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# Effects of orally administration free and Liposomal Levamisole on hematological and biochemical parameters in Sheep

## Efecto de la administración oral de levamisol libre y liposomal sobre parámetros hematológicos y bioquímicos en ovejas

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## ABSTRACT

Analysis of haematological and biochemical parameters monitors animal health and guides diagnosis and treatment. This study compared the haematological and biochemical effects of free and liposomal levamisole given to sheep orally. The study included twenty-one female Curly breed sheep. The animals were divided into three groups: free levamisole (7.5 mg/kg), liposomal levamisole (7.5 mg/kg), and control (physiological serum 7.5 mL/kg). Blood samples were obtained on day (d) 0 (control), d 1, and d 3. Haematological parameters (WBC, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD, and PCT) were assessed utilizing a haematology analyzer, while biochemical parameters (urea, creatinine, AST, ALT, BUN) were evaluated using an autoanalyzer. On d 1, the liposome group exhibited the highest white blood cell measurements. Despite a reduction in PCT values on d 1 within the liposome group, an increase was observed again on d 3. Urea levels on d 1 and 3 were elevated in the control, liposomal levamisole, and free levamisole groups. On d 3, creatinine measurements indicated that levels in the control group were significantly elevated compared to those in the liposomal levamisole and free levamisole groups. On d 3, BUN measurements indicated that the mean for the control group was significantly elevated compared to the liposomal levamisole and free levamisole groups. The Neutrophil, Lymphocyte, and Monocyte counts in the liposomal and free levamisole groups of animals were significantly elevated compared to other measurements recorded on the d 3. This study's findings demonstrate that liposomes affect haematological and biochemical parameters. The results demonstrate that liposomal levamisole causes no harmful effects on animals. It produces advantageous results. Further investigation is necessary to elucidate the effects of Liposomal Levamisole on hematological and biochemical parameters among various animal species.

**Key words:** Biochemical; haematological; levamisole; liposome; sheep

## RESUMEN

El análisis de los parámetros hematológicos y bioquímicos facilita el monitoreo de la salud animal y guía el diagnóstico y tratamiento. Este estudio comparó los efectos hematológicos y bioquímicos del levamisol libre y liposomal administrado por vía oral an ovejas. Veintiuna ovejas hembras de la raza Curly participaron en el estudio. Los animales fueron clasificados en tres grupos: levamisol libre (7,5 mg/kg), levamisol liposomal (7,5 mg/kg) y control (solución salina 7,5 mL/kg). Se recolectaron muestras de sangre en el día (d) 0 (control), el d 1 y el d 3. Los parámetros hematológicos (WBC, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD y PCT) fueron analizados mediante un analizador hematológico, mientras que, los parámetros bioquímicos (urea, creatinina, AST, ALT, BUN) fueron evaluados con un autoanalizador. En el primer día, el grupo del liposoma exhibió las mediciones más elevadas de Leucocitos. A pesar de una disminución en los valores de PCT en el d 1 en el grupo de liposomas, se registró nuevamente un incremento en el d 3. Los niveles de urea en los d 1 y 3 fueron elevados en los grupos de control, levamisol liposomal y levamisol libre. En el tercer d, las mediciones de creatinina revelaron que los niveles en el grupo de control eran significativamente más altos en comparación con los grupos de levamisol liposomal y levamisol libre. El d 3, las mediciones de BUN revelaron que la media del grupo de control era significativamente superior en comparación con los grupos de levamisol liposomal y levamisol libre. Los recuentos de neutrófilos, linfocitos y monocitos en los grupos de animales tratados con levamisol liposomal y libre fueron significativamente superiores en comparación con otras mediciones registradas el tercer día. Las conclusiones de este estudio evidencian que los liposomas influyen en los parámetros hematológicos y bioquímicos. Los resultados evidencian que el levamisol liposomal no produce efectos adversos en los animales. Generar resultados favorables. Es imperativo continuar la investigación para esclarecer los efectos del levamisol liposomal en los parámetros hematológicos y bioquímicos de diversas especies animales.

**Palabras clave:** Bioquímica; hematológica; levamisol; liposoma; oveja

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## INTRODUCTION

Levamisole is an anthelmintic that is used against in the treatment of lung and gastrointestinal nematode infections in both humans and animals [1]. Levamisole can be administered orally, intramuscularly, subcutaneously, parenterally, and transdermally. A variety of formulations has been developed to deliver the drug through multiple routes. These comprise tablets, oblets, wettable powder, and solutions for injection, pouring, or dripping. Upon oral administration, the drug is swiftly absorbed from the gastrointestinal tract and disseminated throughout the body. Approximately 95% of levamisole undergoes biotransformation in the body and is excreted predominantly in feces and urine. The plasma half-life of the drug is 4 to 6 hours (h) in ruminants and 1.8 to 2 h in canines [2]. Approximately 40% of the drug is eliminated in the urine within 12 h post-oral administration. Thereafter, the urinary excretion rate diminishes, with roughly 8% excreted within an 8-h timeframe [2]. The elimination of the drug via feces is a process that exceeds eight d. Approximately 40% of the drug is eliminated from the body in this manner, with a substantial portion occurring within the first 12-24 h [2]. Approximately one percent of the administered levamisole is detected in the kidneys and liver within the initial 12 to 24 h. Levamisole is administered to cattle (*Bos taurus*), sheep (*Ovis aries*), and goats (*Capra hircus*) at doses of 7.5 mg/kg for oral use and 3.5-8 mg/kg for intramuscular and subcutaneous administration [3 , 4].

The immunomodulatory and stimulatory effects of levamisole on the immune system are well recognized; however, the exact mechanism by which this occurs remains uncertain. Research indicates that stimulating phagocytes sensitizes them to mitogens and antigens, subsequently augmenting the quantity of T cells. Levamisole reportedly does not directly affect B lymphocytes; however, it indirectly stimulates the humoral immune response by influencing T lymphocytes and phagocytic cells, leading to elevated antibody levels [5].

