



Hematological parameters after oral administrated of Levamisole with its activity on nonspecific immunity and total serum protein in sheep

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Abstract

In this study (20) ewes were examined and subdivided into two groups, the 1st group test group (n=10) were treated with Levamisole at dose 2.5 mg/kg while the 2nd group was untreated and considered control group (n=10) blood samples were collected into EDTA – containing tubes. Blood samples from all were continued at the day 0,7,14 and 21.

The level of erythrocytes count, PCV, Hb, total leukocyte count, differential leukocytes count (manual standard method), total serum protein, albumin and globulin were measured to evaluate this study.

Appropriate statistical methods were used for data analysis, and (P<0.05).was considered as significant. No significant differences were seen for PCV, RBCs, and Hb. Neutrophil level at day 14 and monocyte level at day 21 were significantly higher than in the control group (P<0.05).

The results show insignificant increase in the total protein and Globulin and insignificant decrease in the albumin.

Levamisole enhance nonspecific immune response due to induce leukocytosis, neutrophilia and monocytosis .

Key words: Leukogram, Levamisole, immunosuppression, Sheep.

القيم الدموية بعد اعطاء الليفاميزول في الاغنام وتأثيراته على المناعه غير المتخصصة وقيم
البروتين الكلي

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الخلاصة:

اجريت هذه الدراسة من خلال استخدام 20 نعجة تم فحصها سريريا والتأكد من سلامتها من الامراض, قسمت هذه النعاج الى مجموعتين كل مجموعة تتكون من 10 نعاج. عولجت المجموعة الاولى بالليفاميزول وبجرعة (2.5 mg/kg). بينما تركت المجموعة الثانية بدون علاج وعدت مجموعة سيطرة. جمعت عينات الدم من الوريد الوداجي ووضعت في انابيب حاوية على مانع تخثر الدم وعملية جمع الدم في الايام (21,14,7,0).

اجريت فحوصات الدم على العينات وشملت العد الكلي لخلايا الدم الحمراء والبيضاء وفحص الهيموكلوبين وفحص خلايا الدم المرصوص وكذلك العد التقريبي لخلايا الدم البيض واجري فحص البروتين الكلي وفحص الالبومين والكلوبيولين. وبعد اجراء الاختبار الاحصائي للكشف عن الفروقات المعنوية بين المجاميع حيث اظهرت النتائج انه لا توجد فروقات معنوية بين مجاميع العد الكلي لكريات الدم الحمراء والهيموكلوبين وحجم خلايا الدم المرصوص في حين سجلت ارتفاعا معنويا في خلايا العدلات في اليوم 14 وخلايا الاحاديه النوى في اليوم 21 وكذلك سجلت انخفاضاً معنوياً في الالبومين وارتفاعاً غير معنوياً في البروتين الكلي والكلوبيولين. اظهرت الدراسة ان استخدام الليفاميزول في الاغنام له تاثير ايجابي على مناعة الجسم وخاصة المناعة غير المتخصصة من خلال زيادة العد الكلي لخلايا الدم البيض بسبب زيادة اعداد الخلايا العدلات والخلايا الاحادية.

Introduction:

Levamisole is a highly acceptable anti-nematodal drug has broad range of activity in a large number of host animals (sheep, cattle, horse, pig, dog, chicken), widely used as veterinary anthelmintic, and was recognized that concurrent enhancement of immune responsiveness sometimes accompanied anthelmintic treatment, it has an immunostimulant agent [1].

The major components of the innate immune system are monocytes, granulocytes and humoral elements, such as lysozymes or the complement system [15]. Its immunostimulant effect may be related to T-cell activation and proliferation, augmentation of monocyte and macrophage activity and an increase in neutrophil mobility, adherence, and chemotaxis [3, 4].

Levamisole has no cytotoxic effects [5]. Immunostimulants can be administered by different routes such as injection, bathing or orally, with the latter appearing to be the most practicable [6]. The ability of this agent is to enhance the response of mammalian T-lymphocytes and macrophages of both healthy [23] and

immunocompromised individuals is well documented [1, 7, 8].

Levamisole is rapidly absorbed from the GI tract and extensively metabolized in the liver and excreted mainly by the kidneys (70% over 3 days). Its plasma elimination half-life is 3-4 hours. Due to short elimination half-life, blood levels of levamisole fall more rapidly and go unnoticed in toxicological examination. Data from few clinical studies indicate that the consumption of 50-200 mg/day of levamisole causes agranulocytosis in 0.08 – 5% of the studied population (WHO, FAS 33).

