Histological study of effect of Leishmania donovani on ileums mice

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KEYWORDS
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ABSTRACT
Background: *Leishmania Donovani* Is A Form Of Internal Parasite That Belongs To The Genus Leishmania And Is The Type Of Hemo-Flagellate Kinetoplastid That Causes Leishmaniasis. **Objective:** The Present Study Investigated The Effects Of Inoculation With *L. Donovani* On The Morphology Of The Ileum In Mice. **Methods:** Balb/C Mice Were Intraperitoneally Inoculated With *L.Donovani*(3 Animals/ 4group). After 1,2,3 Month, The Ilea Were Collected And Histologically Processed. **Conclusions:** *Donovani* Is Induced Histological Change In Ileum Section After The First Month Infection.

1. Introduction

Leishmaniasis is an illness spread by vectors that is contracted through the bite of female sand flies carrying various strains of Leishmania parasites. The disease presents itself in three primary clinical variations: cutaneous, mucocutaneous, and visceral forms of Leishmaniasis. (Reithinger & Dujardin., 2007). Leishmaniasis comprises a set of overlooked tropical illnesses resulting from the presence of the parasite genus Leishmania. (Armitage et al., 2018). Visceral leishmaniasis is an overlooked disease prevalent in tropical regions, resulting from parasitic infection by members of the Leishmania genus that invade macrophages located in various tissues including the spleen, liver, lymph nodes, bone marrow, and intestine. (Cavallone, I. N., 2022) . Leishmania donovani is a form of internal parasite that belongs to the genus Leishmania and is the type of hemo-flagellate kinetoplastid that causes leishmaniasis. (L. Thakur *et al*., 2020). Kala-azar is the common name for visceral leishmaniasis caused by L. donovani (Siriwardana et al., 2019). In most situations, the incubation time lasts between three and six months, although it may drag on for a year or more in rare circumstances (Zheng et al., 2020).

All Leishmania parasites have two main life stages and require two hosts to complete their life cycle, the first of which is the invertebrate host (sand flies), known as the promastigote stage. The second is the amastigote stage of the vertebrate host (human), which invades the vertebrate host's macrophages (Abdulla et al., 2018). In later stages of development, the gastrointestinal system (GI) may become impacted. (Costa et al., 2010), Despite not traditionally being recognized as a primary site for the survival of Leishmania sp., the gastrointestinal tract (GIT) provides favorable conditions for the persistence of amastigote forms. The GIT is home to around 70% of the body's lymphocytes and contains high concentrations of macrophages, dendritic cells, as well as T and B lymphocytes within a specialized area known as the gut-associated lymphoid tissue (GALT). (Flach and Diefenbach, 2015; Greenwood-Van Meerveld et al., 2017; Takiishi et al., 2017; Yap and Mariño, 2018), that can be modulated by different stimuli, allowing the establishment of the parasites.

Although there have been numerous studies documenting the morphophysiological alterations in certain organs due to infections by vascularizing strains of Leishmania, there is a scarcity of literature discussing the potential underlying factors for chronic symptoms such as diarrhea, melena, weight loss, and malnutrition seen in visceral leishmaniasis (VL) patients who are actively ill (Benbella *et al*., 2016; Feleke, 2019), and consequently with GIT dysfunction. Some studies have indicated that Leishmania sp. has the capability to infect mononuclear cells found in the duodenum (Chattopadhyay *et al.*, 2020), jejunum (de Lima *et al*., 2021), ileum (Santos *et al*., 2021), and colon (Passos et al., 2020) of diverse hosts. The proliferation of amastigote forms has been linked to structural alterations in the intestinal segments' walls. Notable modifications included deeper crypts

and taller villi in the ileum (Santos *et al*., 2021), villi and crypt atrophy in the jejunum (de Lima *et al*., 2021), and a marked thickening of the submucosa in the colon (Passos *et al*., 2020). Although research has been conducted on the histopathology of certain nontraditional organs like the jejunum and colon (de Lima *et al*., 2021; Passos *et al*., 2020), there is a scarcity of data regarding the morphological alterations that transpire in the duodenum during L. (L.) infantum infection. This particular intestinal section is intricately linked to the gastrointestinal-associated lymphoid tissue (GALT) and possesses distinct features, including the presence of Brunner glands that generate and discharge mucus capable of neutralizing the acidic pH of the chyme (Greenwood-Van Meerveld *et al*., 2017; Jankowski *et al*., 1994; Madara, 1991). However, little is known about the effects of the *Leishmania* infection on the gastrointestinal tract. Thus, the present study investigated the effects of inoculation with *L. donovani* on the morphology of the ileum in mice.

