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Histopathological and Heamatological Study in Experimentally Infected Rabbits with *Leishmania tropica*

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Abstract

This study was conducted in the laboratories of the Department of Parasitology, Faculty of Veterinary Medicine, University of Baghdad, during the period from the beginning of February 2022 to the end of February 2023. Iraq is an endemic country with a high frequency of both types, visceral and cutaneous leishmaniasis, with several clinical manifestations. This study was designed to describe the histopathological alterations of skin infection and compare the hematological parameters between two groups of rabbits. In this study used two groups of rabbits one of which was experimentally infected with *L. tropica*. *Leishmania tropica* isolate was obtained from AL-Nahrain University, Biotechnology Research Center, isolated from human infection with cutaneous leishmaniasis, and identified by PCR technique. The histopathological study showed as follows: The first group (control group) shows normal skin tissue; there is a thin epidermis covered by a thick stratum corneum, and the dermis contains an eosinophilic network of collagen fibers, sweat glands, and hair follicles. The other section showed higher magnification, normal epidermal layers with a thick keratinized layer above the epidermis; however, in the second group (infected with *L. tropica*) there is incomplete healing of the epidermal layer except for a few hyperplasia of stratum basale and destruction of the keratinocyte layer. Few infiltrations of inflammatory cells, mainly macrophages and eosinophils, and there is no healing and complete separation of epidermal layers and destruction of hair follicles and sweat glands. Find an irregular collagen network; in Lymphocytes, WBCs, and platelets were all significantly affected by cutaneous *Leishmania* infection. The increase of granulation led to decreased the number of amastigote stages.

Keywords: Histopathological, heamatological, *Leishmania tropica*, rabbit.

1. Introduction

Infection with a unicellular parasite from the genus of *Leishmania* that results in the worldwide parasitic disease leishmaniasis (1). *Leishmania* is a protozoan parasite of the

Trypanosomatidae family that causes a wide range of clinical syndromes and produces significant levels of mortality and morbidity (2). The disease transmits to humans when humans and sand flies shares the same environment (3). The most prevalent type of leishmaniasis is cutaneous leishmaniasis (CL), often regarded locally as (tropical sore, oriental sore, and Baghdad sore). It is a social and public health issue in many developing nations. *Leishmania major* in desert regions and *Leishmania tropica* are the main causes of the Old-World disease. (4). *Leishmania* is naturally spread by species of the genus *Phlebotomus*. (5). Anthroponotic CL caused by *L. tropica* has been detected in Iraq and Iran, according to epidemiological studies conducted in the region. (6). In both Iraq and Iran, both countries have reported cases of CL caused by *L. tropica.* (7). The primary mode of parasite elimination was noted to be macrophage activation leading to epithelioid granulomas, but the histopathological spectrum of changes seen in cutaneous leishmaniasis ranges from a diffuse infiltrate of macrophages, lymphocytes, and plasma cells to ill-defined granulomas to granulomatous inflammation with well-defined granuloma necrosis, which was not a prominent feature (8, 9). Hematological changes are closely related to the severity and prognosis of the disease; however, little is known about how visceral *leishmania* (VL)-infected patients' blood characteristics change over time. (10). Hematological characteristics Leukopenia, neutropenia, and lymphocytosis with hypochromic and microcytic anemia were discovered during the evaluation of children who had leishmaniasis (11).

2. Materials and Methods

2.1. Culture of *Leishmania tropica* in RPMI 1640 by using vacuum tubes.

Culture of *Leishmania tropica* in vacuum tubes by using RPMI 1640 media with the addition of 10% Calf Serum and 2 mg Gentamycin 1 IU Penicillin and 2 mg Nystatin per 100 ml. Cultivation was carried out using vacuum tubes with a volume of 5 ml using the hood and after sterilization of all tools by autoclave. After the cultivation is completed, we put the tubes in the tube rack and incubate in the incubator at a temperature of 28°C for a period of 24 to 48 hrs. (12).

