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ORIGINAL ARTICLE

Biochemical and Histological Changes of Liver in Male Albino Rats Infected by *Leishmania donovani*

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ABSTRACT

Background: *The protozoan parasite Leishmania is the cause of leishmaniasis. Humans contract it by the bite of sand flies, which are bloodsucking insects. Iraq is among the many places on the globe where visceral leishmaniasis (VL) is prevalent.* ***Objective:*** *The study's goal was to look at the histological and biochemical effect caused by Leishmania donovani in male albino rats.* ***Methodology****: Twenty mice were divided into 4 groups (n = 5): G1 and G3 were considered as the control group that was given distilled water once at 30 and 60 days, respectively, and G2 and G4 were infected with (2 × 10^7) parasites per mouse once at 30 and 60 days, respectively. These animals were sacrificed on days 31 and 61 post-infection (Pi.).* ***Results****: When compared to control groups, biochemical data demonstrated a significant rise in glutamic pyruvic transaminase (GPT) activity and a considerable decrease in glutamic oxaloacetic transaminase (GOT) activity. Histological results of liver in G2 showed interface hepatitis and hyaline degeneration while G4 showed ballooning degeneration of hepatocyte as compared with control group.* ***Conclusion****: Through the involvement of the liver, leishmaniasis causes alterations in tissue form and function in the host. Understanding these modifications is crucial in finding the effective mechanisms of the parasite and host interaction.*

INTRODUCTION

Approximately 12 million individuals worldwide are at danger from *Leishmania* species, which are trypanosomatids that are endemic to 88 countries, according to the WHO. Different *Leishmania* species are responsible for the various manifestations of Leishmaniases, which might be cutaneous, subcutaneous, or visceral. Visceral Leishmaniasis, often known as kala-azar, is the most common type of the disease in South Asia and is brought on by L.donovani. Although there are treatments for visceral Leishmaniasis, they are time-consuming, costly, and have harmful side effects[1].

The vector and intermediate host is Phlebotomus. The primary disease vector is a female sand fly. An amastigote found in blood meal from an infected individual changes into a promastigote, doubles in the midgut, and travels to the pharynx to prepare for transfer to another host. A sand fly's life cycle lasts roughly ten days[2]. Promastigote, the infectious stage of the *Leishmania*, is injected into an uninfected host while it is feeding. The promastigote is consumed by the macrophage cells, which then transform it into an amastigote that grows inside the macrophages and bursts to release stages that infect more cells. Promastigote stage: Sandfly vector flagellated, extracellular form. Infected female sandflies inject promastigotes into humans when they bite. After entering the human host, promastigotes are phagocytized by macrophages and become non-flagellated amastigotes. Visceral leishmaniasis is caused by amastigotes multiplying in spleen, liver, and bone marrow macrophages. The life cycle continues when another sandfly bites the infected human and ingests macrophages with amastigotes, which then become promastigotes to infect another host[3]. Thus, when another sand fly arrives and feeds on the blood meal from the sick person, the parasite's life cycle inside the human is finished[4].

METHODOLOGY

Leishmania donovani was obtained from department of life sciences at Babylon University and cultivated in vitro using Novy–MacNeal–Nicolle medium (NNN) medium and nutritional broth medium supplemented with 10% fotal calve serum. The culture was then incubated at 26°C. After being collected and treated with normal saline, the parasite was counted (1-2× 10^7/ 250µl)[5].

Animals:

Twenty mature male rats were employed in this investigation. The weight of the animal were range from 224 to 294 g. The animals were kept in cages made of metal. Five albino rats were split up into four groups: 20 mice were divided among 4 groups, control 30, control 60, infected 30 and infected 60. Each group included 5 mice. The treated group received injections of 1-2× 10^7 parasite/rats for 30 and 60 days, respectively, whereas

the control groups received oral D.W. (1 ml) for 30 and 60 days. The animals were euthanized on days 31 and 61 after infection.

Blood Collection:

Using sterile syringes for control and treated male 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 then placed in gel tubes to separate the serum using centrifuges (3000 cycle/5 minutes), and the serum was stored in a refrigerator until it was needed. Using a Spectrophotometer SANYMED diagnostics kit, Italy, the serum was utilized to measure the GOT and GPT following the manufacturer's procedures

Histological Study:

Stained many livers weighted and apiece of tissue was kept in formalin fixative for 24 hrs. Following fixation, the tissue samples (Liver, spleen and kidneys) were dehydrated using serial alcohol. After being cleaned in xylene, tissue samples were embedded in paraffin. Using a microtome, the paraffin blocks were divided into sections that were 5 microns thick. For histological analysis using a light microscope, the acquired tissue sections were loaded on glass slides and stained with hematoxyline and eosin stain[6].

Photography:

A Nikon digital camera and a light microscope were used to take pictures of the tissue.