Material and method

Leishmania Parasite

Iraq isolate (MHOM / IQ /2005 /MRU15) of *L. donovani* was kindly provided by the laboratory of parasitology Graduate studies, Department of Biology, College of science, university of Baghdad, it was previously diagnosed by PCR.

Culture media

A biphasic culture medium was used, in this study for the development and maintenance of Promastigote forms of *L. donovani* at a temperature of 26 ° C. This medium consists of two phases the solid phase and the liquid phase

Animal

Twenty robust adult male mice (BALB / c), weighing approximately 25 g on average, were allowed to acclimate for three to four weeks at 25 °C and 50% relative humidity while being given a commercial diet. Water for drinking set up in the animal house of the University of Babylonia's College of Science. Each group consisted of five mice that received an intraperitoneal inoculation. They were split into three groups, A, B, and C. *L. donovani* promastigote ($1x10^6$) in it. The mice were euthanized in three batches at 30, 60, and 90 days following the injection. N.S. was intraperitoneally administered to five mice in the control group.

4 sample collection

20 adults' males of mice (5 of each group) dissected in the laboratories of the Department of Biology / College of Education for Pure Sciences, University of Babylon and conditions for animal welfare and euthanasia observed during the dissection process. After the ileum extraction and samples taken were fixed in 10% buffered formalin solution, dehydrated, cleared, embedded in paraffin wax, cut (5mm thick) and stained with hematoxylin and eosin for the histopathological characterization.

Blood smear

EDTA anti-coagulated blood samples were collected from animal after euthanasia. Place a drop of blood near one end of a clean glass slide and using another slide (spreader slide) at a 30–45-degree angle, touch the drop of blood and allow it to spread along the edge. Push the spreader slide forward quickly and smoothly to spread the blood across the slide in a thin, even layer. Allow the smear to air dry completely. Do not blow on the slide or use any heat source. Once the smear is dry, fix the cells by covering the slide with methanol for about 2-3 minutes. Allow the slide to air dry completely after removing the methanol, stained according to Leishman's method. after which the slides were air-dried. Leishman working solution (S DFine-Chem Ltd, Product no 44042, batch no Lo4x/0504/0212/71) was diluted 1:6 with phosphate buffered water (pH7.2). Every day a fresh filtered working solution was used. The slides containing the thin smear were then submersed in Leishman stain for 12–15 min. Finally, slides were washed gently under running tap water and air dried prior to microscopic assessment.

Ethical approval

This study was approved by the regional ethical committee of the University of Babylon, Faculty of Science Institutional ethics board (Z231001 in 8-10-2023), informed consent was obtained from participants or their parents / legal guardians plan of the study.