Liposomes represent a substantial advancement in drug delivery systems. Considering that liposomes are deemed the premier carriers for the administration of various agents, such as anticancer drugs, antibiotics, anti-inflammatory, antiparasitic, and antifungal agents, research on liposomes has gained prominence in the pharmaceutical, biological, and medical fields. Liposomes are the first closed microscopic phospholipid sheet systems, which were approved in 1965 [6 , 7 , 8].

Liposomes possess considerable potential as drug delivery systems owing to their biocompatibility and modular characteristics. Moreover, they provide the significant benefit of diminishing the risk of systemic toxicity associated with the encapsulated therapeutic agent. Specifically, liposomes can engage with plasma proteins, resulting in opsonization and consequently modifying the healthy cells they encounter during circulation and elimination [9 , 10]. Moreover, the pharmacokinetics of circulating liposomes may lead to the sequestration of drugs within the organs of the mononuclear phagocyte system, potentially affecting liver and spleen functionality. Liposomal agents can either stimulate or suppress the immune system, depending on their physicochemical properties such as size, lipid composition, pegylation, and surface charge [11 , 12].

Biochemical and hematological blood parameters are essential indicators for evaluating the health status of humans and animals. Certain biological parameters, detected in blood or other biological fluids, are essential for the initial evaluation of potential tissue and organ damage. Haematological parameters, such as leukocytes, erythrocytes, haemoglobin, haematocrit, and platelets, are primarily linked to bone marrow function but are also evaluated as indicators of infection and electrolyte imbalances [13 , 14]. The assessment of aspartate aminotransferase and alanine aminotransferase levels determines the degree of damage to the liver and bile ducts. The assessment of blood urea nitrogen and creatinine levels is utilized to determine the degree of damage to the kidneys [15 , 16].

There is no literature available regarding the impact of free and liposomal levamisole on hematological parameters, specifically (white blood cells (WBC) count, The red blood cells (RBC) count, hemoglobin (HGB) concentration, Hematocrit Value (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), Red Cell Distrubition (RDW-CV), Platelet Value (PLT), Mean platelet volume (MPV), Platelet Distribution Width (PDW), Procalcitonin (PCT), Platelet Large Cell Ratio (P-LCR), platelet activation (P-LCC)) and biochemical parameters (plasma alanine transaminase (ALT), aspartate transaminase (AST), creatinine (Cr), urea (U), blood urea nitrogen (BUN)) in sheep.

The aim of this study was to determine the effects of free and liposomal levamisole on hematological and biochemical parameters in sheep at a standard dosage.

## MATERIALS AND METHODS

### Animals

The research involved 21 female Merino of the origin of Türkiye (*Ovis aries*), aged one to two years, with an average weight of 60 kg, selected randomLy. The sheep were relocated to various pens ten days before the study began to aid their acclimatization to the research environment. They were housed in these pens for the duration of the study. The animals received specialized feed tailored (Energy Sources (Cereals): 50-60%, Protein Sources: 12-16%, Fibre Sources (Roughage): 30-40%, Mineral and Vitamin Premixes: 1-2%, Metabolic Energy (ME): 2.5-3 Mcal/kg, Crude Protein (HP): 12-14%, Calcium: 0.8-1%, Phosphorus: 0.4-0.6%, Salt: 0.5-1%, Vitamin A: 4000-5000 IU/kg, Vitamin D: 800-1000 IU/kg, Vitamin E: 20-40 IU/kg, Crude Cellulose: 15-20%, Fat (Crude Oil): 3-5%) to their weight and age. Hay and water were provided ad libitum.

### Experimental protocol

In this study, liposomes were prepared with special techniques and equipment [17]. A total of 21 female sheep, each weighing 60 kg, were randomly allocated to one of three groups, comprising seven animals per group. In the first group (n=7), a dose of free levamisole at 7.5 mg/kg was administered orally to the sheep; in the second group (n=7), liposomal levamisole at 7.5 mg/kg was administered orally; and in the third group (n=7), serum physiological at 7.5 mL/kg was administered orally to the sheep. The drug was administered as a single dose. Prior to administration, 5 mL blood samples were obtained in Tripotassium Ethylenediaminetetraacetic acid (K3EDTA) and gel tubes on d 0 (Control) and subsequently on d 1 and 3. The analysis of haematological parameters was conducted

promptly (15 min) after the collection of blood samples. Blood samples designated for biochemical analysis were subjected to centrifugation (ALLEGRA–X64R, USA, 3500 G for 15 min), after which the serum was isolated and transferred into 2 mL Eppendorf tubes, subsequently stored in a deep freezer (Arçelik 270530EB, Türkiye) at -80°C until the analysis date.

### Analysis of haematological and biochemical parameters

Hematological parameters were assessed using a Veterinary blood counting device (HASVET VH3 Automatic Hematology Analyzer, Türkiye) from samples collected in K3EDTA tubes. Biochemical analyses were conducted using the RX Daytona+ fully automated veterinary biochemistry apparatus (HASVET, Türkiye) on serum samples thawed at ambient temperature.

### Ethical aspects of the research

The research procedure was conducted with the approval of the Balıkesir University Animal Experiments Local Ethics Committee (Date: 28.11.2024, Decision Number: 2024/11-1).

### Statistical analysis

Friedman's test was applied for intra-group comparisons, while the Kruskal-Wallis test was applied for inter-group comparisons in the statistical analysis of hematological and biochemical parameter results. A p-value below 0.05 indicates that the difference is statistically significant [18].

