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Original Article

Histopathological Study to Evaluate the Effect of Aqueous Extract of *Portunuspelagicus* and Mebendazole on Hydatid Cysts in Mice

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Abstract

Hydatid disease is a parasitic infestation by a tapeworm of the genus *Echinococcus sp*., which has a global distribution. The current study was conducted to evaluate the effectiveness of the crustacean aqueous extract of *Portunuspelagicus* for 2 weeks of treatment compared to mebendazole on hydatid cyst in laboratory mice male Balb / C strain. Mice were infected intraperitoneally with 2000 protoscolices. After 12 weeks of infection, each mouse was treated with mebendazole (50mg/kg) and the hot aqueous extract of *p. pelagicus (*8, 16 g/kg). Samples of infected organs (liver, spleen, and lungs) were examined under a microscope to evaluate the morphological and histopathological changes of hydatid cysts and tissues. The study confirmed macroscopically that there were a number of hydatid cysts of different sizes in the liver, spleen, and lungs, splenomegaly, and congestion of the lungs of the positive control group. The histological changes in the organs of the group treated with the crustacean extract were represented by the vacuolation of hepatocytes in the centrilobular area of the liver. At the same time, the lungs show intensive peri-bronchiolar inflammation, pulmonary vascular congestion, and in the spleen, the deposition of amyloid-like material in the white pulp, extramedullary hematopoiesis, While the histopathological changes in the organs of mice treated with mebendazole, were represented by the presence in the mild liver vacuolation of the centrilobular area. In contrast, the lungs show mild pulmonary vascular congestion and emphysema, and the spleen shows normal white pulp, the normal red pulp of mice. The aqueous extract *Portunuspelagicus* and mebendazole are effective in controlling the contamination in the intermediate hosts.

Keywords: hydatid cysts, *Portunuspelagicus*, aqueous extract mebendazole, Histopathology

1. Introduction

Hydatid disease is a worldwide zoonosis caused by the larval stage of the *Echinococcus sp.* tapeworm (1). Parasitism is one of the most important factors in creating restrictions and economic losses in livestock production. Cystic echinococcosis (CE), caused by unilocular hydatid cysts (HC), is one of these factors. (2). *Echinococcus* has a worldwide distribution, particularly in rural districts where the dog (definitive host)-livestock (intermediate hosts) synanthropic cycle is prevalent, which causes severe economic losses via condemnation of livers in slaughterhouses as well as direct effects on the health of animals and humans Thompson (3), (4). The disease is generally asymptomatic; the most common complication is compression or rupture of pericystic structures (5). The organ most commonly involved is the liver (50- 70%), followed by the lungs (20-30%) and other organs (like

the spleen, kidney, heart, bones, central nervous system, etc.) in less than 10% of the cases (6). The effect of infection with CE on tissues of the body depends on the affected organ and the degree of growth and development of the hydatid cyst, which is determined by factors related to the immune reaction of the host and the vitality of the larvae and tissues (7, 8).

Treatment for HC liver can include medication or surgery. So far, various medications have been used to treat HC. This study aimed to compare the therapeutic effects of an aqueous extract of *Portunuspelagicus* and mebendazole in controlling and treating mouse hydatid cysts.

2. Materials and Methods

2.1. Preparation of Aqueous Extract of *P. pelagicus* (Blue Crab):

The *P. pelagicus* (blue crab) is brought from the old Basrah market, Basra province in southern Iraq, washed, cleaned and then grilled in the oven over gentle heat to cook and burn any part of it while keeping it soft. After it is finished, it is ground in the electric grinder to become a fine powder and then was prepared by placing 50 g of crab powder in a 500 ml glass beaker and adding to 500 ml of boiled distilled water and stirring for 30 mint and left for 24 hours, then filtered with two layers of boring cloth, packed in test tubes and placed in a centrifuge for 10 minutes at a speed of 3000 rpm, then the precipitate was left, and the filter was collected in glass dishes and dried by lyophilizer and kept in the refrigerator until use.

2.2. Collection of Hydatid Cysts

Cystic *of Echinococcosis. granulosus* was obtained from the infected liver of sheep at a slaughterhouse Al-Basrah, Iraq. *E. granulosus* protoscoleces were obtained by an aseptic puncture of the cysts (9).

2.3. Viability of Protoscolices

The viability of protoscolices was determined according to Shahnazi, Azadmehr (10) by using an eosin stain to identify the alive protoscolices under microscopic.

