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Visceral Leishmanisis Affecting Some Physiological And Histological Nature Of Kidney And Spleen In Male Albino Rats

Bushra Husein Atia[1], **Amel Ali Altaee**[2], **Maher Ali Alquraishi**[3]
[1,23]Babylon University,College of Science

Abstract:

Background: *A group of diseases affecting both humans and animals, leishmaniasis is caused by the protozoan parasite Leishmania, which is a member of the Trypanosomatidae family. Iraq is among the countries with the highest prevalence of visceral leishmaniasis (VL). The study aimed to investigate the biochemical and histological effect on kidney and spleen of male albino rats infected with Leishmania donovani.*

Methodology: *20 rats were divided into 4 groups (n=5): G1 and G3 regarded as control administrated distilled water for 30 and 60 days respectively and G2 and G4 infected by ($2x10^7$) parasites/ rat for 30 and 60 days respectively. The animals were sacrificed at 31 and 61 day post infection (Pi.).*

Results: *The results showed that there was no significant change in means of total protein, globulin and albumin levels in male albino rats treated with parasite for 30 days and 60 days. Histological data of kidney showed focal glomerular atrophy and revealed hyaline cast in the renal tubule in rats treated with parasite for 30 days, as compared with control group. While the histological section of kidney in male rats treated with parasite for 60 days showed normal architecture of tissue as compared with control group. Spleen section revealed white pulp atrophy, secondary lymphoid follicles with blurring of the boundaries between white pulp and red pulp in rats treated for 30 days, on the other hand there was blurring of the boundaries between white and red pulp in rats treated for 60 days.*

Conclusion: *L.donovani causes several changes in tissue form (kidney an spleen) including focal glomerular atrophy and revealed hyaline cast in the renal tubule in kidney and white pulp atrophy, secondary lymphoid follicles with blurring of the boundaries between white pulp and red pulp in spleen and its function in the host.*

Key word: *Visceral Leishmaniasis (VL), male albino rats, spleen, kidney, total protein, globulin, albumin,.*

INTRODUCTION:

The protozoan Leishmania causes visceral leishmaniasis (VL), an infectious, systemic, and zoonotic illness that is mostly spread by infected female sandflies. Leishmania, a protozoan parasite that is a member of the Trypanosomatidae family, is the cause of a group of diseases that affect both humans and animals. Over 20 *Leishmania* species distributed globally have been identified as the causative agents for human infection, transmitted by infected female sandflies during their hematophagic feeding and it's the main vector of Leishmania. Different Leishmania species cause different clinical manifestations of the disease, leading to at least three different syndromes: cutaneous leishmaniasis (CL), the most common form with skin ulcerations; mucocutaneous leishmaniasis (MCL), which affects the mucous membranes of the mouth, nose, and throat; and visceral leishmaniasis (VL), also known as kala-azar, which is the most severe form due to its high fatality rate if treatment is not received. (Roatt *et al.*,2020; PAHO,2023; WHO, 2024).

MATERIAL AND METHODS:

NNN medium was used to cultivate the Leishmania parasite, which was then grown in nutritional broth medium supplemented with 10% fotal calf serum and incubated at 26°C. After being collected and treated with regular saline, the parasite was counted. (1-2× 10^7/ 250µl) (Mullen *et al.,* 1998)

Animals: 20 animals were used in this study (mature male rats). Animal's weight arranged between (224–294 g). The animals were housed in metal cages. Albino rats were divided into 4 groups (n=5), control and treated group. The control groups treated orally with D.W (1 ml) for 30 and 60 days respectively, while the treated group injected with 1-2× 10^7 parasite/rats for 30 and 60 days respectively. At the end of experiment the animals scarified at day 31 and 61 post infection.

Blood Collection: Using sterile syringes for control and treated pregnant rats, blood samples were taken directly from the heart of experimental animals (after treatment was finished) using the heart puncture method. Five milliliters of fresh blood were placed in gel tubes to separate serum using centrifuges (3000 cycle/5 minutes), and the serum was stored in a refrigerator until it was needed. Spectrophotometer kit, was used to measure the total protein, globulin and albumin in serum.