## RESULTS AND DISCUSSIONS

In this study, the effects of different forms of levamisole on hematological and biochemical parameters were examined in detail. The findings indicate that levamisole formulation leads to significant differences in certain parameters. This study reported that oral administration of free and liposomal levamisole to sheep at a dosage of 7.5 mg/kg did not result in any adverse effects in the animals. The reference intervals of haematological and biochemical parameters in sheep are presented in TABLE I [19]. The haematological and biochemical parameters of sheep are displayed in TABLE II and III, respectively.

A statistically significant difference was observed between the groups regarding the d 1 WBC measurements (P<0.05). The mean WBC on d 1 in the control group (0.83±0.24) was significantly lower compared to that in the liposomal levamisole group (4.63±4.34) and the free levamisole group (1.46±0.37). In the liposomal levamisole group, white blood cell (WBC) values were observed to increase significantly on the d 1. This may be related to the potential effects of liposomal carrier systems on immune modulation. However, by the d 3, a balancing in WBC values was observed, suggesting that the immune system is regulated by a homeostatic mechanism. On d 1, a statistically significant difference in PCT values was observed between the groups (P<0.05). The mean PCT of the control group animals on d 1 (0.38±0.10) was significantly higher compared to that of the animals in the liposomal levamisole group (0.23±0.13) and the free levamisole group (0.24±0.08). On d 3, a statistically significant difference was observed between the groups regarding PCT measurements (P<0.05). The mean PCT values on d 3 for the control group (0.30±0.07) were higher compared to those for the liposomal levamisole group (0.29±0.07) and the free levamisole group (0.20±0.07). Regarding PCT values, although a decrease was observed in the liposomal levamisole group on the d 1, an increase was recorded again on the d 3. This change may be associated with an inflammatory response and requires further investigation. A statistically significant difference was identified in the MCV values recorded on d 0, 1, and 3 for the control group animals (P<0.05). The value recorded on d 3 was significantly higher compared to the other values. A statistically significant difference was identified in the MCV values recorded on d 0, 1, and 3 for the animals in the liposomal levamisole group (P<0.05). The value on d 3 demonstrated a significant increase relative to the other values.

The results indicated no statistically significant difference in intra and inter-group comparisons concerning the remaining variables (P>0.05).

**TABLE I. Reference values of some haematological and biochemical parameters in sheep [19]**

| Parameters | Reference range |
|---|---|
| WBC (10^9/L) | **5,10-15,80** |
| Lym% | **40-75** |
| Neu% | **10-50** |
| Mono% | **0-6** |
| Eos% | **0-10** |
| RBC (10^12/L) | **5,50-14,20** |
| HGB (g/L) | **63- 132** |
| HCT | **0,200- 0,390** |
| MCV (fL) | **25,0- 41,0** |
| MCH (pg) | **8,0-12,3** |
| MCHC (g/L) | **290- 360** |
| RDW-CV (%) | **11,0-17,0** |
| RDW-SD (fL) | **20,0-35,0** |
| PLT (10^9/L) | **100 -800** |
| MPV (fL) | **3,5 -6,0** |
| PDW-CV (%) | **0,133- 0,185** |
| PDW-SD (fL) | **12,0- 17,5** |
| PCT (%) | **0,50- 4,20** |
| P-LCC (10^9/L) | **15 -278** |
| P-LCR (%) | **0,150- 0,650** |
| Urea (mg/dL) | **8-30** |
| Creatinine (mg/dL) | **0,6-1,2** |
| BUN (mg/dL) | **10-30** |
| ALT (U/L) | **26-34** |
| AST (U/L) | **60-280** |

U/L: Units per liter, mg: milligram, dL: deciliter, fL: femtoliters, (g/L): grams per liter, pg: picograms, WBC: White blood cell, Lym: Lymphocyte, Neu: Neutrophil, Mono: Monocyte, Eos: Eosinophil, RBC: Red blood cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW-CV: Red cell distribution width-coefficient of variation, RDW-SD: Red cell distribution width-standard deviation, PLT: Platelet count, MPV: Mean platelet volume, PDW-CV: Platelet distribution width-coefficient of variation, PDW-SD: Platelet distribution width-standard deviation, PCT; Procalcitonin, P-LCC: Platelet large cell coefficient, P-LCR: Platelet large cell ratio, BUN: Blood urea nitrogen, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase.