Statistical analysis:

Using the Analysis of Variance (ANOVA) test at Least Significant Differences (L.S.D.) and Duncan test in accordance with the Statistical Package for Social Science (SPSS) system version 23, the collected data was statistically analyzed. Under level probability 0.05, the significance threshold was approved.

RESULTS

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

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

Table 1: Effect of *L. donovani* on liver weight of male albino rats treated for 30 and 60 days.

Groups	liver weight (gms) Mean±S.D	
	30 days	60 days
Control	B 2.70±0.97	a 2.469±0.27
treatment	B 2.59± 0.33	a 2.992±0 .21
Significant	N.S	N.S
P value	**≤0.05**	

* Different symbols mean significant differences. N.S. not significant differences (P≤0.05)

Effect of *Lieishmania donovani* on Liver Enzyme Function (GOT, GPT) of Male Albino Rats

The results showed that there was significant decrease in means of GOT levels in male albino rats treated with parasite for 30 days and 60 days (33.35±7.92 and41.88±16.21 (U/L)) respectively as compared with control group (60.12±3.96 and 21.69±6.26(U/L)) respectively. While there was significant increase in means of GPT levels in treated rats for 30 days and 60 days (34.12±20.22 and 56.61±13.96 (U/L)) respectively in comparison with control group (15.90±8.25 and 27.72±25.63(U/L)) (Table 2).

Table 2: Effect of *Lieishmania donovani* on GOT of Male Albino Rats treated for 30 days.

Groups	GOT(U/L) Mean±S.D			
	30 days	60 days	P value	Significance
Control	C 60.12±3.96	a 21.69±6.26	**≤0.05**	0.008
Treatment	Ab 33.35±7.92	bc 41.88±16.21		
Significance	S	S		
	GPT(U/L) Mean±S.D			
	A 34.12±20.22	a 15.90±8.25		
	B 56.61±13.96	B 27.72±25.63		
Significance	S	S		
P value	**≤0.05**			

* Different symbols mean significant differences. ***S** = significant.

Histology of liver

Histological section of liver in male rats treated with parasite for 30 days showed interface hepatitis and hyaline degeneration as compared with control group (Fig.1).

The histological section of liver in rats treated with *L. donovani* for 60 days showed ballooning degeneration of hepatocyte as compared with control group (Fig. 2).

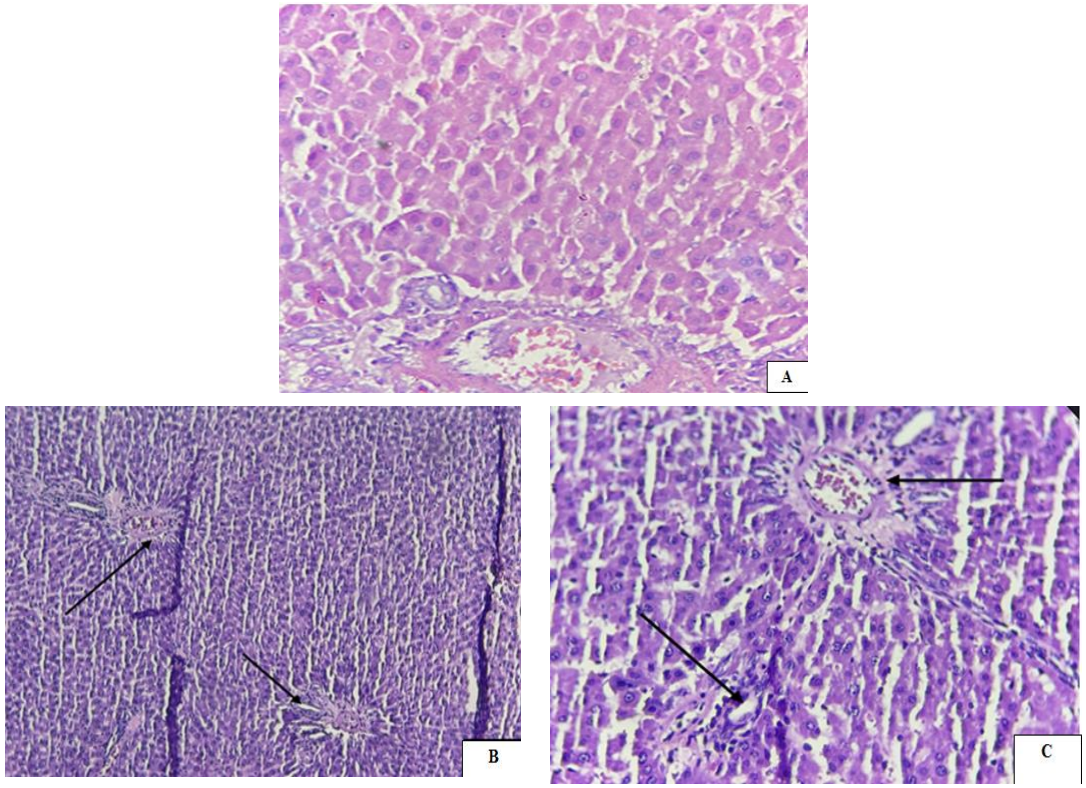

Fig. 1: transvers section of male albino rats liver treated with *L. donovani* for 30 days. A: control GROUP (40X), B: demonstrates interface hepatitis (10X) C: hyaline degeneration (40X), H&E–stain.