Histological Study: Kidney was weighted and apiece of tissue was kept in formalin fixative for 24 hrs. After fixation, tissue section prepared and stained according to procedure of Bancroft and Steven (2010). Using a light microscope and a digital camera (Nikon), tissue sections were photographed. According to the Statistical Package for Social Science (SPSS) system version 23, the Analysis of Variance (ANOVA) test at Least Significant Differences (L.S.D.) and Duncan was used to statistically analyze the collected data. Under level probability 0.05, the significance threshold was approved.

RESULTS:

1- Effect of *Lieishmania donovani* on organs weight of male albino rats (Treated for 30 and 60 Days).

The results in Table (1and 2) revealed that the liver, kidney and spleen weight means in male albino rats treated with *L. donovani* (1× 10^7 parasite/ 250µl) for 30 and 60 days revealed no significant changed (P≤0.05) as compared with control groups.

Table (1): Effect of *Lieishmania donovani* on organs weight of male albino rats treated for 30 days.

Groups	Organ weight (gms) Mean±S.D			
	Kidney		Spleen	
	30 days	60 days	30 days	60 days
control	a 0.56± 0.16	a 0.42±0.03	a 0.4± 0.13	a 0.46 ±0 .21
treatment	a 0.45 ± 0.05	a 0.49±0 .16	a 0.46± 0.08	a 0.44 ± 0.09
Significance	0.119		0.514	
P value	≤0.05			

* Different symbols mean significant differences.

2. Effect of *Lieishmania donovani* on Total Protein Levels of Male Albino Rats Treated for 30 and 60 Days

The obtained data in the present study (Table 2) revealed that there was no significant change in the level of total protein in serum of male albino rats treated with parasite for 30 and 60 days (5.58± 0.61 and4.603±0.624) respectively as compared with control groups(5.50±0.75 and 5.043±1.24)

Table (2): Effect of *Lieishmania donovani* on concentration of total protein (gm/dL) of male albino rats treated for 30 and 60 days.

groups	Total Protein (gm/dL) Mean±S.D	
	30 days	60 days
control	a 5.50±0.75	a 5.043±1.24
treatment	a 5.58± 0.61	a 4.603±0.624
	N.S	N.S
Significance	0.487	
P value	**≤0.05**	

* Different symbols mean significant differences ***N.s** = not significant. Significance =0.487

3. Effect of *Lieishmania* on Globulin of Male Albino Rats Treated for 30 and 60 Days

Table (3) revealed that there was no significant change in means of glouline levels in male albino rats treated with parasite for 30 days and 60 days (2.146±0.815 and 27.72±25.63 (g/dL) respectively in comparison with control groups (1.99±0.697 and 1.83±0.97 (g/dL) respectively.

Table (3): Effect of *Lieishmania donovani* on concentration of globulin (g/dL) of male albino rats treated for 30 and 60 days.

groups	Globulin (g/dL) Mean±S.D	
	30 days	60 days
control	a 1.99±0.697	a 2.27±0. 45
treatment	a 2.146±0.815	a 1.83±0.97
	N.S	N.S
Significance	0.901	
P value	**≤0.05**	

* Different symbols mean significant differences . ***N.s** = not significant. Significant =0.901

4. Effect of *Lieishmania* on albumine (g/dL) of Male Albino Rats Treated for 30 and 60 Days

Albumine concentration means of male albino rats treated with parasite for 30 and 60 days showed no significant differences (3.44±0.22 and 2.50±0.80) respectively as compared with control groups (3.50±0 .16 and 2.77±0.37) respectively (Table 4).