2.2. Counting *Leishmania* parasites:

Leishmania promastigotes were counted according to (13).

2.3. Experimental animals

Ten (New Zealand) white rabbits aged 2-3 months and weighing 1500-2000g were used in this study. They are housed in a typical animal facility with regulated temperatures (20 ±5C°). Each group of rabbits was maintained in a plastic cage for the period of the studies and fed on a special formula feed and water. A clean area was maintained by the regular changing of the bedding. At the Baghdad University College of Veterinary Medicine animal shelter.

According to (14), *Leishmania* promastigotes were counted by adding 20 µl from the PBS in a 1.5 ml Eppendorf tube with 20 µl of 2% formaldehyde, completely mixed. 10 µl of the fixed promastigotes are put on the Neubauer hemocytometer, and they are then left at room temperature for 5 minutes to enable the parasites to settle. The value indicates the number of promastigotes in one milliliter of culture when five squares are counted and multiplied by 10^5. Using PBS, the parasite concentration was increased to 12×10^6 promastigotes/ml before being injected intradermally into rabbit groups two, using 0.25 ml of a solution containing 3×10^6. Group one (n=5): rabbits were left without any infection and treatment (considered as the control

group). Group two (n=5): rabbits were injected into the skin with 3×10^6 promastigotes of *Leishmania tropica* (considered as the infected group). Laboratory animals were injected into the ear region and the nasal region.

2.4. Hematological study:

A hematological study of two groups of rabbits after the infection was carried out to detect the hematological changes that occur in the blood components after infection and study the difference between the two groups by statistical analysis (15).

2.5. Histopathological Study :

After rabbit euthanized skin Specimens with dimensions (1 x 2 cm) were taken from the affected area; the tissues were fixed in 10% buffered formaldehyde solution immediately after removal. After 48 hrs. of fixation, the specimens were washed with tap water, and then processing was routinely done with a set of upgrading alcoholic concentrations from 70% to absolute 100% for 2 hrs in each concentration to remove water from the tissues. Then clearance was done by xylol, and then the specimens were infiltrated with semi-liquid paraffin wax at 58°C in two stages, and then blocks of specimens were made with paraffin wax and sectioned by rotary microtome at 5 µm for all tissues. All tissues were stained with Hematoxylin and Eosin (H&E) stain, and the histopathological changes were observed under a light microscope (16, 17).

2.6. Statistical Analysis:

To determine how various factors affect the study, a statistical analysis system was applied (18). In this study, the chi-square test was applied to compare percentages in a significant way.

3. Results

3.1. Hematological study:

A hematological study done by use of a veterinary blood analyzer of two groups of rabbits after the infection was carried out to detect the hematological changes that occur in the blood components and the study the difference between groups by statistical analysis (**Table 1**).

Table 1. Compare the effect of *L. tropica* on some blood parameters between the Control and infected group.

Blood parameter	Groups	Mean ± SE	*P value*
WBC 10^9/L	Control	8.268 ± 0.384	0.000*
	Infected	5.172 ± 0.325	
LYM 10^9/L	Control	4.00 ± 0.371	0.002*
	Infected	1.904 ± 0.198	
MON 10^9/L	Control	2.40 ± 0.40	0.018*
	Infected	0.588 ± 0.114	
GRA 10^9/L	Control	5.720 ± 0.265	0.010*
	Infected	3.352 ± 0.355	
LYM %	Control	56.00 ± 2.880	0.001*
	Infected	28.740 ± 1.33	
MON %	Control	3.480 ± 0.320	0.002*
	Infected	6.60 ± 0.223	
GRA %	Control	59.80 ± 2.408	0.038*
	Infected	48.02 ± 2.410	
RBC 10^{12}/L	Control	5.918 ± 0.238	0.005*
	Infected	4.344 ± 0.083	
HGB g/dl	Control	12.44 ± 0.419	0.035*
	Infected	10.90 ± 0.314	