**TABLE II. Intragroup and intergroup comparisons of hemogram values**

| | | Control | | Liposomal Levamisole | | Free Levamisole | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean±SD | Median (Min-Max) | Mean±SD | Median (Min-Max) | Mean±SD | Median (Min-Max) | H | p |
| WBC (10^9/L) | Day 0 | 1.13±0.77 | 0.90 (0.50-2.70) | 1.53±0.88 | 1.30 (0.60-3.10) | 0.89±0.35 | 10 (0.40-1.30) | 2.433 | 0.296 |
| | Day 1 | 0.83±0.24 | 0.80 (0.50-1.10) | 4.63±4.34 | 3.30 (0.10-11.7) | 1.46±0.37 | 1.50 (0.90-1.80) | 7.897 | **0.019** |
| | Day 3 | 1.61±0.85 | 1.70 (0.70-2.70) | 1.81±0.75 | 1.60 (1.30-3.50) | 1.70±0.79 | 1.30 (0.70-2.90) | 0.237 | 0.888 |
| | | F=2.741 | p=0.254 | F=1.143 | p=0.565 | F=5.429 | p=0.066 | | |
| RBC (10^12/L) | Day 0 | 9.38±1.25 | 9.69 (7.88-11.22) | 9.48±1.56 | 9.39 (8.03-12.4) | 9.48±0.52 | 9.34 (8.75-10.3) | 0.096 | 0.953 |
| | Day 1 | 9.02±1.21 | 9.18 (7.15-10.81) | 9.76±3.53 | 9.29 (3.36-13.7) | 9.98±0.82 | 9.91 (9.18-11.3) | 2.111 | 0.348 |
| | Day 3 | 9.26±0.95 | 9.53 (7.91-10.32) | 8.26±0.88 | 8.32 (7.07-9.75) | 9.36±1.09 | 9.18 (7.74-11.1) | 4.219 | 0.121 |
| | | F=1.143 | p=0.565 | F=3.714 | p=0.156 | F=3.714 | p=0.156 | | |
| HGB (g/L) | Day 0 | 9.60±1.56 | 9.50 (7.80-11.60) | 10.09±2.24 | 9.90 (7.80-14.4) | 9.91±0.70 | 10.10 (9.10-10.7) | 0.424 | 0.809 |
| | Day 1 | 9.35±1.51 | 9.40 (7.20-12) | 11.11±4.56 | 9.90 (3.40-16.2) | 10.76±1.07 | 11.10 (9.40-12) | 2.291 | 0.318 |
| | Day 3 | 9.76±1.09 | 10.10 (8.50-11.30) | 9.06±1.13 | 8.90 (7.30-10.8) | 10.09±1.19 | 9.90 (8.50-11.7) | 2.498 | 0.287 |
| | | F=2.000 | p=0.368 | F=1.143 | p=0.565 | F=3.714 | p=0.156 | | |
| HCT | Day 0 | 33.36±4.45 | 34.30 (28.20-39.0) | 35.21±6.88 | 35.30 (28.1-48.9) | 33.93±2.21 | 34.10 (30.9-37.4) | 0.067 | 0.967 |
| | Day 1 | 32.64±4.92 | 33.30 (25.60-40.1) | 37.94±15.55 | 33.20 (12-55.20) | 36.70±3.77 | 37.80 (31.6-40.6) | 1.244 | 1.785 |
| | Day 3 | 34.60±3.98 | 36 (29.90-39.1) | 32.17±3.35 | 31.60 (27.3-36.6) | 34.90±3.66 | 35.40 (30.1-40.5) | 1.785 | 0.410 |
| | | F=0.857 | p=0.651 | F=0.667 | p=0.717 | F=1.143 | p=0.565 | | |
| MCV (fL) | Day 0 | 35.60±0.84 | 35.50 (34.50-37) | 37.07±1.35 | 36.90 (35.1-39.4) | 35.49±1.33 | 35.30 (33.9-37.9) | 5.138 | 0.077 |
| | Day 1 | 36.20±1.26 | 35.90 (34.4-38.1) | 38.34±3.28 | 37.80 (35.2-44.3) | 36.86±3.07 | 36.10 (34.1-43.1) | 1.806 | 0.405 |
| | Day 3 | 37.40±1.14 | 37.20 (35.9-39.4) | 39.07±2.64 | 38.70 (36.4-44.1) | 37.39±1.79 | 37.10 (35.1-40.5) | 2.601 | 0.272 |
| | | F=7.185 | p=**0.028** | F=8.000 | p=**0.018** | F=5.429 | p=0.066 | | |
| MCH (pg) | Day 0 | 10.16±0.58 | 10 (9.80-11.40) | 10.51±0.60 | 10.40 (9.7-11.5) | 10.23±0.25 | 10.30 (9.9-10.6) | 2.560 | 0.278 |
| | Day 1 | 10.31±0.46 | 10.30 (9.70-11.10) | 11.13±0.98 | 11.10 (10.1-12.9) | 10.74±0.83 | 10.40 (10-12.40) | 3.381 | 0.184 |
| | Day 3 | 10.47±0.26 | 10.50 (10.2-10.9) | 10.89±0.50 | 10.70 (10.3-11.8) | 10.71±0.42 | 10.50 (10.30-11.40) | 3.369 | 0.186 |
| | | F=2.571 | p=0.276 | F=4.692 | p=0.096 | F=4.000 | p=0.135 | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| MCHC (g/L) | Day 0 | 28.67±1.35 | 27.80 (27.6-31.0) | 28.49±1.18 | 28 (27.7-30.8) | 29.11±0.73 | 29.40 (28-29.90) | 2.723 | 0.256 |
| | Day 1 | 28.66±1.03 | 29.10 (27.2-29.9) | 29.20±0.48 | 29.30 (28.3-29.8) | 29.29±0.41 | 29.30 (28.8-29.8) | 1.269 | 0.530 |
| | Day 3 | 28.19±0.67 | 28.40 (26.9-28.9) | 28.06±1.15 | 27.80 (26.7-29.7) | 28.84±1.06 | 28.30 (27.9-30.7) | 1.496 | 0.473 |
| | | F=1.407 | p=0.495 | F=6.000 | p=0.050 | F=2.000 | p=0.368 | | |
| RDW-CV (%) | Day 0 | 15.89±0.65 | 16.10 (14.8-16.7) | 16.07±0.56 | 16 (15.3-16.9) | 15.70±0.71 | 15.80 (14.7-16.4) | 1.013 | 0.603 |
| | Day 1 | 15.81±0.67 | 15.60 (15-16.80) | 15.21±0.42 | 15.10 (14.9-16.1) | 15.97±0.92 | 16.20 (14.5-17.1) | 4.945 | 0.084 |
| | Day 3 | 15.91±0.60 | 15.70 (15.20-17) | 15.66±0.50 | 15.90 (14.7-16.1) | 16.26±0.81 | 15.90 (15.3-17.8) | 1.484 | 0.476 |
| | | F=1.556 | p=0.459 | F=6.000 | p=0.050 | F=2.000 | p=0.368 | | |
| PLT (10^9/L) | Day 0 | 693.57±181.88 | 704 (417-903) | 652.29±153.14 | 652 (442-818) | 515.57±189.98 | 538 (197-716) | 3.058 | 0.217 |
| | Day 1 | 786±207.42 | 800 (485-1086) | 530±313.28 | 533 (209-1040) | 493.43±207.79 | 529 (259-757) | 4.695 | 0.095 |
