levamisole groups were negatively affected (in the BCG group more than the levamisole group) while, they were ameliorated in the E and Se group for 14 days. In addition, the iron profile of the BCG group was markedly affected and non-significantly changed in the levamisole and E and Se groups.

Discussion

The appearance of resistant strains of different pathogens and the antibiotic usage futility, steered the researchers' attention toward immunostimulants (3). Among them levamisole and BCG, which are potent immunostimulants widely used in human and animal medicine. According to the current data, both of them succeeded in improving the estimated immunity parameters. This was represented here by the neutrophilic leukocytosis and increased neutrophils phagocytic index, hyperglobulinemia, hypoalbuminemia, and increased APPs concentrations observed in levamisole and BCG groups, and the increased neutrophils phagocytic activity detected in BCG group only throughout the research.

The levamisole and BCG non-specific immunopotentiating effect was mainly attributed to their ability to enhance the expression of the pro-inflammatory cytokines (IL-1, TNF-α, TNF-γ) (2,3,23). The activation of the pro-inflammatory cytokines (by levamisole and BCG) evokes neutrophils maturation and release from bone marrow and increases their activity and function. Neutrophilic leukocytosis with enhanced activity and function was noticed before with levamisole administration by Refat (24), Sadigh-Eteghad (25), and BCG administration by Brook (26). The pro-inflammatory cytokines also stimulate immunoglobulin production (γ-globulin) and arrange acute phase response (α and β-globulin), leading to the outstanding hyperglobulinemia (and the subsequent hyperproteinemia and decreased A/G), hypoalbuminemia (negative acute phase reactant) and increased positive APPs (Fb, Hp, SAA, Cp) in levamisole and BCG groups along the research. Hyperglobulinemia, hypoalbuminemia, and acute

phase response were recorded before with levamisole and BCG administrations by many authors (24,27-31). In addition, the pro-inflammatory cytokines increase free radicals' formation and accumulation causing the oxidative stress noted in both groups (represented by the decreased TAC). Unfortunately, the accumulated free radicals attack liver and kidney cells leading to a prominent increase in the liver and kidney function tests in levamisole and BCG groups. Previous reports pointed to oxidative stress and subsequent elevated liver and kidney function tests accompanying levamisole and BCG administrations (3,24,32-37). Drug metabolism may be an additional cause of oxidative stress and associated elevated liver and kidney functions in levamisole group (34-37).

On the other hand, a transit depression in RBCs, Hb, and PCV was noted on the 3rd day in the levamisole group and decreased RBCs, Hb, PCV, MCV, MCH, MCHC (from the 7th day) values were detected in BCG group till the end of the study. This agreed with previous data referred to anemic changes caused by levamisole and BCG administration (23,24,38,39). They assigned these changes to the above-mentioned oxidative stress as the released free radicals attack RBCs causing their destruction and lysis. Additionally, the activated pro-inflammatory cytokines inhibit erythropoietin synthesis and subsequent RBC production and release from bone marrow (2,3,23).

Interestingly, the aforementioned clinicopathological and immunological changes were more prominent and persistent in the BCG group than in the levamisole group. As BCG evokes the production of the pro-inflammatory cytokines by a powerful unique mechanism called trained immunity using the mycobacterium lipoproteins, LPS, and CpG oligonucleotide, its effect may sustain up to 3 months (3,29,33). In addition, BCG motivates adaptive immunity against unrelated pathogens and enhances T-helper1 and T-helper17 responses by another mechanism, referred to as heterologous immunity (33,40). This explains why the iron profile didn't vary in the levamisole group and it markedly changed in the BCG group in the current data. Meanwhile, the activation of the prior pro-inflammatory cytokines was more powerful in the BCG group than in the levamisole group. These cytokines trigger marked hypoferremia, hypotransferrinemia, and hyperferritinemia to reduce the iron bioavailability for the pathogens in order to prevent their growth. Thus, the immunopotiniating action of BCG increases and a subsequent increase in TIBC, UIBC, and decreased Tf sat. % were obtained in the BCG group throughout the study. Similar observations were recorded before in BCG-vaccinated neonates (38).