Modern veterinary and human medicine used levamisole to modify immune response primarily; levamisole is a potent broad-spectrum anthelmintic, which is active against most pathologic nematodes in animals and man. It is modulates immune function at a dose of 2-3 mg/kg body mass intermittent rather than continuous treatment within three days or as a single dose once a week (16,17).

The primary action of levamisole seems to be a cell-mediated immune response through enhancing immune response and increase serum antibody

titers after immunization, the number of leucocytes, phagocyte activities, expression of cytokines by monocyte (macrophages), lymphocyte proliferation and antitumor responses [18].

The objective of the present research work is to check effect of levamisole on nonspecific immunity of sheep by monitoring several parameters; Erythrogram, Leukogram, Total protein, albumin and Globulin.

Material And Methods

Animals Experimental design.

This study was conducted by defining a group of Ewes(20) after confirming free of the disease , The animals divided into two groups, each group consist of 10 animals, the first group was treated with levamisole at a dose of 2.5 ml / 10 kg of body weight, and left the second Group Untreated control group promised.

Blood samples were collected to study the immune state Of animals before and after treatment for a period of three periods (0, 7, 14 and 21 days).

The parameters evaluated after treatment

- 1-Total Leukocyte Count.
- 2-Diffrintial Leukocytic Count.
- 3-Total serum protein.
- 4-Albumin
- 5-Globulin
- 6-Erythrocyte count,Hb and PCV.

Collection of blood samples:

Blood samples were collected from the jugular vein (5 ml) from each animal in EDTA tubes; two separated blood samples were collected.

One sample was taken in EDTA tubes for TLC by using heamatocytometer chamber and differential leucocytic count by using blood smear on slide and fixation with concentrated Methyl alcohol for 3-5 minutes then stained with Giemza stain for 45 minutes.

The second sample was taken in test tubes without anticoagulant(Sheep red blood cells (SRBCs) were separated by centrifugation)for clear serum separation which is carefully collected and stored in epindorf tubes for total protein, albumin and globulin (by subtraction of albumin from total protein), which estimated spectrophotometer used commercial kits.

Drug

Levamisole HCL (Levamisole[®]) injectable solution 7.5% obtained from veterinary and Agricultural products MFG.CO.LTD. Amman- Jordan. CID Co., Egypt.

Statistical Analysis

Hematological values and serum biochemical parameters were analyzed by one way analysis of variance followed by Dunn's multi range test (ANOVA) using software program SPSS version 20 for window.

Results And Discussion

Erythrogram results:

The results of hematology (RBCs,Hb and PCV) are shown in the table (1), there is no significant changes ($P<0.05$)in test and control groups. these results agree with result recorde by [19] .but disagreed with the researcher [20] stated that levamisole induced insignificant decrease in erythrogram in sheep.

Table 1 Erythrogram of sheep treated with levamisole (n=10)

TIME/DAY	Groups	RBCs 10 ⁶ /mm ³	Hb (Gm %)	PCV (%)
0 Day	control	11.3± 0.84a	12.9± 0.84 a	30.09± 0.70a
	Test group	11.5± 0.64a	12.8± 0.97 a	29.21± 0.30a
7Day	control	11.7± 0.85a	12.8± 0.73 a	31.11± 0.60a
	Test group	11.1± 0.52a	12.2± 0.52 a	30.43± 0.10a
14 Day	control	12.2± 0.21a	12.5± 0.82 a	30.41± 0.30a
	Test group	12.6± 0.09a	12.2± 0.71 a	30.32± 0.50a
21 Day	control	12.7± 0.83a	12.7± 0.60 a	30.61± 0.11a
	Test group	12.3± 0.89a	12.4± 0.13 a	31.22± 0.80a

Different small letters represent the significant differences (P<0.05).

Leukogram results: The results of Leukogram Total and differential Leukocyte count are shown in table 2.

Total Leukocytic count: The Levamisole induce significant increase (P<0.05) in the test group in the 2nd and 3rd weeks compared with the control group.

Differential leukocyte count:

Neutrophil cells: The results show significant increase (P<0.05) in the test group in the 2nd and 3rd weeks compared with the control group.

Lymphocyte cells: The results of Lymphocyte cells show there is insignificant increase in the test group in the 21 Day as compared with control group in same day.