Blood parameter	Groups	Mean ± SE	*P value*
HCT pg	Control	35.620 ± 1.223	0.018*
	Infected	30.682 ± 0.404	
MCV fl	Control	64.20 ± 1.019	0.014*
	Infected	59.0 ± 0.707	
MCH %	Control	31.08 ± 8.006	0.466
	Infected	29.820 ± 6.880	
MCHC g/dl	Control	33.840 ± 0.169	0.027*
	Infected	30.0 ± 1.086	

3.2. Histopathology:

3.2.1. First group (control group):

The lesion in the skin of rabbits in the control group is characterized by normal skin tissue; there is a thin epidermis covered by a thick stratum keratinizum (**Figure 1**) at higher magnification, normal epidermal layers with the thick keratinized layer above the epidermis with thick and regular collagen fibers and normal hair follicles and sweat glands (**Figure 2**).

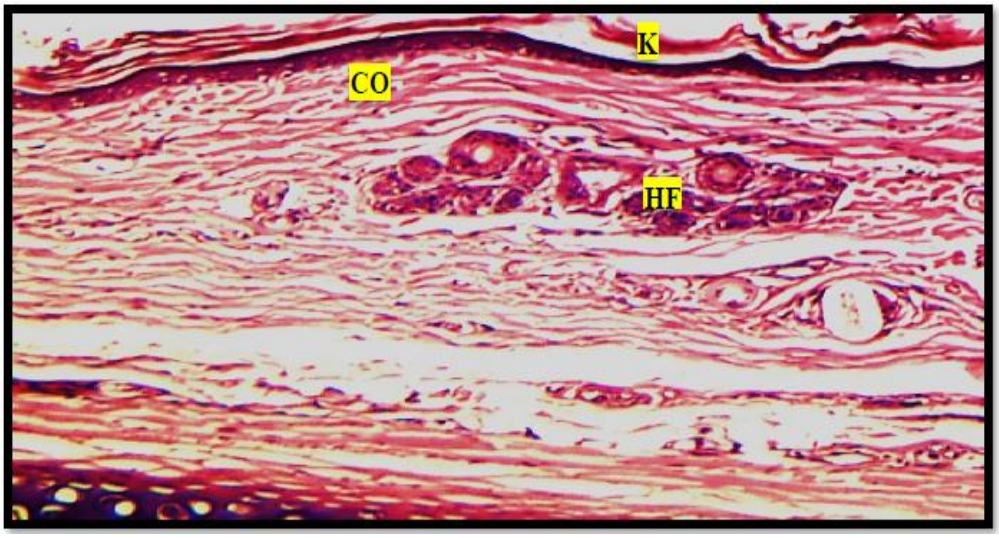

Figure 1. Normal skin tissue, there is thin epidermis which covered by thick stratum Keratinized (K). The dermis contains eosinophilic network of collagen fibers (CO), sweat glands and hair follicle (HF).20X H&E stain.