Results

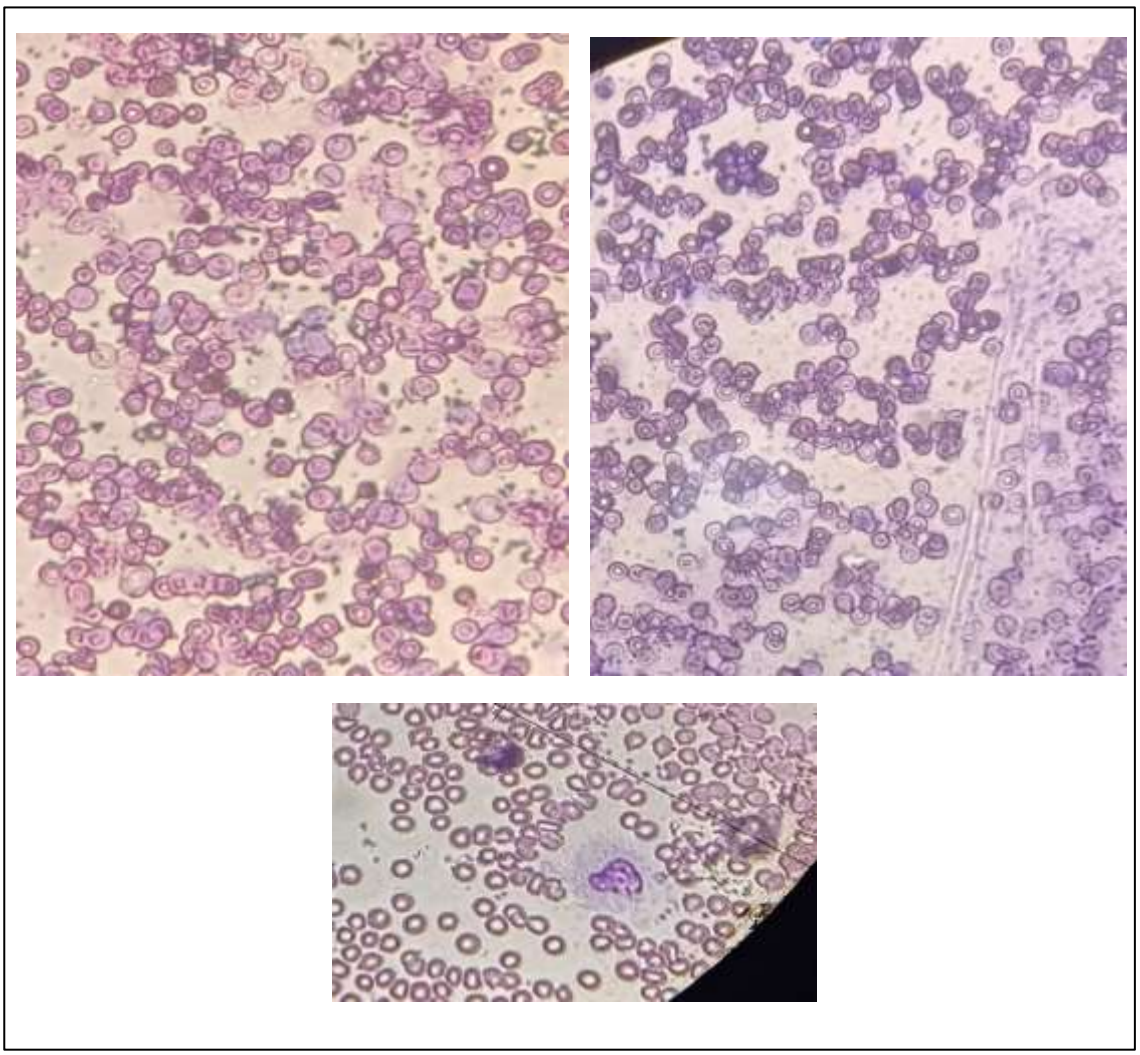

Figure (1) section of blood smear showing amastigote form of *L. donovani*

Control group

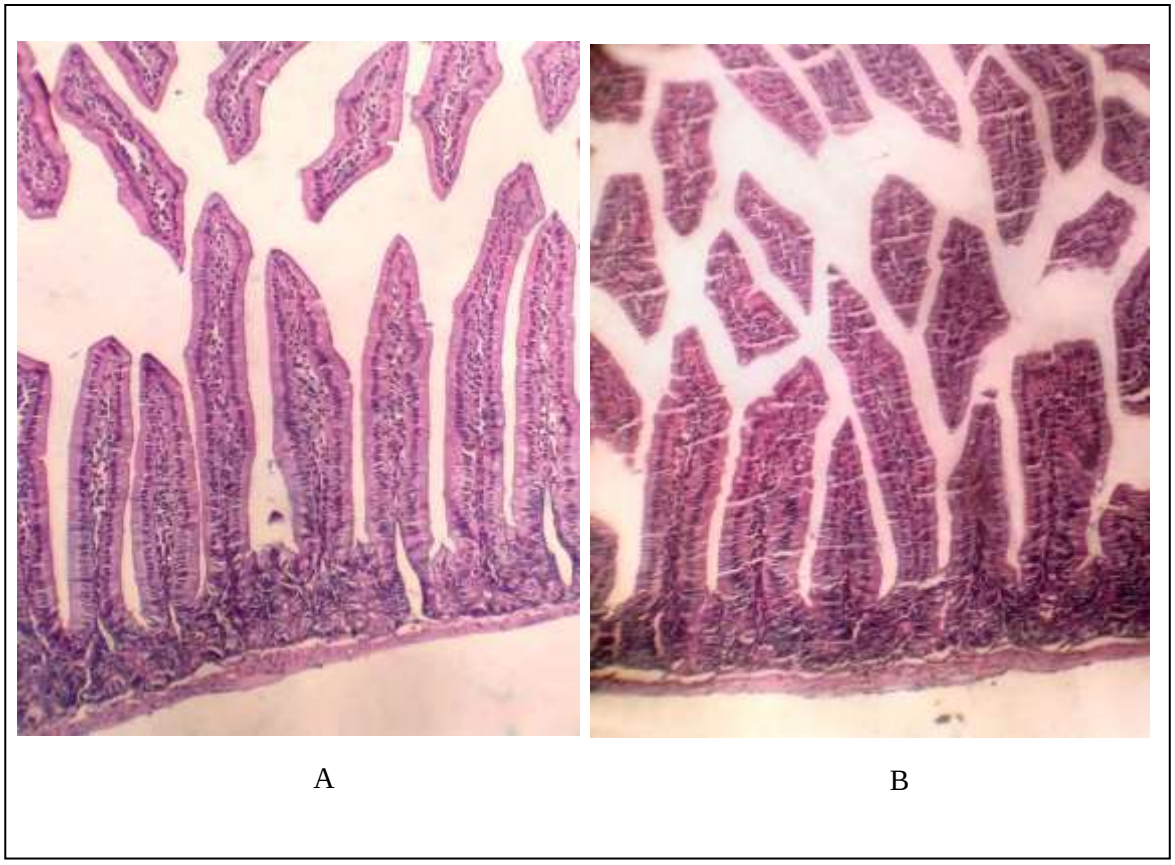

Figure (2) cross section of control group of ileum sample from mice, stained with H&E, 10X.

One month-post infection

The histopathological change in ileum of mice infected with *L. donovani* after one months in figure (3) the section (A), there is evidence of mild destruction of mucosal cells, characterized by the presence of areas where the epithelial lining appears damaged indicated by the red arrows. The villi seem slightly blunted or shortened**,** In the section (B) This section shows inflammatory cells within the lamina propria, which is indicative of an ongoing immune response to the *L. donovani* infection. The immune cells include lymphocytes, macrophages, and possibly other cells, such as neutrophils. This is the body's fight against *L. donovani*.