| | Day 3 | 664.29±121.21 | 685 (495-771) | 641.29±219.80 | 692 (342-946) | 443.14±159.89 | 418 (244-715) | 5.450 | 0.066 |
| | | F=1.143 | p=0.565 | F=2.571 | p=0.276 | F=0.286 | p=0.867 | | |
| MPV (fL) | Day 0 | 4.90±0.73 | 4.90 (3.90-6) | 4.54±0.25 | 4.50 (4.30-5) | 4.87±0.98 | 4.60 (4.10-7) | 0.788 | 0.674 |
| | Day 1 | 4.97±0.58 | 5 (4.10-5.90) | 4.63±0.41 | 4.50 (4.20-5.40) | 5.23±1.05 | 4.90 (4-6.90) | 1.815 | 0.403 |
| | Day 3 | 4.66±0.44 | 4.50 (4.10-5.20) | 4.79±0.50 | 4.80 (4.10-5.40) | 4.64±0.25 | 4.60 (4.30-5) | 0.275 | 0.872 |
| | | F=1.143 | p=0.565 | F=0.308 | p=0.857 | F=2.296 | p=0.317 | | |
| PDW (fL) | Day 0 | 6.81±1.50 | 6.80 (4.90-8.90) | 5.97±0.59 | 5.80 (5.50-7.10) | 6.37±1.61 | 5.80 (5.20-9.90) | 0.919 | 0.632 |
| | Day 1 | 6.77±1.34 | 6.80 (4.90-8.90) | 6.31±0.99 | 5.80 (5.20-7.70) | 6.30±2.12 | 6.80 (3.10-9.90) | 0.510 | 0.487 |
| | Day 3 | 6.10±0.92 | 5.80 (4.90-7.40) | 6.54±1.58 | 5.80 (4.90-9.60) | 6.40±0.67 | 6.50 (5.20-7.40) | 0.487 | 0.784 |
| | | F=0.286 | p=0.867 | F=1.000 | p=0.607 | F=0.857 | p=0.651 | | |
| PCT (%) | Day 0 | 0.33±0.09 | 0.37 (0.20-0.44) | 0.29±0.07 | 0.31 (0.20-0.37) | 0.25±0.08 | 0.28 (0.08-0.32) | 2.249 | 0.325 |
| | Day 1 | 0.38±0.10 | 0.42 (0.24-0.50) | 0.23±0.13 | 0.23 (0.10-0.43) | 0.24±0.08 | 0.24 (0.15-0.39) | 7.046 | **0.030** |
| | Day 3 | 0.30±0.07 | 0.32 (0.21-0.38) | 0.29±0.07 | 0.32 (0.18-0.38) | 0.20±0.07 | 0.20 (0.12-0.32) | 7.069 | **0.029** |
| | | F=1.143 | p=0.565 | F=2.571 | p=0.276 | F=1.143 | p=0.565 | | |
| P-LCR (%) | Day 0 | 0.27±0.72 | 0 (0-1.90) | 0.00±0.00 | 0 (0-0) | 2.51±6.65 | 0 (0-17.60) | 1.057 | 0.589 |
| | Day 1 | 0.39±0.69 | 0 (0-1.70) | 0.69±1.42 | 0 (0-3.80) | 5.21±8.41 | 0 (0-18.70) | 0.959 | 0.619 |
| | Day 3 | 0.16±0.42 | 0 (0-1.10) | 1.46±2.17 | 0 (0-5.70) | 0.00±0.00 | 0 (0-0) | 4.594 | 0.101 |
| | | F=0.500 | p=0.779 | F=2.800 | p=0.247 | F=3.500 | p=0.174 | | |
| P-LCC (10^9/L) | Day 0 | 1.86±4.91 | 0 (0-13) | 0.00±0.00 | 0 (0-0) | 8.29±21.92 | 0 (0-58) | 1.057 | 0.589 |
| | Day 1 | 2.71±5.09 | 0 (0-13) | 2.00±3.61 | 0 (0-9) | 16.86±25.24 | 0 (0-58) | 1.161 | 0.560 |
| | Day 3 | 1±2.65 | 0 (0-7) | 6.43±9.24 | 0 (0-24) | 0.00±0.00 | 0 (0-0) | 4.594 | 0.101 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | F=0.500 | p=0.779 | F=2.800 | p=0.247 | F=3.500 | p=0.174 | | |
| Neutrophills | Day 0 | 20.70±1.26 | 20.60 (19.3-22.4) | 21.50±1.37 | 21.40 (19.6-22.3) | 22±001.10 | 20.00 (20.3-24.1) | 4.124 | 0.088 |
| | Day 1 | 20.50±1.14 | 20.30 (20.1-22.3) | 31.60±1.24 | 31.10 (28.7-33.4) | 20.60±1.66 | 20.70 (20.1-21.9) | 1.223 | **0.030** |
| | Day 3 | 20.40±1.35 | 20.10 (18.5-22.6) | 32.70±1.64 | 32.30 (31.8-32.6) | 20.50±1.14 | 22.40 (19.5-22.1) | 2.401 | **0.028** |
| | | F=1.164 | p=0.578 | F=6.000 | **p=0.017** | F=2.644 | **p=0.032** | | |
| Lympho-cytes | Day 0 | 52.40±1.73 | 52.10 (48.7-55.2) | 53.50±1.61 | 53.10 (48.7-56.2) | 52.50±2.04 | 53.40 (52.6-56.4) | 3.126 | 0.125 |
| | Day 1 | 52.50±1.64 | 52.40 (50.3-53.8) | 65.40±2.10 | 65.50 (58.3-69.6) | 53.30±2.11 | 53.50 (52.1-55.8) | 5.154 | **0.043** |
| | Day 3 | 50.10±1.26 | 50.40 (50.1-51.6) | 70.60±1.36 | 70.20 (66.8-73.4) | 53.60±1.68 | 52.40 (50.2-53.5) | 5.076 | **0.035** |
| | | F=3.195 | p=0.074 | F=2.441 | **p=0.021** | F=1.144 | **p=0.043** | | |
| Monocytes | Day 0 | 1.30±0.62 | 1.20 (0.90-2.10) | 1.60±1.01 | 1.40 (1.20-2) | 2.40±0.86 | 2.30 (1.50-3.30) | 2.129 | 0.136 |
| | Day 1 | 1.80±0.81 | 1.60 (1.20-1.90) | 4.20±1.04 | 2.30 (1.60-3.10) | 1.10±1.24 | 1.70 (1.60-2.30) | 6.074 | **0.038** |
| | Day 3 | 1.60±1.03 | 1.50 (1-1.60) | 6.00±1.14 | 4.70 (3.40-6.60) | 3.70±0.86 | 2.70 (1.90-4.20) | 5.854 | **0.026** |
| | | F=4.574 | p=0.765 | F=2.741 | **p=0.028** | F=3.496 | **p=0.035** | | |
| Eosinophils | Day 0 | 4.40±1.37 | 4.20 (3.10-5.60) | 6.60±1.05 | 6.30 (5.80-7.40) | 6.50±0.74 | 6.40 (5.70-7.70) | 3.805 | 0.064 |
| | Day 1 | 4.80±1.58 | 4.50 (3.90-6.10) | 4.50±0.74 | 4.10 (4-5.30) | 5.00±1.23 | 5.00 (4.80-6.20) | 2.259 | 0.191 |
| | Day 3 | 3.30±0.84 | 3.20 (3.10-4.80) | 3.60±0.86 | 3.50 (3.30-4.20) | 3.70±0.84 | 3.80 (3.50-4.90) | 3.716 | 0.225 |
| | | F=2.000 | p=0.373 | F=1.153 | p=0.675 | F=2.364 | p=0.125 | | |