Table (4): Effect of *Lieishmania donovani* on concentration of albumine (g/dL) of male albino rats treated for 30 and 60 days.

groups	albumin (g/dL) Mean±S.D	
	30 days	60 days
control	a 3.50±0 .16	a 2.77±0.37

treatment	a 3.44±0.22	a 2.50±0.80
	N.S	N.S
Significance	0.152	
P value	**≤0.05**	

* Different symbols mean significant differences. ***N.s** = not significant. Significance =0.152

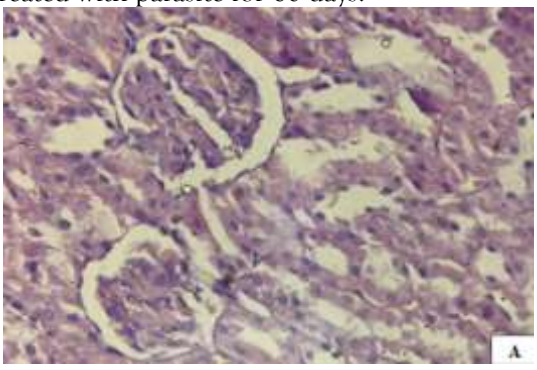
3- Histological Study

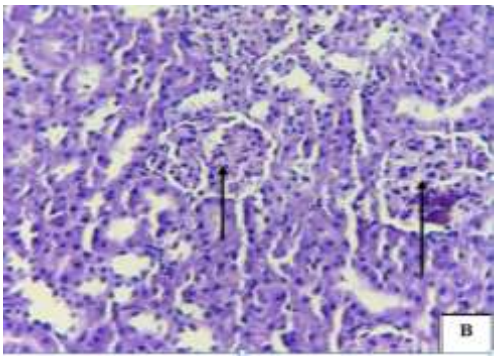
3-1 Kidney:

As shown in Figure (4-10),the histological section of kidney in male rats treated with parasite for 30 days showed focal glomerular atrophy and revealed hyaline cast in the renal tubule as compared with control group. While the histological section of kidney in male rats treated with parasite for 60 days showed normal architecture of tissue as compared with control group (Fig.1,2).

3-2 Spleen

The histological section of spleen in rats treated with *L. donovani* for 30 days revealed white pulp atrophy, secondary lymphoid follicles with blurring of the boundaries between white pulp and red pulp as compared with control group (Fig.3,4). On the other hand, Figure(4) showed blurring of the boundaries between white and red pulp as compared with control group in the spleen of male rats treated with parasite for 60 days.





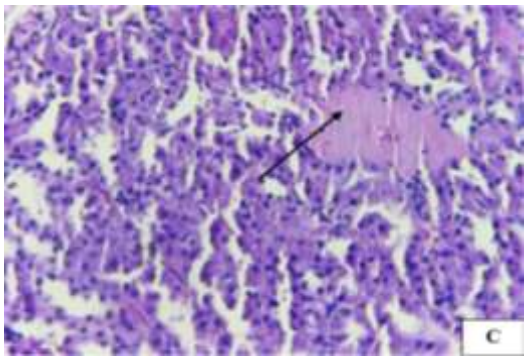


Figure (1): Transvers section of male albino rat's kidney. A: Control group, B,C:Treated with *L. donovani* for 30 days demonstrate focal glomerular atrophy and hyaline cast in the renal tubule respectively .H&E–stain. (X40)

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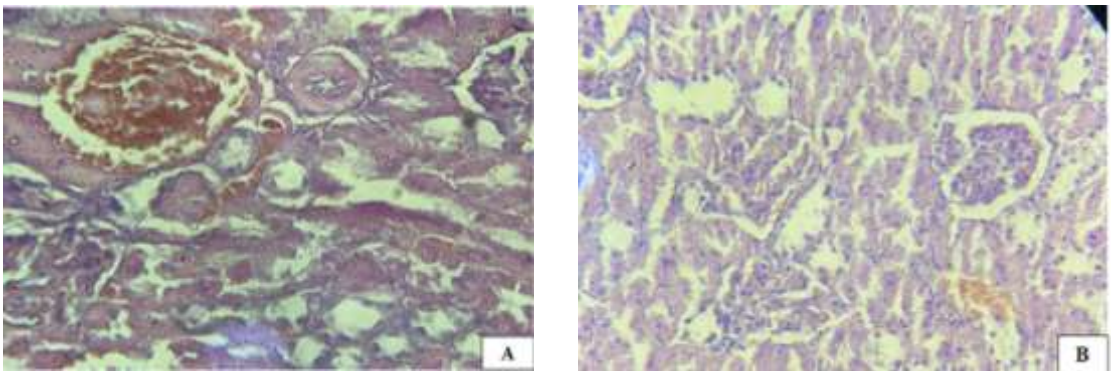


Figure (2): Transvers section of male albino rats Kidney. A: Control, B: Treated group with *L .donovani* for 60 days showing normal appearance of tissue, H&E–stain (X10).

