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Pathological Changes of Visceral Leishmaniasis on Liver and Spleen in Experimentally Infected BALB/C Mice

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Abstract: Visceral leishmaniasis is a zoonotic protozoal disease caused by Leishmania donovani in which the pathogen disseminates to visceral organs inside the macrophage that survive within phagolysosome & evade host defense mechanism result in potential fetal infection associated with hepatosplenomegaly, lymphadenopathy and progressive anemia.The present study include study of pathological changes in reticuloendothelial system (liver and spleen).The result detected Different pathological lesions showed in different passages. Mild pathological changes noticed in liver in mice of passages one and two associated with granuloma formation with centrilobular necrosis in liver parenchyma, also the liver is disorganized and hypocellularity, with hepatic sinusoidal dilation and congestion with slight congestion of hepatic central veins. While in spleen, the pathological changes were mild to moderate suppression in lymphoid tissue of white pulp. The more splenic lesions showed in mice of passages three and four were granuloma formation with presence of amastigotes of Lishmania donavani

KEYWORD: *Visceral leishmaniasis, pathological changes, BALA/c mice*

Introduction: Visceral leishmaniasis remains a major health issue in Iraq, the disease is mostly prevalent in the alluvial plain of the central part of Iraq (Desjeux, 2004). About half of the number of patients were in their first year of age and the incidence decreased with age increment. Both sexes are equally affected, and the disease is commonest in winter and (.Gani *et al.,*2008).Visceral leishmaniasis (VL) cases have increased during the last few years, in the southern governorates of Iraq, the total numbers of listed VL cases were 1009 in 2008 and reached 1549 in 2009 in Iraq (Majeed *et al.,* 2013). The mononuclear phagocyte system is the natural habitat of *Leishmania donovani* in men (spleen, liver, bone marrow, intestinal mucosa and mesenteric lymph nodes), this parasite can be contained in kidney endothelial cell (Al-Hassani 2016). In the spleen, liver, bone marrow and other centers of the mononuclear phagocyte system, some of the parasitized macrophages are set free into the blood stream or lymphatic where they lodge and the parasites multiply rapidly, amastigotes

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are now taken up by fixed macrophages, for example Kupffer, cells in the liver, multiply in these cells and kill them (Beaver *et al.*, 1985).

Materials and Methods

Experiment design: The study has included 20 Balb/c mice, males, (6~8) weeks-old divided into 4 majer passages, each mice injected intra-peritoneal with ($3x10^6$) promastigotes of *L. donovani*, were the first passage was after 30 days post infection, the second passage was after 30 days post infection, the third passage was after 30 days post infection, finally the fourth passage was after 30 days post infection, samples of spleen and liver from every animal were isolated for prepare histopathological sections to demonstrate the pathological changes.

Histopathological Sections: Samples of 200 mg from spleen and liver from each animal were fixative in Bouin's fluid to prepare histopathological sections. The tissue was trimmed and the specimens were washed by using tap water for (1-2 hr.) and transferred to the following steps [6].The yellow stain of Bouin's Fluid can be removed with multiple washes in buffered ethanol. Current protocols suggest that several changes of 70% ethanol are used to wash the tissue until the yellow color disappears.

1. Dehydration:Specimens were passed through ascending grades of ethanol alcohol (50%, 60%, 70%, 80%, 90%, 95%and 100%) for 1 hour in each concentration. 2. Clearing:Two changes of xylol used for clearing. The specimens rested 1 hour in each step. 3. Impregnation with paraffin wax:The specimen were transferred from the clearing agent to a bath of melted paraffin wax for two hours at 58 ºC in oven. 4. Blocking:To be prepared for cutting, the tissue poured into blocks of pure wax. They were about 24 hours away to give paraffin time to solidify. 5. Sectioning:Cutting of blocks by rotary microtome. Parts were cut (5-6) μm thick. And each segment will then be moved to a water bath set at 52 ^oC. Using Mayer's albumin, each segment fixed to a glass slide. During 24 hours slides were dried by oven at 40 ^oC. 6. Staining: The sections stained after dipping in xylol for two minutes to dissolve remaining paraffin, transfer to 100% ethanol for 2 minutes to remove xylol. Rehydration of tissue by passing through descending grades of ethanol alcohol (90%, 70%) for 2 minutes, then dipping for one minute in distil water, transfer to staining solution of Haematoxylin for 5 min., washing by tap water till deep blue color appeared. Dipping slides in 1% eosin staining solution for 3 min. then washed by distil water then dehydration through ascending grades of ethanol alcohol (70%) for half min. and (100%) for 2 min.