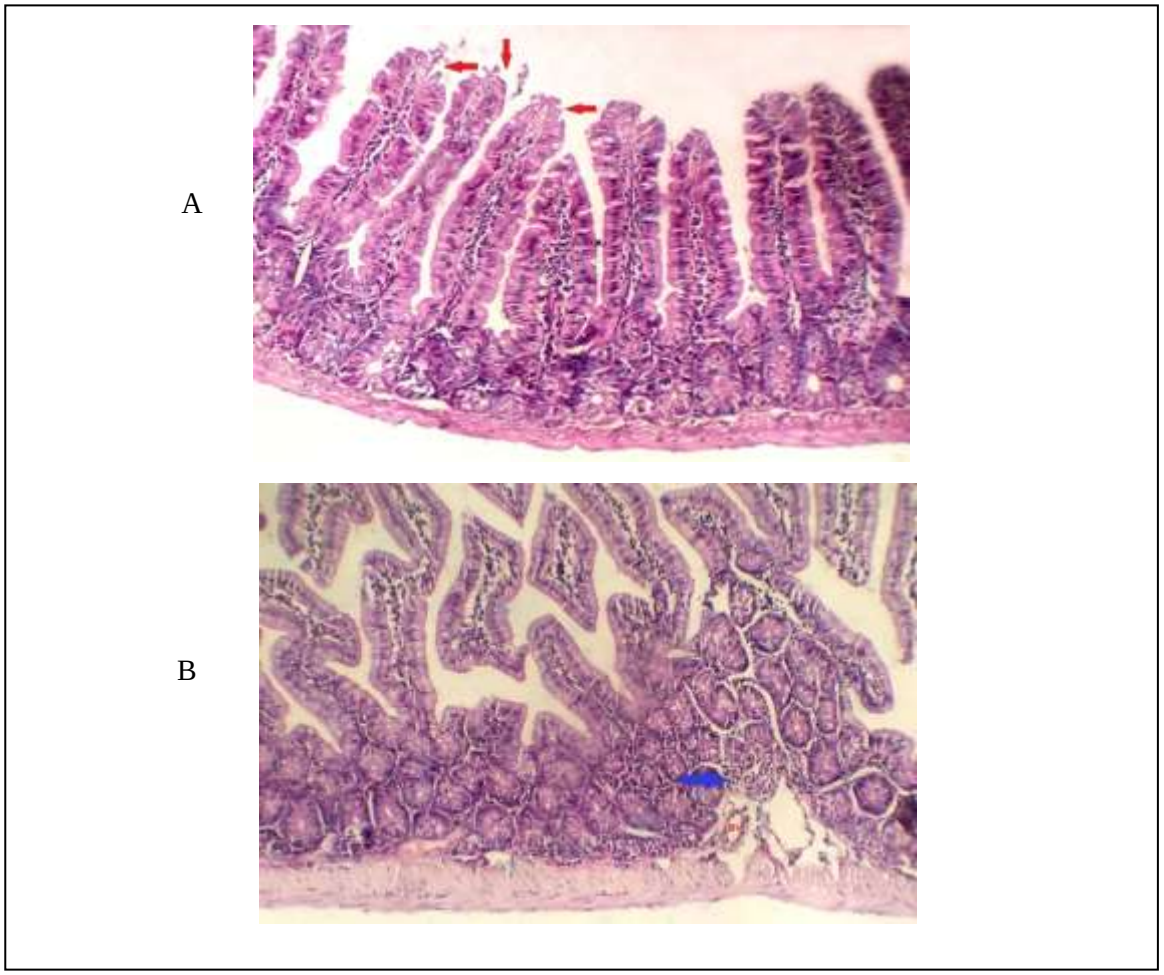

Figure (3) cross section of ileum in mice infected with *L. donovani* after one months of infection. (A) show mild destruction of mucosal cells (B) show inflammatory cells, Blood vessel, stained with H&E, 10X.

Two months-post infection

The histopathological change in ileum of mice infected with *L. donovani* after two months in figure (4) the section (A) shows a significant inflammatory cell in the lamina propria, which is indicative of a robust immune response against the *L. donovani* infection. These inflammatory cells include lymphocytes, macrophages, and possibly neutrophils, which are commonly recruited to sites of infection to fight off the parasite. The presence of a blood vessel (BV) is marked, which may show signs of dilation or congestion, reflecting the vascular changes that occur during inflammation. This can be a result of the increased blood flow and permeability needed for immune cell recruitment to the site of infection. The entire tissue section (B) exhibits significant changes, suggesting extensive remodeling due to chronic inflammation by parasite. There might be an increase in the number and depth of the crypts (crypt hyperplasia), which is often seen in response to epithelial injury and reflects an attempt to regenerate the damaged mucosal lining because of infection with parasite. Section (c) This section shows a marked destruction of the villi, characterized by blunting, shortening, and in some areas, complete loss of the normal finger-like projections. This severe villus atrophy suggests that the infection has led to substantial damage to the intestinal mucosa, impairing the normal absorptive function of the ileum.