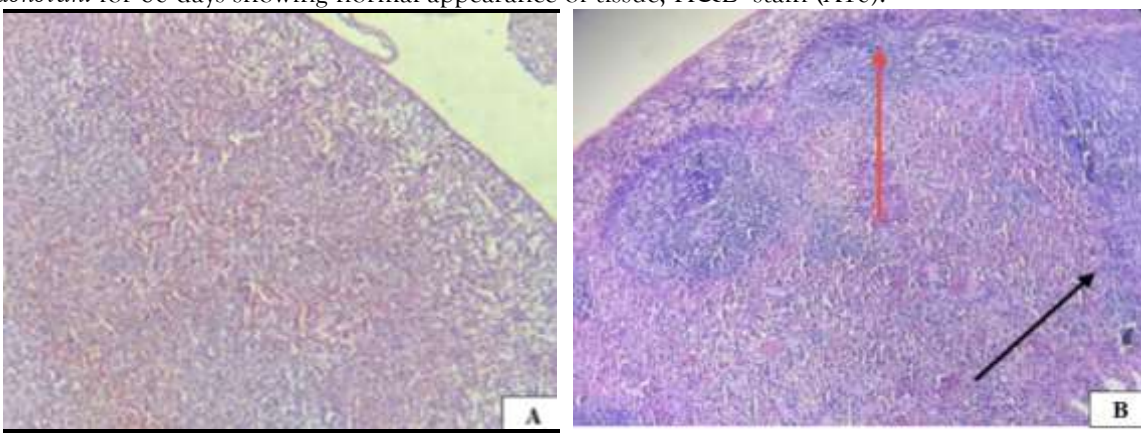
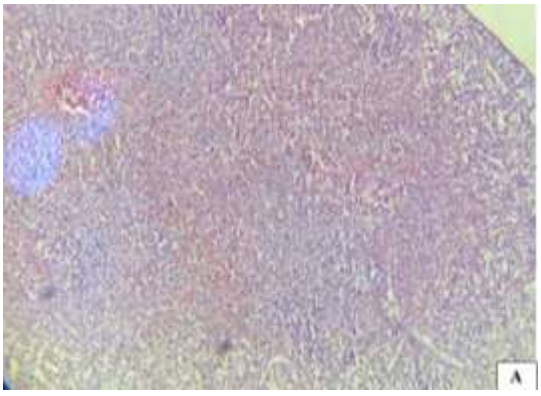


Figure (3): transvers section of male albino rats spleen treated with *L. donovani* for 30 days revealed (A) normal histology of spleen (control) and (B)white pulp atrophy, secondary lymphoid follicles (red arrow) with blurring of the boundaries between white pulp and red pulp (black arrow). H&E–stain (X10)