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RESULTTS: Different pathological lesions showed in different passages. Mild pathological changes noticed in liver in mice of passages one and two. While in spleen, the pathological changes were mild to moderate suppression in lymphoid tissue of white pulp. The more splenic lesions showed in mice of passages three and four were granuloma formation with presence of amastigotes of *Lishmania donavani* and sever hemorrhage with massive hemosidrosis deposition together with evidence lesions noticed in liver associated with extensive areas of centrilobular necrosis and sever congestion in central vein with hepatic sinusoidal dilation.

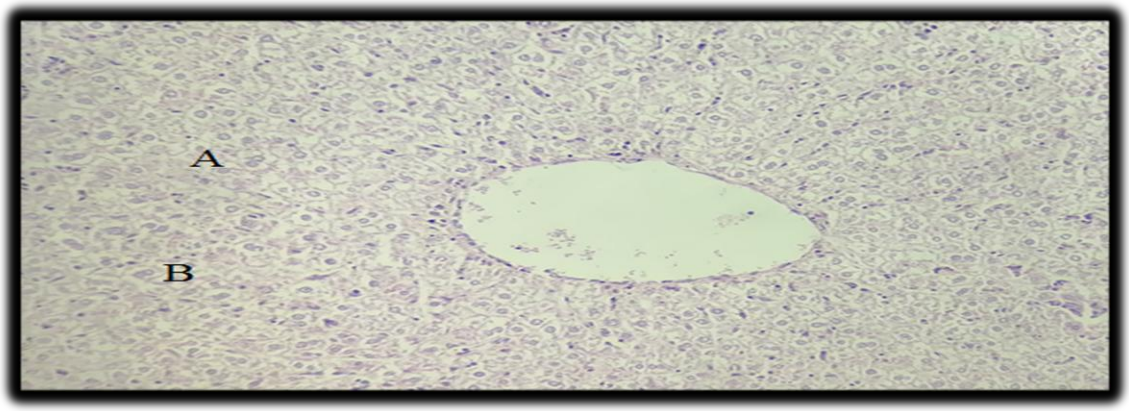

Figure 1: Hhistopathological lesions in the liver of mice in passage one H&E 20 dpi (X40) A-Disorganized of liver& hypocellularity of hepatocytes due to centrilobular necrosis in hepatic tissue which characterized by pyknosis ,karyorrhexis and karyolysis of their nuclei. B-Hepatic sinusoidal dilation C- central vein dilation D- congestion.

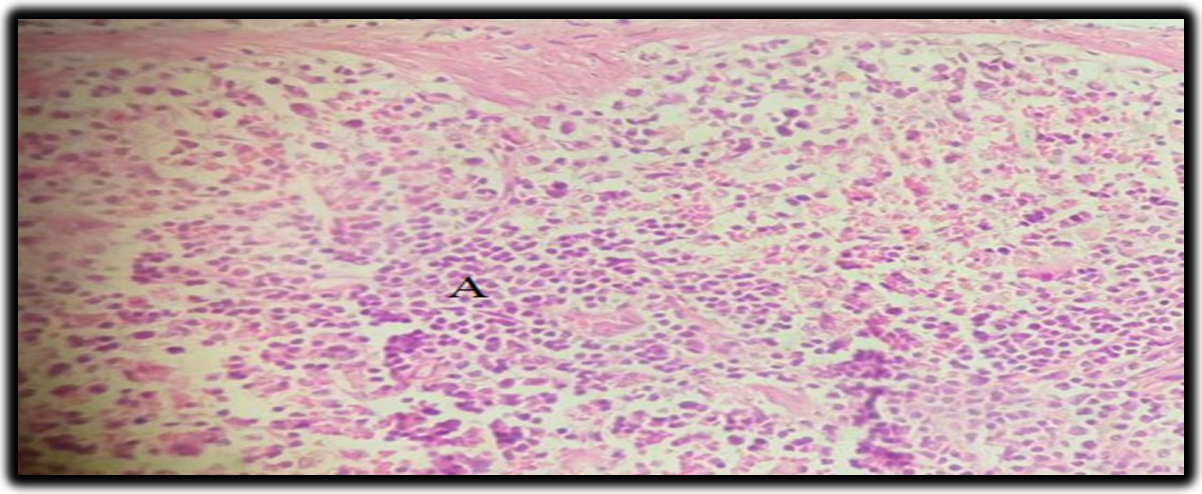

Figure2: Hematoxylin and eosin stained representative images of histopathological lesions in the Spleen of mice in passage 1 20 dpi (X20) :A- Mild suppression in lymphoid tissue of white pulp due to decrease population in the lymphocytes , macrophages& plasma cells in the area around the central artery of lymphatic nodules (white pulp) of spleen.