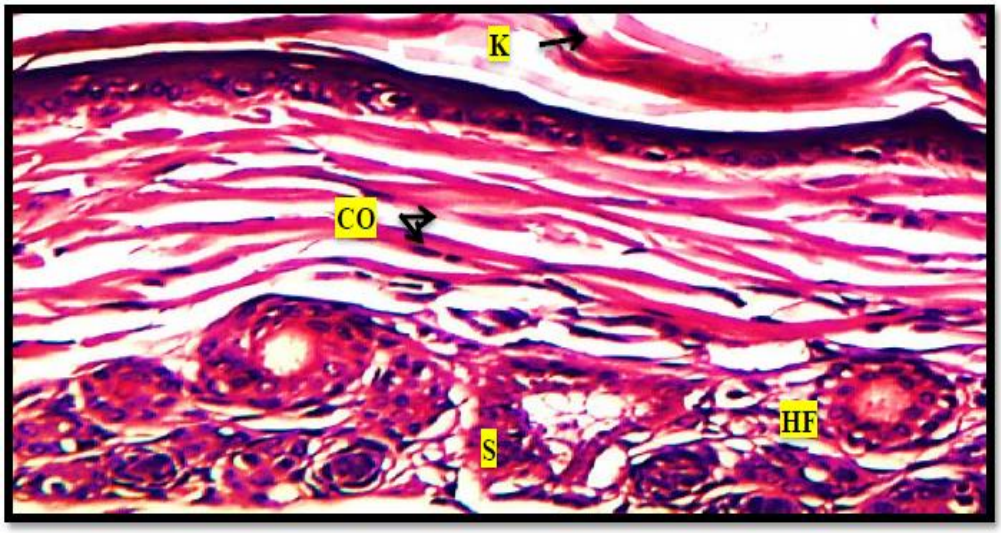

Figure 2. Higher magnification, Normal epidermal layers with thick keratinized layer above the epidermis (K). Thick and regular collagen fibers (CO) and normal hair follicles (HF) and sweat glands(S) .50X. H&E stain.

3.2.2. The 2nd group (infected with *L. tropica):*

In this group, 2 weeks post-infection, there is severe destruction of the infected skin represented by complete loss of the epidermal layer with severe infiltration of inflammatory cells in the dermis and congestion of blood vessels (**Figure 3**). There is no healing and complete separation of epidermal layers. (**Figure 4**). Marked degeneration of epidermis and destruction with sloughing of a keratinized layer in the epidermis and infiltration of macrophage (**Figures 5** and **6**). The destruction of hair follicles is evident in **Figure (7).**

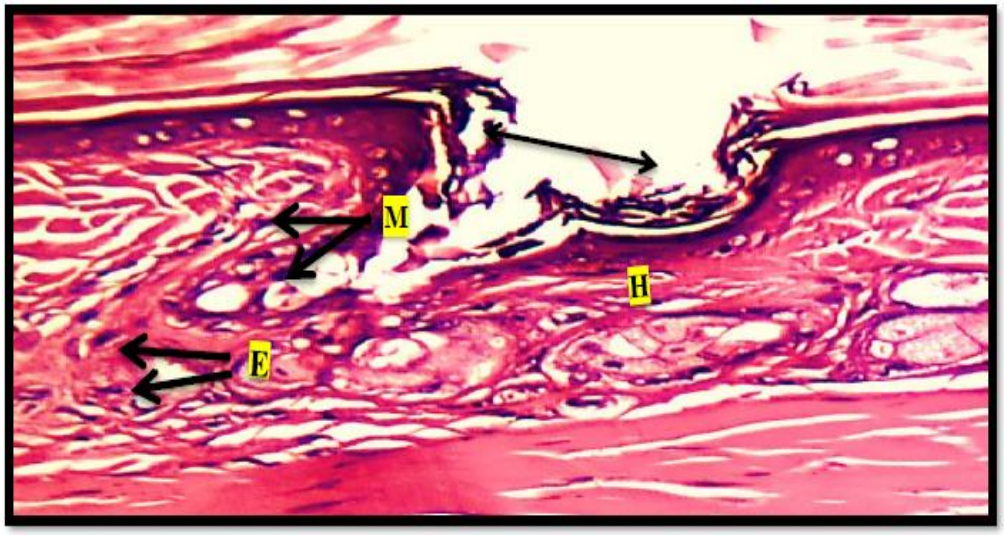

Figure 3. There is incomplete healing of epidermal layer except few hyperplasia of stratum basale (H), destruction of stratum keratinizum. Few infiltrations of inflammatory cells mainly macrophages (M), and eosinophils (E). 50X H&E. stain.