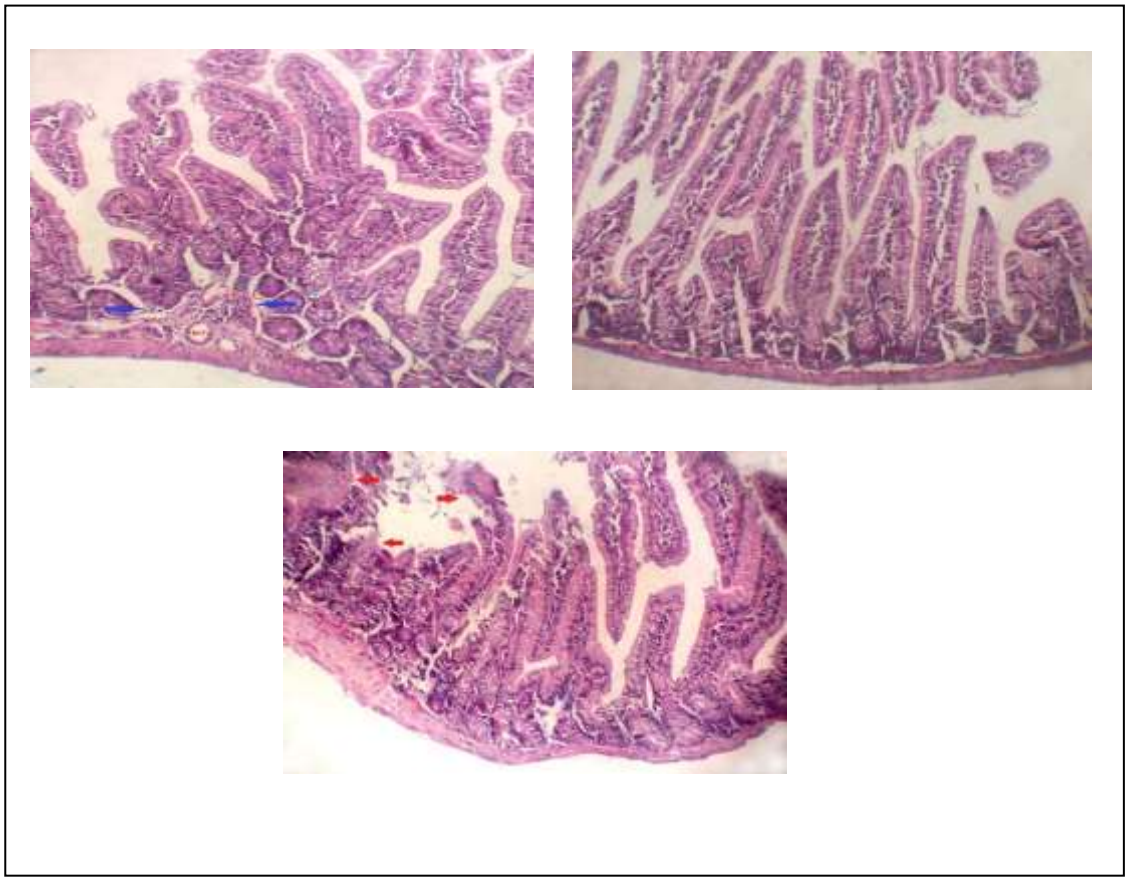

Figure (3) Cross section in ileum of mice infected with *L. donovani* after two months of infection. (A) show the inflammatory cells, Blood vessel (BV) , (B) show the whole tissue change (C) show sever villus distraction . stained with H&E, 10X .

Three months – post infection

The histopathological change in ileum of mice infected with *L. donovani* after three months in figure (5) The section (A) shows severe lymphocyte within the lamina propria, indicating an intense and chronic immune response. The dense accumulation of lymphocytes and possibly other immune cells, such as macrophages and plasma cells, reflects a sustained attempt by the host to combat the persistent infection. There is also destruction of villi evident in this section. The villi appear blunted, shortened, or completely destroyed in some areas, with loss of the normal finger-like architecture. The epitheli A g may be significantly compromised, with B ntial loss of enterocytes and goblet cells. This disruption suggests advanced tissue damage o ongoing inflammation and direct effects of the parasite. The section (B) reveals a hemorrhagic area, indicating the presence of extravasated red blood cells (RBCs) in the lamina propria or submucosa. This suggests that there has been damage to the blood vessels, either directly from the parasite or as a result of the inflammatory response causing increased vascular permeability or rupture. The surrounding tissue appears disrupted,