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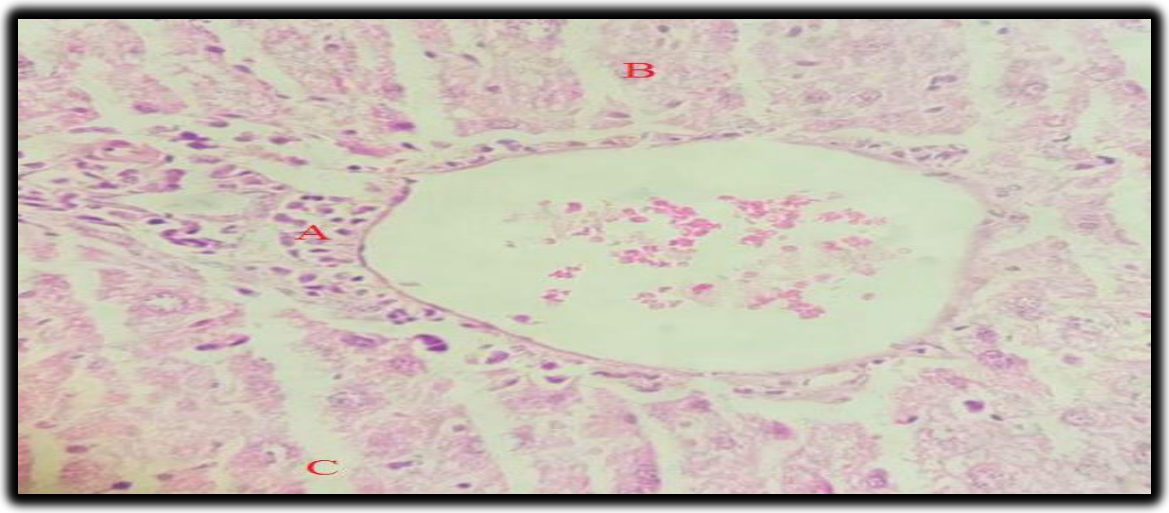

Figure 3: Hematoxylin and eosin stained representative images of histopathological lesions in the liver of mice in passage 2 20 dpi (X40) : A-Granuloma without presence of amastigotes of *L.* which characterized by moderate MNCs infiltrations especially lymphocytes , macrophages & plasma cells. B-Centrilobular necrosis in areas around central vein of liver C- Hepatic sinusoidal dilation and congestion, and destruction of hepatic tissue.

Also the study observed histopathological lesions in the Spleen of mice in passage 2 included A-White pulp was disorganized with moderate suppression in lymphoid tissue due to decrease in the lymphocytes , macrophages &plasma cells in the area around the central artery of lymphatic nodules (white pulp) of spleen.

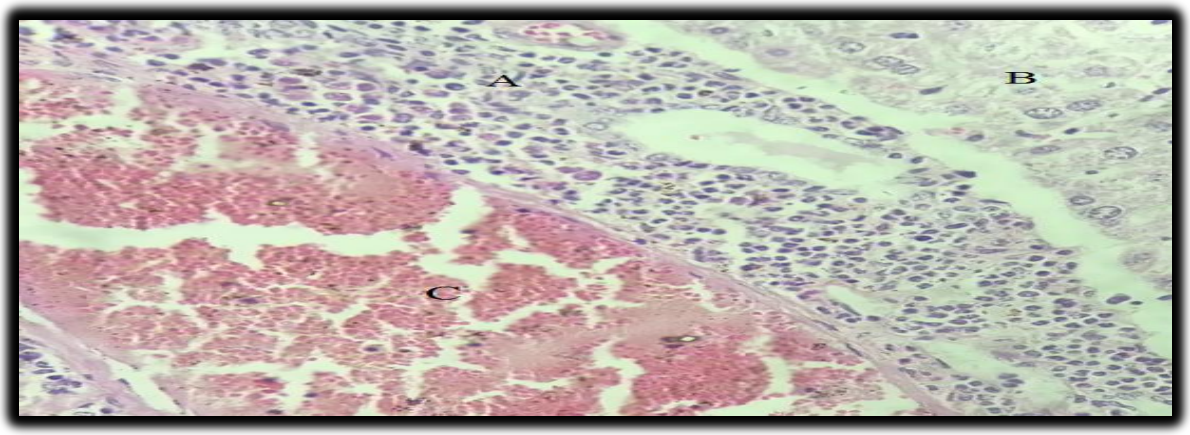

Figure 4: Hematoxylin and eosin stained representative images of histopathological lesions in the liver of mice in passage 3 20 dpi (X40) :A- Granuloma formation without presence of amastigotes of *Leshmania donovani* consisting of high population of macrophages and lymphocytes and plasma cells scattered throughout hepatic parenchyma &portal areas of liver. B-Centrilobular necrosis in areas around central vein of liver. C-sever congestion in portal vein.