F: Firedman Test, H: Kruskal-Wallis H Test, P<0.05, SD: Standart deviation, Min: Minumum, Max: Maximum, WBC: White blood cell, RBC: Red blood cell, HGB: Haemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW-CV: Red blood cell distribution width, PLT: Platelet count, MPV: Mean platelet volume, PDW: Platelet distribution width, PCT: Procalcitonin, P-LCR: Platelet large cell ratio, P-LCC: Platelet large cell coefficient

The results from d 1 of urea measurements demonstrated a statistically significant difference between the groups (P<0.05). The mean urea level on d 1 was significantly elevated in the control group (52.43±7.55) compared to the liposomal levamisole group (43.71±4.82) and the free levamisole group (46.14±5.34). On d 3 of the study, a statistically significant difference was found between the groups (P<0.05). The mean urea level on d 3 was significantly elevated in the control group (52.43±6.45) compared to the liposomal levamisole group (50.00±3.11) and the free levamisole group (45.43±2.37).

A statistically significant difference was found in the urea values of the animals in the liposomal levamisole group on d 0, 1, and 3 (P<0.05). On d 1 the recorded value was markedly lower than the subsequent tests. On d 3, a statistically significant difference in creatinine values was found between the groups (P<0.05). The mean creatinine level on d 3 of the study was significantly elevated in the control group (0.67±0.05) in comparison to both the liposomal levamisole group (0.56±0.05) and the free levamisole group (0.57±0.02). The analysis of the intragroup values demonstrated a statistically significant disparity among the three measurements across all three groups (P<0.05). The minimum creatinine levels were recorded in all three groups on d 3. Similarly, significant differences were detected in urea and creatinine levels between the groups. In particular, the significantly higher creatinine levels in the control group on the d 3 suggest the potential effects of levamisole on kidney function. This finding indicates the need for further research on the elimination mechanisms of levamisole.

On d 3 of the study, a statistically significant difference was observed between the groups regarding BUN values (P<0.05). The mean BUN value on d 3 for the control group (23.88±2.57) was significantly higher than that recorded for the liposomal levamisole group (23.29±1.28) and the free levamisole group (21.32±1.12). A statistically significant difference was identified in the BUN values of the animals in the liposomal levamisole group on d 0, 1, and 3 (P<0.05). On d 1, a markedly lower value was recorded. A statistically significant difference was found in the AST values of the control group animals on d 0, 1, and 3 (P<0.05). The data collected on day 0 exhibited markedly higher levels than those recorded on d 1 and 3. A statistically significant difference was observed between the groups in the ALT values on d 1 (P<0.05).

The mean ALT value on the initial observation d for the control group (14.57±3.46) was significantly lower than that

of the liposomal levamisole group (20.14±2.85) and the free levamisole group (17.43±4.43). A statistically significant difference was found among d 0, d 1, and d 3 counts of neutrophils, lymphocytes, and monocytes in both the liposomal and free levamisole groups (P<0.05). The measurement on d 3 demonstrated a significantly higher value compared to the other measurements. No statistically significant differences were found in the other variables when comparing intra- and inter-group data (P>0.05) (TABLE III). Significant increases were observed in neutrophil, lymphocyte, and monocyte counts in the liposomal and free levamisole groups. This increase is thought to be associated with the stimulatory effects on the immune system. In this context, analyzing similar parameters in different animal species will contribute to a better understanding of the biological effects of levamisole.