2.4. Infection of Mice

Healthy and safe laboratory Male mice, aged 8-10 weeks and weighing 30 to 40 grams, were infected intraperitoneally with 2000 protoscolices dissolved in 0.5 ml normal saline (0.9%). Infected animals were divided into two groups: control and treatment. All the research animals were fed, given tap water, and placed at a temperature of 24 to 25°C.

2.5. Experimental Design

After 12 weeks of injecting the mice with *E. granulosus* protoscolices, each mouse in groups A, B, and C was treated with mebendazole and the hot aqueous extract of *p. pelagicus* according to the doses which had referred to, into five experimental treatment groups:

Group A (10 mice) was used as the negative control group (without infection) given distilled water, B (10 mice) was used as the positive control group (infected without treatment)given distilled water, group C (10 mice) received mebendazole at 50mg/kg BW /day, Group D (10 mice) received treated with 8 g/kg BW/day of *P. pelagicus* (Blue crab), group E (10 mice) received treated with 16 g/kg BW /day of *P. pelagicus* (Blue crab) orally for 2 weeks.

All mice from groups A, B, C, and E were euthanized after 14 weeks of infection, and a necropsy was carried out immediately after that.

2.6. Histopathological Examination

Samples were examined under a microscope to evaluate the morphological and histopathological changes of hydatid cysts and tissues. The tissue samples from the infected organs (liver, spleen, and lungs) were immediately fixed in 10% neutral buffered formalin for at least 24 hours. In progressive degrees of ethyl alcohol, fixed samples were trimmed, washed, dehydrated, cleared in xylene, and embedded in paraffin wax. According to the method described by Suvarna, Layton (11), thin slices of 4-5 m thickness were cut and stained with hematoxylin and eosin for general examination under a microscope.

3. Results

The main characteristic lesions that are observed in the macroscopical section in the infected without treatment mice (group B) included: the presence of several hydatid cysts with a variable size in the liver parenchyma, the number of hydatid cysts in the spleen with splenomegaly, and the number of hydatid cysts with congestion in the lungs.

The histological examination of some sections of these organs taken from the liver, lung, and spleen of non-infected mice showed that the tissues were normal without any histological changes.

The histological sections of the effect of infection with HC, which was caused by larvae of *E. granulo*us in males of Balb/c without treatment, observed that the liver hydatid cyst, a massive area of inflammation around the hydatid cyst (Figure 1), vacuolation of hepatocytes in the centrilobular area, vacuolation of hepatocytes in the peri-portal area and peri-portal inflammation (Figure 2). While lungs show hydatid cyst, a massive area of inflammation around the hydatid cyst, inflammatory exudate filling the alveolar spaces (Figure 3), and intensive peri-bronchiolar inflammation, massive interstitial haemorrhage (Figure 4) while in spleen shows hyperplasia of the white pulp, deposition of amyloid-like material around the white pulp (Figure 5), also hydatid cyst surrounded by a massive inflammatory infiltrate (Figure 6).

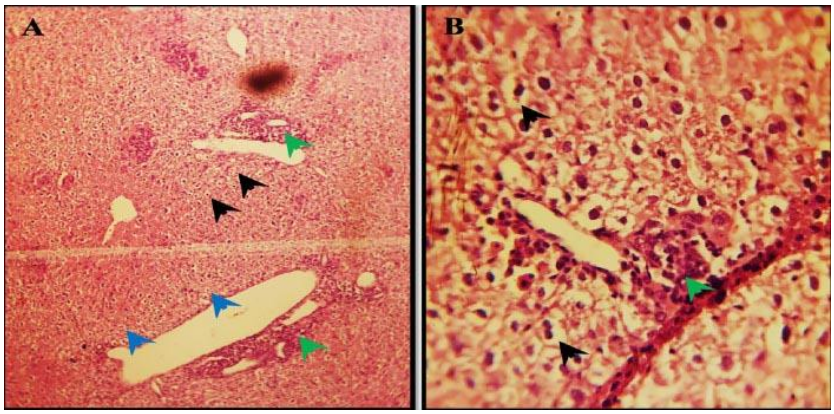

Figure 1. A section of the positive control liver of mice shows vacuolation of hepatocytes in the centrilobular area (black arrow), vacuolation of hepatocytes in the peri-portal area (blue arrow), peri-portal inflammation (triditis) (green arrow) H&E **A**) 125×, **B**) 500×