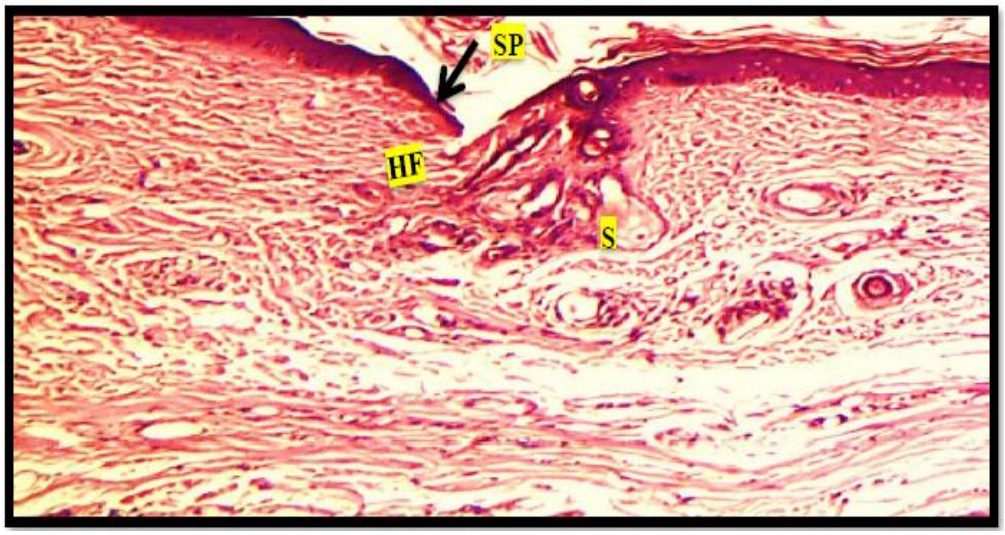

Figure 4. There is no healing (SP) and complete separation of epidermal layers (SP), infiltration of inflammatory cells (M), and destruction of hair follicles (HF) and sweat glands (S). 20X H&E Stain.

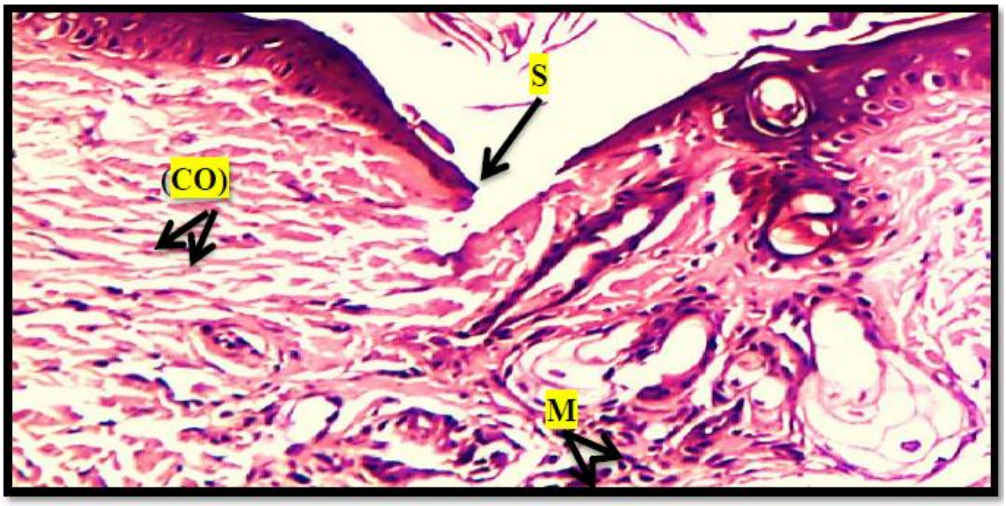

Figure 5. Complete separation of epidermal layer (S), with fine and irregular collagen network (CO), infiltration of inflammatory calls mainly macrophage (M).50X H&E stain.