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While the histopathological lesions in the Spleen of mice in passage 3 observed that : A-Parasite phagocytized by red pulp macrophages. B-Granuloma formation in areas of red pulp.

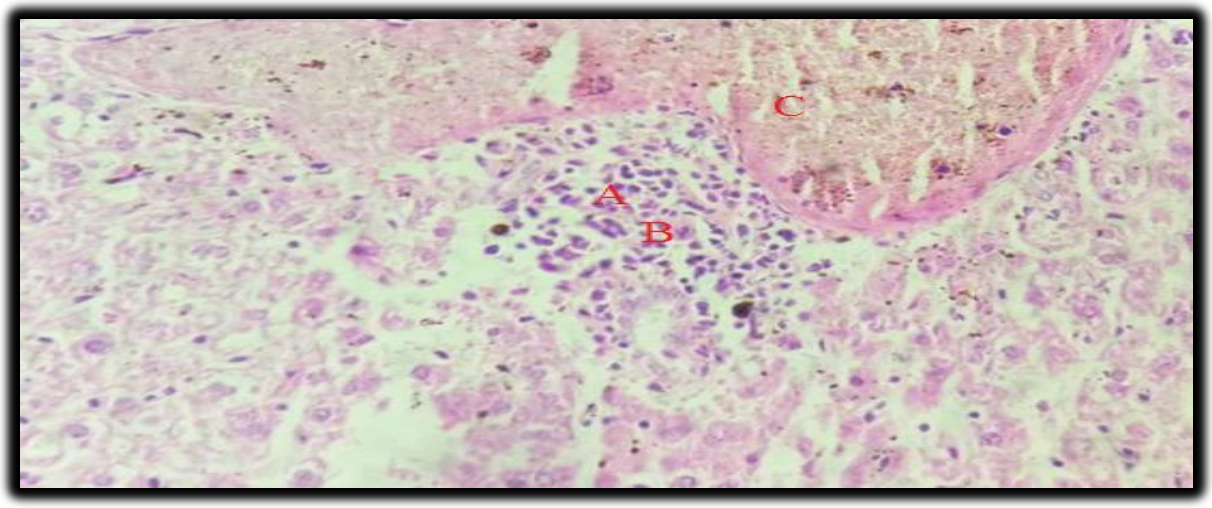

Figure 5: Hematoxylin and eosin stained representative images of histopathological lesions in the liver of mice in passage 4 20 dpi (X20) : A-Mature granuloma with sever MNCS infiltrations mainly macrophages, lymphocytes and plasma cells. B-Kupffer cells engulfing amastigotes of *Leshmania donovani* C-Sever congestion in the central vein of liver.

Also observed that in the histopathological lesions in the liver of mice in passage 4 : A-Granuloma formation due to sever MNCs aggregations mainly macrophages, lymphocytes and plasma cells. B-Sinusoidal dilation and congestion. C-Congestion of central vein D-Extensive hepatic necrosis in areas around the central vein .

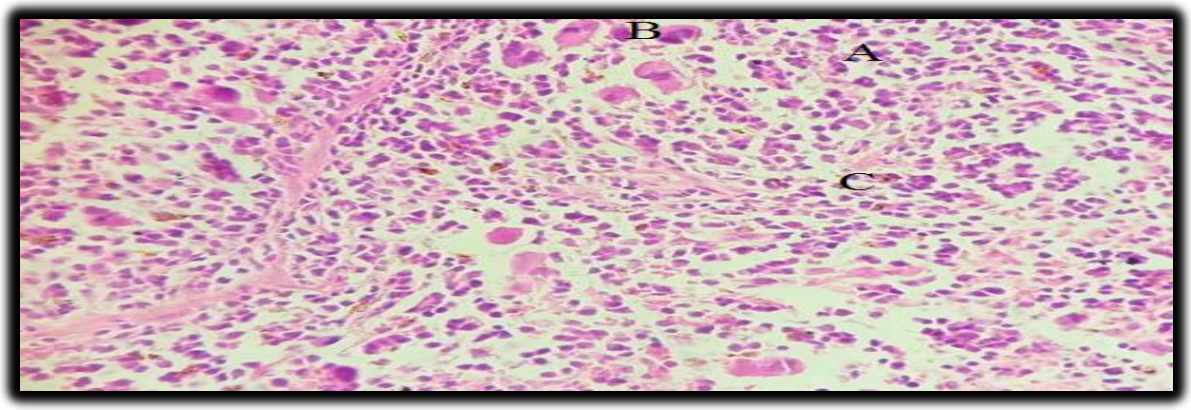

Figure 6 : Hematoxylin and eosin stained representative images of histopathological lesions in the Spleen of mice in passage 4 20 dpi (X40) : A-Granuloma formation due to hyperplasia of mononuclear cell such as lymphocytes and macrophages and plasma cells in areas of red pulp. B-Parasite containing & engulfed by red pulp macrophages. C-Massive hemosidrosis deposition.