Eosinophil cells: The results showed significant decrease in Eosinophil cells in the 14, 21 days in the test group compared with control group.

There is no significant change in the Basophil cells in the all groups.

Monocyte cells: The results showed significant increase (P<0.05) in the

monocyte in the day 21 as compared with control groups.

Our study results showed that Levamisole induced significant increase in the TLC therefore cause leukocytosis in the 14 and 21 days, neutrophilia, lymphocytosis and Monocytosis.

Elevation in leukocytes after treatment with levamisole was supported by [9,24] mentioned that levamisole induced leukocytosis in dairy cows and this elevation may be due to increase in lymphocytes and monocytes. These results agreed with (21) that lymphocytes, monocytes and eosinophil were significantly increased after administration of Levamisole. Some researcher demonstrated that Levamisole enhances the innate immune response as it does with the acquired response (acting as an adjuvant) [10,19].

Table 2 Leukogram sheep treated with levamisole(n=10) Absolute differential count (10³/cmm)

TIME/DAY	Group	TLC	N	L	M	E	B
0 Day	control	7.69±0.95a	2.28±0.764	4.210±0.43626a	0.535±0.17841a	0.885±0.13316a	0.800±0.12274a
	Test group	8.94±0.95a	2.380±0.764a	4.910±0.43626a	0.635±0.17827a	0.885±0.12316a	0.885±0.10574a
7Day	control	8.60±0.95a	2.012±0.784a	4.310±0.43626a	0.431±0.478a	1.885±0.14316a	0.985±0.14374a
	Test group	8.30±0.98a	2.500±0.85	4.700±0.44845a	0.850±0.245a	0.871±0.23469a	0.750±0.10574a
14 Day	control	7.94±0.95 a	3.412±0.764a	4.110±0.436a	0.736±0.57823a	0.986±0.153a	0.875±0.1647a
	Test group	11.39±0.58b	4.001±1.13 b	5.220±0.470a	0.898±0.270a	0.871±0.235a	0.852±0.2409a
21 Day	control	8.64±0.95a	3.412±0.713a	4.810±0.43626a	0.515±0.67814a	0.855±0.15316a	0.865±0.18574a
	Test group	12.20±0.71b	4.197±1.31 b	5.515±0.31935a	1.380±0.20591b	0.725±0.12024a	0.438±0.08113a

Different small letters represent the significant differences (P<0.05).

Total serum protein:

The results of total serum protein ,globulin and albumin is shown in the table 3 indicates insignificant increase in the total plasma protein ,globulin compared with control group in the Day 21 while no significant change in all control and test group in the day 0,7 and Days 14.The result of albumin show significant decrease in the 21days as compensatory mechanism duo to increases globulin and insignificant Decrease in the total protein in the Day 7.

In the present experiment, a significant increase in total serum protein, globulin at day21 due to improving immunity and increase total leukocytic count and antibodies.

Same effect was obtained by [11], these obtained results nearly agree with

those observed by [34] who found an increased in total protein following levamisole treatment. Also, [12,14,22] found that levamisole caused an increase in immunoglobulin and immunity of the animals due to increase in globulin.

On the other hand, the above mentioned results were also supported by the previous studies [13] mentioned that rats treated with levamisole show significant increase in total serum protein and globulin and such findings may be due to the ability the levamisole to enhance both cellular and humeral immune responses to restore depressed immune function through stimulating antibody formation and enhance T-cell response by stimulating T- cell activation and proliferation.

Table 3 Total Protein,albumin and globulin profile of sheep treated with levamisole (n=5)

Time/Day	0 Day		7 Day		14 Day		21 Day	
	control	Test group	control	Test group	control	Test group	control	Test group
Total protein	59.038±	58.038±	52.438±	59.275±	57.037±	60.688±	60.148±	61.312± 4.77277b
	2.651a	2.65167a	2.65167a	5.33756a	2.65167a	3.26687a	2.65167a	
Albumin	28.698±	29.698±	27.697±	26.849±	30.98±	30.988±	28.618±	26.275± 0.94199b
	1.04217a	3.04207a	0.04407a	2.48141a	2.88988a	2.88988a	1.04217a	
Globulin	30.344±	30.940±	29.541±	30.427±	29.945±	30.427±	32.342±	35.037± 4.98537b
	5.11064a	5.10064a	5.13064a	5.10064a	5.12064a	4.80292a	5.14064a	

Different small letters represent the significant differences (P<0.05).

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