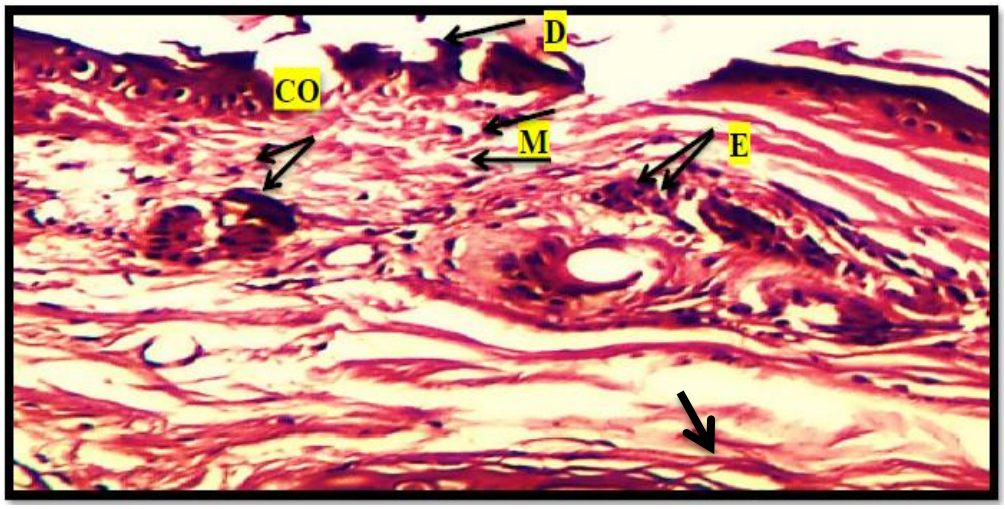

Figure 6. Marked degeneration (D), of epidermis and destruction with sloughing of keratinized layer in the dermis, there is infiltration of macrophage (M), with few eosinophils (E), and fine network of collagen (CO). 50X H&E stain.

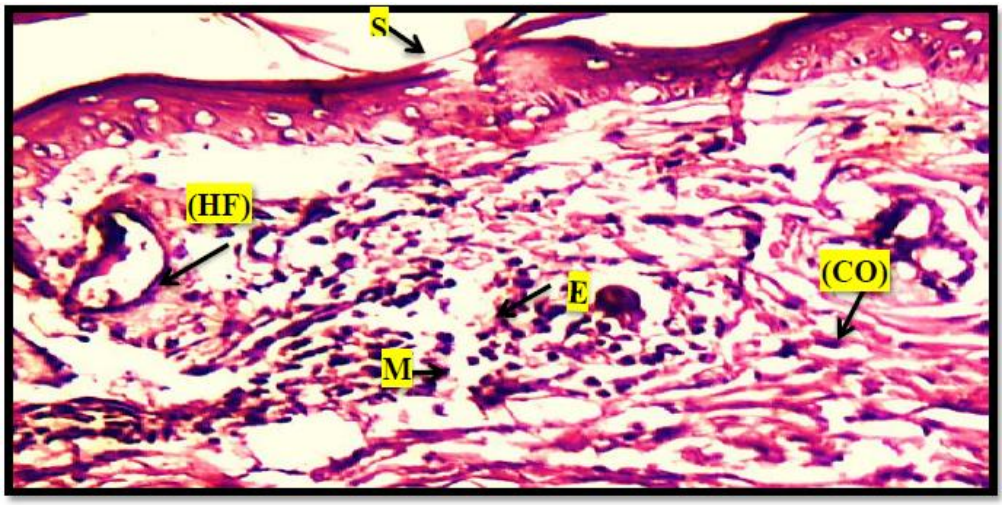

Figure 7. Incomplete healing of epidermal layers (S), marked infiltration of inflammatory cells (macrophage (M) and eosinophils (E)). In the dermis fine network of collagen (CO), with the destruction of hair follicles (HF). 50X H&E stain.