C

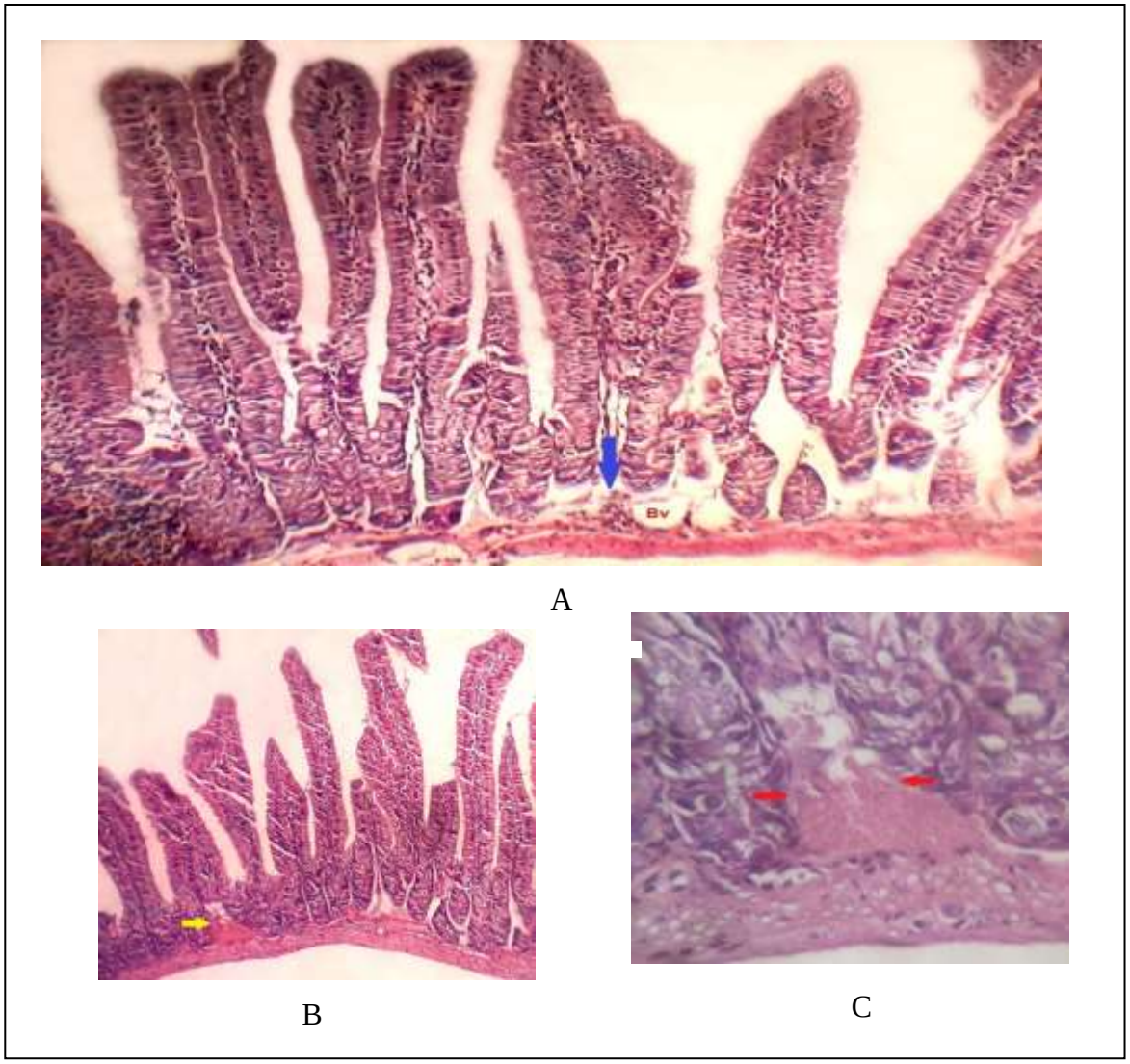

Figure (5) Cross section in ileum of mice infected with *L. donovani* after three months of infection. (A) show the sever lymphocyte infiltrations, show destruction of villi, (B) show hemorrhagic area, stained with H&E, 10X ,(C) show hemorrhagic area in 40X

Discussion

The intestine is an endocrine organ that plays a crucial role in nutrient response and mediates systemic immune responses (Cavallone, *et al* 2022). In the recent study, have observed the histopathological changes in the ileum of BALB/c mice infected with *L. donovani*. In one, two, three months post infection. These results were similar to another study that's reveal in the early stage of infection the section shows mild destruction of mucosal cells. The villi appear somewhat blunted or shortened, and there is evidence of disruption in the epithelial lining. Some areas show partial loss or damage to enterocytes, which can compromise the intestinal barrier and absorption capacity. This mild damage is an early sign of the impact of *Leishmania donovani* infection, causing epithelia damage and loss. while inlate stage of infection in the two and three months There is severe disruption, characterized by blunting, shortening, and partial destruction of the villi. This suggests that the infection has caused extensive damage to the mucosal surface, leading to a loss of the normal finger-like architecture. The epithelial lining is compromised, with possible loss of enterocytes and damage to the mucosal barrier, which is a marked deviation from the normal appearance seen in the control tissue, The severe villus disruption observed in the infected tissue indicates advanced disease progression and damage caused by the infection. The destruction of villi suggests a profound impact on the mucosal architecture and function, which could contribute to malabsorption, diarrhea, or other