**TABLE III. Intragroup and intergroup comparisons of biochemical parameters**

| | | Control | | Liposomal Levamisole | | Free Levamisole | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean±SD | Median (Min-Max) | Mean±SD | Median (Min-Max) | Mean±SD | Median (Min-Max) | H | p |
| Urea | Day 0 | 45.71±3.99 | 45 (40-52) | 50.29±7.36 | 52 (40-62) | 47.71±5.65 | 49 (37-53) | 2.265 | 0.322 |
| | Day 1 | 52.43±7.55 | 51 (42-67) | 43.71±4.82 | 45 (35-49) | 46.14±5.34 | 46 (38-56) | 6.714 | **0.035** |
| | Day 3 | 52.43±6.45 | 49 (47-63) | 50.00±3.11 | 49 (46-55) | 45.43±2.37 | 46 (42-49) | 8.922 | **0.012** |
| | | F=4.667 | p=0.097 | F=8.074 | p=**0.018** | F=0.963 | p=0.618 | | |
| Creatinine | Day 0 | 0.78±0.08 | 0.76 (0.99-0.91) | 0.78±0.06 | 0.78 (0.67-0.84) | 0.83±0.06 | 0.84 (0.76-0.89) | 2.051 | 0.359 |
| | Day 1 | 0.81±0.06 | 0.81 (0.73-0.92) | 0.73±0.11 | 0.76 (0.62-0.94) | 0.76±0.09 | 0.74 (0.65-0.92) | 3.588 | 0.166 |
| | Day 3 | 0.67±0.05 | 0.66 (0.62-0.77) | 0.56±0.05 | 0.55 (0.51-0.65) | 0.57±0.02 | 0.57 (0.55-0.61) | 11.890 | **0.003** |
| | | F=8.000 | p=**0.018** | F=10.296 | p=**0.006** | F=11.143 | p=**0.004** | | |
| BUN | Day 0 | 21.97±2.23 | 22.65 (18.69-24.6) | 23.72±3.58 | 24.77 (18.7-28.97) | 22.08±2.54 | 22.08 (17.3-24.8) | 1.732 | 0.421 |
| | Day 1 | 23.50±3.93 | 23.36 (19.6-31.3) | 20.04±2.18 | 19.76 (16.36-22.9) | 21.69±2.58 | 21.96 (17.8-26.2) | 3.770 | 0.152 |
| | Day 3 | 23.88±2.57 | 22.90 (21.9-29.4) | 23.29±1.28 | 22.90 (21.5-25.04) | 21.32±1.12 | 21.50 (19.63-22.9) | 8.543 | **0.014** |
| | | F=0.667 | p=0.717 | F=8.000 | p=**0.018** | F=0.963 | p=0.618 | | |
| AST | Day 0 | 120.29±56.79 | 107 (77-245) | 129.29±9.81 | 130 (113-141) | 131.86±38.30 | 118 (91-191) | 3.684 | 0.159 |
| | Day 1 | 110.29±21.95 | 99 (85-144) | 108.43±19.64 | 101 (86-146) | 113.71±20.01 | 111 (87-143) | 0.387 | 0.824 |
| | Day 3 | 89.71±10.63 | 88 (78-109) | 112.29±21.85 | 108 (81-150) | 103.29±22.69 | 94 (88-152) | 5.286 | 0.071 |
| | | F=7.143 | p=**0.028** | F=2.296 | p=0.317 | F=3.714 | p=0.156 | | |
| ALT | Day 0 | 16.57±4.65 | 16 (11-24) | 22.57±4.24 | 20 (18-28) | 22.29±4.64 | 22 (14-29) | 5.549 | 0.062 |
| | Day 1 | 14.57±3.46 | 16 (10-18) | 20.14±2.85 | 20 (17-23) | 17.43±4.43 | 18 (11-23) | 6.137 | **0.046** |
| | Day 3 | 14.57±3.78 | 16 (8-19) | 17.71±3.90 | 17 (12-23) | 19.29±4.31 | 19 (13-24) | 3.916 | 0.141 |
| | | F=0.538 | p=0.764 | F=3.769 | p=0.152 | F=5.429 | p=0.066 | | |

F: Firedman Test, H: Kruskal-Wallis H Test, P<0.05, SD: Standart deviation, Min: Minumum, Max: Maximum, BUN: Blood urea nitrogen, AST: Aspartate aminotransferase, ALT: Alanine transaminase

Besides its anthelmintic properties, levamisole has been shown to possess immunostimulant effects. The advised dosage is 7.5 mg/kg administered orally once. Adverse effects are linked to a narrow therapeutic index resulting from overdose and activation of nicotinic acetylcholine receptors, leading to a reduced convulsion threshold, respiratory muscle paralysis, and asphyxia [20 , 21]. No adverse effects were observed in this study; however, symptoms related to levamisole toxicity were documented in some animals [22]. Additionally, symptoms linked to depression, anorexia, seizures, ataxia, and frothy salivation in canines were documented [23]. Furthermore, the occurrence of severe nicotinic-type symptoms, such as hypersalivation, oral foaming, muscle tremors, recumbency, and tachypnea in Friesian calves was recorded [24].

A study was performed to examine the effects of levamisole on the haematobiochemical profiles of gastrointestinal nematodosis in sheep. The results indicated a substantial

elevation in the average values of hemoglobin (HGB), packed cell volume (PCV), glucose, iron (Fe), and calcium (Ca) ten days post-treatment. A notable reduction was observed in the average values of total leucocyte count (TLC), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) [25]. Other researchers assessed the therapeutic efficacy of the triclabendazole-levamisole combination in endoparasitic infections in sheep. In hematological findings, it was established that HCT, HGB, and eosinophil ratios were significantly reduced compared to the control group on d 0. Nonetheless, they indicated that these values ascended to the levels of the control group by the 14th d post-treatment, and no statistically significant differences were observed in other hematological parameters when compared to the control group and the baseline (d 0) [26]. In this study, contrary to previous studies, HGB, HCT, eosineophil values were not affected and AST was found to be different in the control group.