Discussion: Generally and according to present histopathological observations, atrophied & disorganized with severe depletion in lymphoid tissue and necrotic lesions were reported in infected mice noticed in splenic and macrophage and hepatic kupffer cells at 30 days post infection associated with development of granulomatous either in liver or splenic tissue, these finding may be due to host response to infection. This agree with the results of (Salguero *et al.*,2017) where they showed that, the histopathological changes may be due to host responses to infection of *Leishmania spp.* also, agrees with Kaye & Beattie (2016), this study report The histopathological hallmark of hepatic may be resistance to visceral species of *Leishmania* by development of functional granulomas. Also, agrees with previous studies (Carrion *et al.*,2006) who observed that the majority of hepatic granulomas were immature, with the presence of amastigotes, and this explains Early amastigote replication in tissue macrophages and high amastigote loads in the livers of mice, also reflect that resident macrophages are the first line of defense against *Leishmania* parasites within this tissue. Moreover, monocytes are recruited into the granuloma (Stanley& Engwerda, 2007) and Hepatic resistance against *L. donovani* infection correlates well with the generation of reactive oxygen and reactive nitrogen (Kaye *et al.,* (2004). An increase in the number of granulomas associated with the increase in parasite load in the liver has been demonstrated previously and the most of the extracellular promastigotes, before entry into macrophages, are killed by complement factors (Mangoud *et al.*,1997).

The lesion low severity may be due to by the presence of antileishmanial antibodies, which are produced in VL this agree with, however, the role of elevated antibody levels in kala-azar patients towards protection or pathogenesis is still unclear (Dirwal, 2019).

In this study, the parasites in spleen section were shown clearer than liver, that may because the spleen was regarded the main organs of peripheral lymphatic system, which have main function filtrated blood from harmful antigens because have large number of macrophages these macrophages are host of parasites in mammals, or that may be due to the difficulty finding parasites in liver because large size of it when compared with spleen, that is agreement with (Jarallah, 2016) who demonstrated that spleen was more sensitive for infection when compared with liver and the number of parasites in spleen was more than for same passage of inoculation, but Santos *et al.*,(2013) found the most histopathological changes detected in liver with granuloma and infiltrations especially lymphocytes , macrophages & plasma cells. The more splenic lesions showed in mice of passages 3 and 4 (30 days post-infection) were granuloma formation due to hyperplasia of the phagocytic

MNCs s such as lymphocytes and macrophages and plasma cells with presence of amastigotes of *Lishmania donavani* which phagocytized by red pulp macrophages , with sever hemorrhage with massive hemosidrosis deposition together with evidence lesions noticed in liver associated with extensive areas of centrilobular necrosis and sever congestion in central vein with hepatic sinusoidal dilation.

In other study observed that hyperplasia in kupffer cell with hepatosplenomegaly and infiltrations mainly macrophages, lymphocytes with congestion in the central vein of liver, also confirmed presence hyperplasia og Kupffer cells engulfing amastigotes of *Leshmania donovani* , granuloma and infiltrations in lymphocytes as immune response for liver tissue (Gutierrez *et al.*,1984).

Abreu-Silva *et al*.(2004) confirmed that the increasing size of liver and spleen due to accumulation of amastigotes in this organs and happens hyperplasia in the cells of the endothelial reticulum, the hepaosplenomegaly appear after 30 days post infection and led to increase protection of macrophages as a defense against parasite and the spleen have the largest role in immune defense in the host body. Squires *et al.*,(1989). demonstrate the presence of large changes in the spleen tissue, with a greater dilation in the white pulp than the red pulp also, infiltration macrophage and Lymphocyte with severe fibrosis around the white pulp, Congestion of the arterioles and increase their size.

The study concluded that different pathological lesions showed in different passage, sever gross and microscopic lesions in passage 4 than other passages (3, 2 and 1). The histopathological lesions in spleen and liver may due to the effect of virulence factors of parasite. In addition to that the role of immune response of the host against the parasite invasion that cause different lesions in tissue

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