4. Discussion

The CL-infected group had a significantly lower number of lymphocytes, platelets, and mixed WBCs (monocytes, basophils, and eosinophils) compared to the control group. The mean number of lymphocytes for the control and infected groups were (4.000.371) and (1.904 0.198), respectively. The mean mixed WBC values were (8.268, 0.384) and (5.1720.325), respectively. The former finding is in agreement with (19), who observed that the lymphocyte count decreased significantly, while the more recent finding conflicts with Al-Rumaidh who observed no significant differences in WBC count. Differences in virulence between *Leishmania* spp. (or strains) and the immunological response they elicit support our results, which agree with CL findings in Iraq (20). The study by (21) shows red blood cell count (5.7±0.11), hemoglobin concentration (13.2±0.47), and white blood cell count (6.6±1.13) in the control group in male rabbits, and this is so close to our result.

It has been discovered that throughout the hematological studies, there was no significant difference between the values of all blood parameters for a solution when compared with a control, which is in agreement with that found by (22) that found hematological parameters in mice (RBC and WBC, hemoglobin, hematocrit, and platelet count).

The results of the homological analysis were supported (23). In this study, the hematological parameters of the two groups were quite similar, but it was also noted that the differential lymphocyte count tended to decline in the infected group. Despite these differences, our study found that they were not statistically significant. The results were comparable to those of (24). The obtained results were also comparable in terms of hematological parameters (25).

The current study's results were in line with (26) histopathological observations, which showed that infected control groups had severe degenerative and necrotic lesions. These lesions were mostly caused by macrophages responding to challenge and developing a granulomatous inflammatory reaction. These results may be because of how the host responded to infection. The histopathological results could potentially be attributed to the host's response to the infection of *Leishmania* spp.

Also, the findings agreed with (27, 28), which found histopathological changes of the skin during cutaneous leishmaniasis explain the granulomatous reaction in the site of infection together with the presence of the amastigote stage or LD bodies in the lesion based on the current study. The essential feature of CL pathology is the colonization by amastigote of cells of the mononuclear phagocytic system and the resulting granulomatous inflammatory response (29). This study disagrees with our study because in our study, histological section was conducted for the purpose of comparison of healing between infected, non-infected, uninfected, and treated groups to find the difference and which of them healed faster. Additionally, our findings align with the findings of (30), who conducted an investigation into the spectrum of clinical and histological manifestations of cutaneous leishmaniasis. Lesions can manifest as nodules, plaques, or ulcers, typically appearing on exposed sites, which aligns with the findings of our study. The histopathological findings in acute CL reveal a dermal infiltrate predominantly composed of macrophages, which contain a large number of leishmania organisms known as LD bodies. Additionally, plasma cells and dense mixed inflammatory cell infiltrates are also present in the dermis. When ulceration occurs, secondary infiltration with neutrophils occurs. A study by (19) discovered that the morphology of LD bodies in histopathological sections resembled a rounded

shape with a nucleus and kinetoplast; however, in some sections, we detected spindle-shaped forms similar to smear morphology, which contradicted our findings.

Few inflammatory cells, primarily macrophages and eosinophils, have infiltrated the area. Agreeing with that, the presence or absence of necrosis, unorganized or organized granulomas, eosinophils, and polymorphonuclear cells were among the histopathological features that were noted for analysis. We have estimated lymphocytes, plasma cells, and macrophages (histiocytes) using an arbitrary semiquantitative method (from scarce to abundant) (31).

5. Conclusion:

Lymphocytes, WBCs, and platelets, were all significantly affected by Cutaneous *Leishmania* infection. The increase of granulation leads to decreased the number of amastigote stage.

Acknowledgments

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Conflict of Interest

The writers state there are no conflicts of interest.

Ethical Clearance

Ethical approval was granted through the local committee of the animal care and use at the College of Veterinary Medicine within the University of Baghdad (number 577 Data 12/3/2023) before starting this study.

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