The impact of therapeutic and toxic doses of levamisole on thyroid hormone levels and certain biochemical parameters in Akkaraman breed sheep was studied. Administration of levamisole orally at doses of 7.5 mg/kg and 40 mg/kg resulted in a decrease in ALP activities, while activities of AST, ALT, lactate dehydrogenase (LDH), and creatinine kinase (CK) increased significantly [27]. A study was performed on certain haemato-biochemical parameters of sheep subjected to levamisole administration during transport. After the administration of 15 mg/kg intramuscular levamisole, sheep were transported over a distance of 260 km within 30 min. PCV decreased, while total serum protein, total leucocyte count, absolute lymphocyte count, and absolute neutrophil count remained relatively unchanged; however, serum albumin levels increased significantly. The researchers determined that intramuscular administration of levamisole at a dosage of 15 mg/kg, 30 min prior to the transportation of Yankasa sheep, elevated serum albumin levels and the neutrophil-to-lymphocyte ratio [28].

This study yielded results consistent with [27], indicating that ALT activities were elevated in both free and liposomal levamisole groups relative to the control. Likewise, the neutrophil and lymphocyte ratios in the liposomal and free levamisole groups of animals were markedly elevated compared to other measurements recorded on the third day. A study revealed that the percentages of monocytes, erythrocyte sedimentation rate, and packed cell volume at 1 and 24 h, as well as MCV and MCHC concentrations, increased after Sahiwal heifers were administered 7.5 mg/kg of levamisole [29].

Others study was performed on the haematological and serum biochemistry parameters of levamisole in buffaloes (*Bubalus bubalis*), with oral administration of levamisole at a dosage of 15 mg/kg resulted in a significant increase in monocytes, PCV, MCV, and MCHC after day 1 to d 7. On d 1, both WBC and RBC levels rose, but subsequently declined. Significant increases in total serum protein, albumin, and globulin concentrations were observed on d 1 and 7, while serum urea concentration significantly increased on d 14 [30]. The impact of levamisole administration on the immune system of cattle vaccinated for anthrax was studied. They determined that there was no disparity between the groups in neutrophil, eosinophil, monocyte, and lymphocyte counts on d 0; however, the elevation in neutrophil and monocyte counts in the levamisole group relative to the vaccine group was statistically significant, while the increase in lymphocyte counts on d 7 was not statistically significant. On d 14, the levels of neutrophils, monocytes, and lymphocytes in the levamisole-vaccine group exhibited a significant increase compared to the vaccine group. The findings of this study align with the literature regarding certain parameters [31].

A study was carried out on the performance and hematology of dietary levamisole in juvenile *Piaractus mesopotamicus*. It was determined that haematological parameters, including HGB, plasma glucose, WBC, and differential leukocyte count, were influenced by levamisole. They reported that the counts of WBC, lymphocytes, neutrophils, monocytes, eosinophils, and specialized granulocytic cells significantly decreased after 15 d, and that prolonged levamisole administration beyond 15 d exhibited toxicity to lymphopoietic tissues [32].

A study was performed to evaluate the toxicological, hematological, and immunological impacts of dietary levamisole and ivermectin on *Colossoma macropomum* (Serrasalmidae). The study demonstrated that levamisole did not induce mortality or behavioral alterations in fish. Ivermectin was determined to induce 100% mortality at specific feeding concentrations. After 24, 96, and 240 h of administration, there were no changes in erythrocyte parameters or albumin levels in any treatment group administered levamisole. Levamisole, at doses of 900 and 1200 mg/kg, resulted in reductions in the albumin-globulin ratio relative to the control group and the 300 and 600 mg/kg treatment groups. Fish administered diets of 600, 900, and 1200 mg/kg exhibited elevated glucose and total plasma protein levels relative to the control and 300 mg/kg diet groups, alongside an increase in leucocyte activity. The researchers determined that levamisole is effective in the diet of *C. macropomum* for antiparasitic interventions against helminth species and that dietary administration of levamisole can enhance elements of the innate immune system [33].

A study assessed the impact of single and triple levamisole administration on hematological parameters in rats with experimental pleuritis. Three doses of levamisole resulted in a notable reduction in RBC and an increase in MCV 48 h post-administration. The administration of a single dose of levamisole resulted in a notable elevation in hematocrit and neutrophil count at 72 h, as well as an increase in white blood cell count at both 24 and 72 h. The study determined that both single and triple doses of levamisole administration resulted in statistically significant alterations in certain hematological parameters, thereby influencing the inflammatory process [34]. A study on the haematological and biochemical parameters of levamisole in broiler chickens was conducted. Broilers administered levamisole exhibited elevated antibody titres in contrast to those not treated with levamisole. No differences were observed in TEC, HGB, and PCV, and creatinine levels remained within the normal range among the treated groups. ALT and AST levels were elevated in birds treated with a high dose (10 mg/kg) of levamisole. Serum total cholesterol, LDL cholesterol, and triglycerides were elevated, while HDL cholesterol remained unchanged [35].

This study aligns with the findings of existing literature. The variations in certain parameters may be attributable to the differences among animal species. To acquire more comprehensive information, liposomal levamisole should be sourced from various animal species, including fish, broiler chickens, and cattle.

## CONCLUSIONS

In this study, a liposomal levamisole formulation was prepared for use in sheep. After characterisation, it was administered orally. It was established that liposomal levamisole has the potential to induce alterations in haematological and biochemical parameters. A limitation of this study is that it was conducted using only sheep as the subject population. Thanks to this research, studies on liposomal levamisole in other animal species should be carried out and the results should be evaluated comprehensively. If the findings demonstrate positive outcomes, the production of commercially available drugs containing liposomal levamisole for the treatment of parasites and immunological purposes in animals within the domain of veterinary medicine will become a viable option. In conclusion, this study is an important investigation evaluating the effects of different formulations of levamisole on hematological and biochemical parameters. Liposomal levamisole was observed to have the potential to modulate the immune response. Accordingly, liposomal levamisole should be evaluated in large-scale clinical studies, and its long-term effects should be determined by applying different doses. In this regard, the present study will serve as a example for future research in this field.

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**Conflict of Interest**

The authors have stated that they do not have any competing interests.

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