In contrast to levamisole and BCG, E and Se have an inhibitory effect on the pro-inflammatory cytokines and their immunostimulant effect was assigned to their nature as free radicals' scavengers (1,4,13). So, they protected the body cells from free radicals' harmful effects, especially (RBCs, and the liver), and increased TAC for 14 days in the current study. This led to a considerable enhancement in the red blood cells parameters and indices in E and Se group on the 3rd and 7th days, and improved TLC, neutrophils count, phagocytic activity, index, TP, Glob, throughout the research. Besides, E and Se administration ameliorated the liver functions on the 3rd and 7th days and had no adverse effect on kidney functions or iron profile in the study. Similar results were obtained before, with vitamin E and Se injections either combined or separated in different animal species (1,4,13,16,41-46).

Conclusion

BCG had the most powerful and persistent immunomodulatory effect among the studied immunostimulants, with adverse effects on the erthyrogram, liver, kidneys, and iron profile. So, it is better to inject E and Se with BCG.

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Conflict of interest

There is no conflict of interest.

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التقييم السريري المرضي لتأثيرات بعض المحفزات المناعية في حملان البرقي

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الخلاصة

يعد كل من الليفاميزول ولقاح بي سي جي وفيتامين هـ والسيلينوم عوامل تقليدية لتكوين المناعة. وتهدف هذه الدراسة إلى رصد ومقارنة تأثير هذه العوامل على بعض المقاييس السريرية المرضية والمناعية. لذلك، قسمت مجموعة من ستون حملا ذكرا من نوع البرقي، سليمة سريريا وبعمر ٦ أشهر بالتساوي إلى ثلاث مجموعات. حقنت المجموعة الأولى بعقار ليفاميزول ١٠% تحت الجلد لمدة ٣ أيام متتالية، ١مل/٥٠ كجم من وزن الجسم (١٠٠ملجرام من الليفاميزول). بينما حقنت المجموعة الثانية تحت الجلد بلقاح البى سي جي ٠,١ مل. أما المجموعة الثالثة فحقنت مرة واحدة تحت الجلد بفيتامين هـ والسيلينوم بمقدار ٠,٥ مل / ١٠ كجم من وزن الجسم. جمعت عينات الدم في الأيام ٠ و ٣ و ٧ و ١٤ و ٢١ و ٣٥. ثم قدرت المعاملات السريرية المرضية والمناعية وحللت إحصائيا. أظهرت المجموعات الثلاث تحسنا مهما في المعاملات المناعية المقدرة، لكن مجموعة البي سي جي كان لديها أعلى درجة من التأثير المناعي لفترة أطول، بينما كانت مجموعة هـ والسيلينوم ومجموعة الليفاميزول متساويتان تقريبا. ومن ناحية أخرى، تأثرت سلبيا كل من معاملات كرات الدم الحمراء والسعة الكلية لمضادات الأكسدة ووظائف الكبد والكلى في مجموعة البي سي جي، ومجموعة الليفاميزول، في حين أنها تحسنت في مجموعة هـ ولسيلينوم لمدة ١٤ يوما. بالإضافة إلى ذلك، أظهرت صورة الحديد لمجموعة البي سي جي نقصا معنويا في مستويات الحديد في الدم ونقص ترانسفيرين الدم وفرط فيريتين الدم ولم تتغير كثيرا في كل من مجموعة الليفاميزول ومجموعة فيتامين هـ والسيلينوم. في النهاية، خلصت الدراسة الى أن للقاح البي سي جي تأثيرا محفزا مناعيا قويا ومستداما، ويوصى بحقنه مع فيتامين هـ والسيلينوم لتجنب آثاره الجانبية.

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