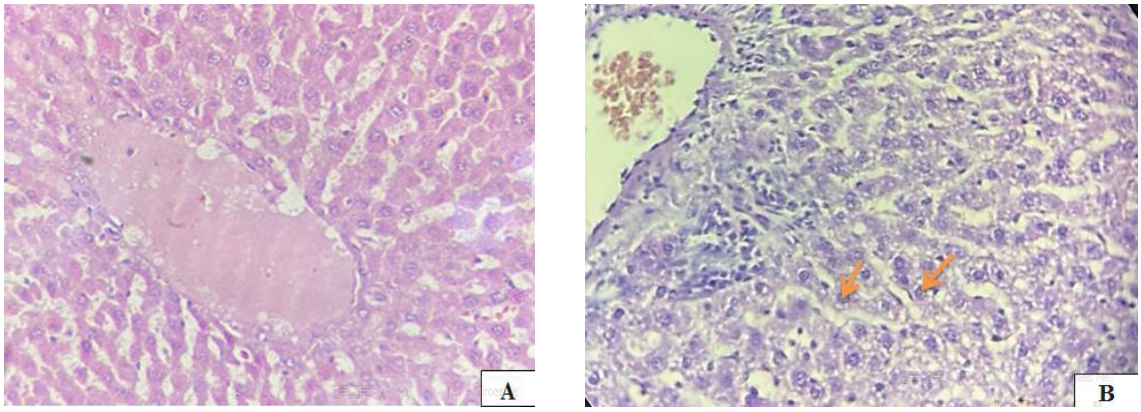

Fig. 2: Transvers section of male albino rats liver treated with *L.donovani* for 60 days. A :Control, B:Treated group demonstrates ballooning degeneration of hepatocyte (red arrow), H&E–stain. (X40).

DISCUSSION

The results of liver weight of present study was not affected significantly which may be due to immune response or to the period of infection, therefor the present study was not agree with results of other study on animals and human who found significant decrease in weight from controls[7].

The results of liver function agree with the study by Davachi, et al.[8] who observed significant differences in serum GOT in *Leishmania* treated groups as compared with control groups. But didn't agree with the results of SGPT which showed significant decreased in test groups in comparison to control groups. Fall of SGOT and SGPT levels as key liver enzymes, might be indicating an anti-parasitic immune response. Liver plays a key role in the metabolic transformation and removal of many enzyme. Also the result of our study agree with the report of Mathur, *et al.* [9] who found significant increase in levels of GPT and disagree in result of GOT, therefore SGOT and SGPT, may be utilized to ascertain whether the liver is operating correctly or whether it has sustained an injury or ailment[8]. The liver is pivotal in the pathogenesis of visceral leishmaniasis. Histplogical alteration of present study agree with Lima, *et al.*[10] who found hepatic modifications, including portal and perivascular inflammation, along with an increased presence of granulomas, which are associated with elevated *Leishmania* burdens. Modabberi, *et al.* [11] revealed that, in comparison to the control group, the Leishmania-infected group had a considerably lower number of hepatocytes and their nuclei volumes. The Leishmania-infected group's liver had more Kupffer cells and a larger volume of them than the control group, and the changes were statistically significant. Also Vianna, *et al.* [12] demonstrated that *L. donovani* caused noticeable ultrastructural alterations in the liver acinus. The portal tracts were characterized by the presence of epithelioid cells encircled by lymphocytes, indicating the presence of an inflammatory infiltrate primarily composed of mononuclear cells. It was discovered that there were noticeable ultrastructural alterations in the liver acinus around the centrilobular vein, as well as an inflammatory infiltrate composed of macrophages, lymphocytes, and plasma cells. The endomembrane system's disruption was one of the major pathogenic alterations in hepatocytes.

CONCLUSION

The single subcutaneous inoculation of 2×10^7 *L. donovani* promastigotes in male albino rats produced measurable liver injury within 24 hours—specifically, an increase in serum ALT, a decrease in AST, and histological features of interface hepatitis, hyaline degeneration, and hepatocyte ballooning. This model reliably captures early hepatic effects of visceral leishmaniasis and can serve as a basis for future studies on its pathogenesis.

Ethical Approval

All animal procedures were carried out in accordance with the guidelines of the National Council for Animal Care and the ARRIVE guidelines, and were approved by the Animal Care and Use Committee of the College of Science, Babylon University (Approval No. Z-240801-2024-08-28).

Conflict of Interest

The authors declare that they have no competing interests.

Consent for Publication

All authors have read and approved the final manuscript and give their consent for publication in Egyptian Journal of Medical Microbiology

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