gastrointestinal symptoms associated with chronic parasitic infections. This result smiler. In studies by Lima *et al* investigating the effects of *Leishmania* infection on the small intestine, significant changes were observed in the villi. For example, in golden hamsters infected with *Leishmania infantum*, researchers documented notable hypertrophy (enlargement) of the villi and the crypts in the jejunum and ileum. This hypertrophy was associated with an increase in intraepithelial lymphocytes and a reduction in goblet cell numbers, which are responsible for mucus production in the intestines. These changes were particularly pronounced at 60- and 90-days post-infection, suggesting that the structural alterations to the villi progress with the duration of the infection. Additionally, there was evidence of mucosal atrophy, or thinning, which further compromised the intestinal lining and absorption capacity. (de Lima, S. K. S., *et al 2021*)

There is a noticeable presence of inflammatory cells within the lamina propria. The inflammatory infiltrate consists of lymphocytes and possibly other immune cells, such as macrophages. This infiltration reflects an initial immune response to the presence of *L. donovani* this is in early stage of infection while in the late stage of infection There is a significant increase in inflammatory cells around the blood vessels, indicating a strong immune response to the *Leishmania* infection. The presence of these cells, such as lymphocytes and macrophages, reflects the body's attempt to control and eliminate the infection. The blood vessels (BV) appear congested and possibly dilated, which suggests an increase in vascular permeability and inflammation. This change is associated with the immune response and contributes to the observed tissue damage. The hemorrhagic areas observed in the three months post infection is more extensive, indicating severe vascular injury. The presence of hemorrhage suggests that the infection has caused significant damage to blood vessels, likely due to a combination of direct parasitic effects and the host's prolonged immune response Smiller Histopathological changes in the ileum caused by the parasite *Leishmania* have been documented in various studies. In dogs naturally infected with *Leishmania infantum*, the presence of the parasite was detected throughout different segments of the gastrointestinal tract, including the ileum. The infection led to significant cellular infiltrations in the lamina propria, muscularis mucosae, and submucosa layers, predominantly composed of macrophages, plasma cells, and lymphocytes. These infiltrations were associated with a chronic inflammatory response, but there were no severe mucosal erosions observed. The histological findings indicated that macrophages containing Leishmania amastigotes were frequently present in the ileum, particularly within the lamina propria and submucosa layers, without significant granuloma formation. (Pinto, A. J., 2011)

Furthermore, A study examining the effects of *Leishmania* species on the ileum of mice found that different species of *Leishmania* (such as *Leishmania (Viannia) braziliensis, Leishmania (Leishmania) amazonensis*, and *Leishmania (Leishmania) major*) can induce various histopathological changes. These changes include an increase in the number of immune cells like intraepithelial lymphocytes (IELs), alterations in enterocytes, hypertrophy of intestinal villi, and modifications in Paneth and goblet cells, which play crucial roles in mucosal defense and inflammation regulation. The extent of these changes depended on the specific *Leishmania* species and the duration of infection, highlighting the complex interactions between the parasite and the host's intestinal tissue. (Santos, A. G. A. D., 2018)

A study on the histopathological effects of *L.* donovani on the liver of infected mice reported that different strains of *Leishmania* could cause significant immune responses in various tissues, including the ileum. The study observed granuloma formation and infiltration of immune cells like macrophages in affected tissues, which suggests similar potential changes in the ileum due to systemic immune activation. (Bodes, F. S.,2017), Furthermore, the jejunum, duodenum, and colon can also be affected. In these areas, changes may include villus atrophy, crypt hyperplasia, increased intraepithelial lymphocytes, and chronic inflammation involving the lamina propria and submucosa. This can result in symptoms like diarrhea, malabsorption, and general gastrointestinal discomfort (Santos, *et al* , 2018)

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