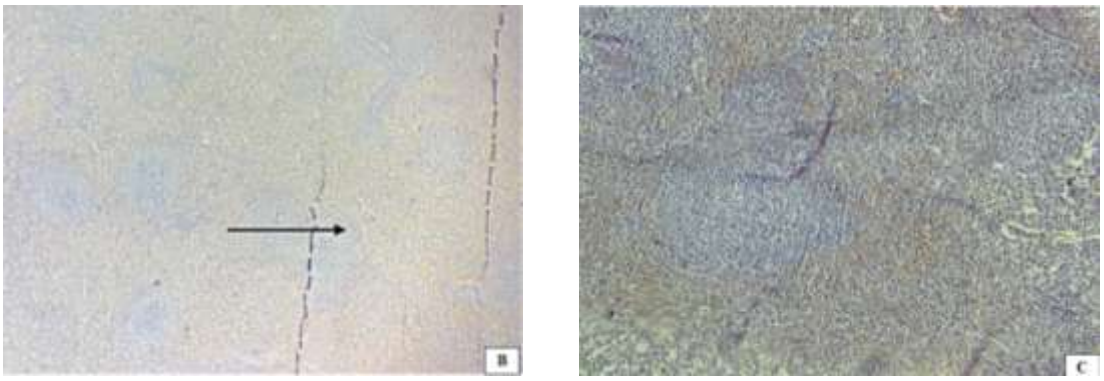


Figure (4): transvers section of male albino rats spleen treated with *L .donovani* for 60 days with blurring of the boundaries between white and red pulp, H&E–stain (X10),

DISCUSSION:

The result of organ weight showed no significant changes of kidney and spleen therefor it no agree with Varma and Naseem (2010) who found enlargement of liver and spleen and weight loss in human infected with *L. donovani.* The data of total protein, globulin and albumin revealed no significant change, there for the study was not corroborate with the data obtained by Ali (2013) who revealed a non-significant drop in albumin levels but a substantial rise ($P < 0.05$) in total protein in patients with L.donovani, which targets the visceral organs. Sahni (2012) said that hypergammaglobulinemia, which raises the level of total protein, is a characteristic of L. donovani infection. Mukerrama *et al* (2016) discovered that a parasite infection raises the level of total protein. Patients also showed a comparable markedly elevated globulin level. Likewise, Paltrinieri *et al.* (2016) demonstrated that total proteins and total globulin are often elevated, particularly during the acute stage of the illness; the severity of the clinical score can be correlated with the increase in total protein. In light of this, patients' albumin:globulin ratio was lower than controls'.Ferreira *et al.* (2021). revealed that whereas albumin and AGR reduced in CanL, serum total proteins (STP) and globulins rose. Hanan *et al.* (2024) observed that after the seventh day, mice treated with L.donovani had higher amounts of total protein than the non-infected group.

Histological results of kidney recognized focal glomerular atrophy and revealed hyaline cast in the renal tubule as compared with control group. While the histological section of kidney in male rats treated with parasite for 60 days showed normal architecture of tissue as compared with control group. These data was no agreement of that reported by Varma and Naseem (2010) who disclosed that the reticuloendothelial system is the target of L. donovani, which results in decreased bone marrow activity, splenic cellular destruction, anemia, leukopenia, and thrombocytopenia, as well as the different hematological manifestations of hepatosplenomegaly, weight loss, and hypergammaglobinemia.Rigo *et al.* (2013) observed membranous and membranoproliferative glomerulonephritis in kidney followed by focal segmental glomerulosclerosis,

Moreover, periglomerular inflammatory infiltration, multifocal and widespread peritubular inflammatory infiltration, tubular and fibrosis enlargement, and cylindruria were discovered.Various histopathological changes in kidney of mice infected with visceral leishmaniasis which includ: damage to the glomerulus, hypertrophied epithelia with pyknotik nuclei, necrotic alterations in the proximal and distal convoluted tubules, glomerular tuft degeneration and infiltration by red blood cells and chronic inflammatory cells, and tubular epithelium swelling and vacuolation were all noted.by Mohimeed and Abdullah (2018). Dias *et al.* (2020) discovered In dogs with symptomatic

CanL, kidney injury is common. Kidney disease is frequently linked to glomerular damage from immune complex deposition and a progressive decrease in peritubular capillary perfusion, which results in tubular and interstitial damage. (Clementi *et al.*, 2011 and Pineda *et al.*, 2017). Modabberi *et al*. (2021) discovered that there were more lymphocytes and macrophages in the spleen of the Leishmania-infected group than in the control group, and that the volume of trabeculae and central arteries in the spleen of the infected group was smaller than that of the control group.While the normal architecture of kidney of male rats treated with parasite for 60 days in comparison with control group may be atributed to the role of recovery from parasite by the action of immune system.

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