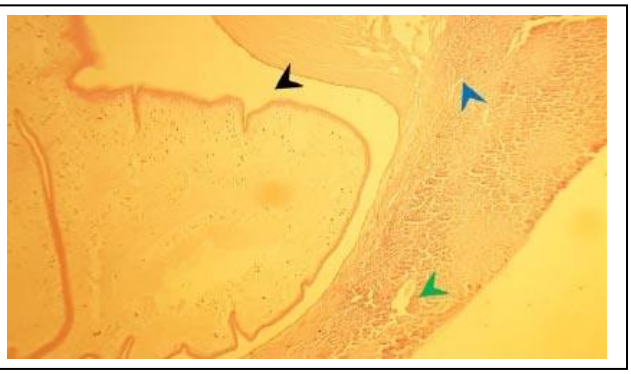

Figure 2. A section of the positive control liver of mice shows a hydatid cyst (black arrow), a massive area of inflammation around the hydatid cyst (blue arrow), central vein (green arrow)

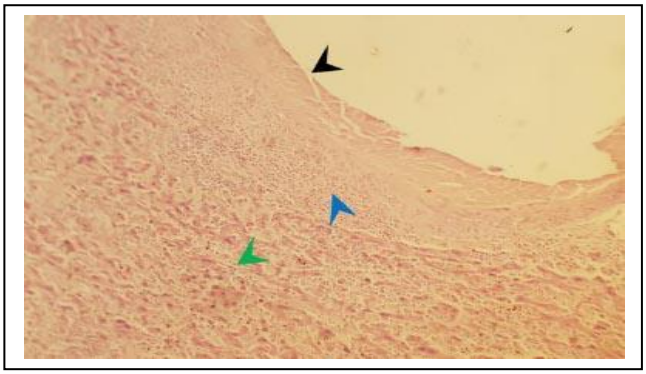

Figure 3. A section of positive control lung of mice shows hydatid cyst (black arrow), massive area of inflammation around the hydatid cyst (blue arrow), inflammatory exudate fills the alveolar spaces (green arrow) H&E 125×

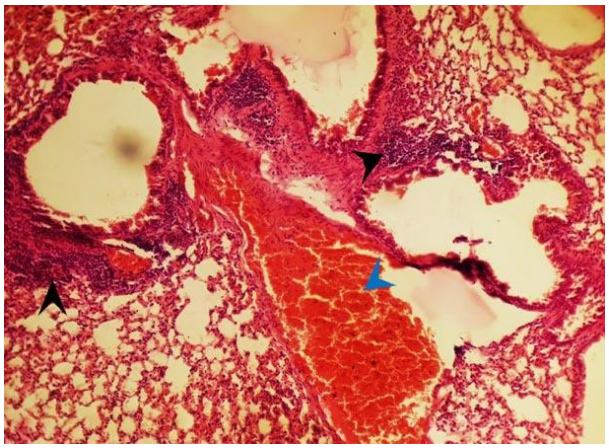

Figure 4. A section of positive control lung of mice shows intensive peri-bronchiolar inflammation (black arrow), massive interstitial hemorrhage (blue arrow) H&E 125×

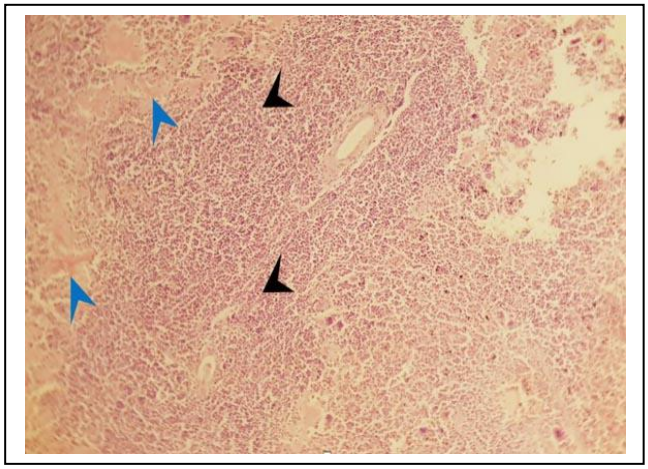

Figure 5. A section of positive control spleen of mice shows hyperplasia of the white pulp (black arrow), deposition of amyloid like material around the white pulp (blue arrow) H&E 125×

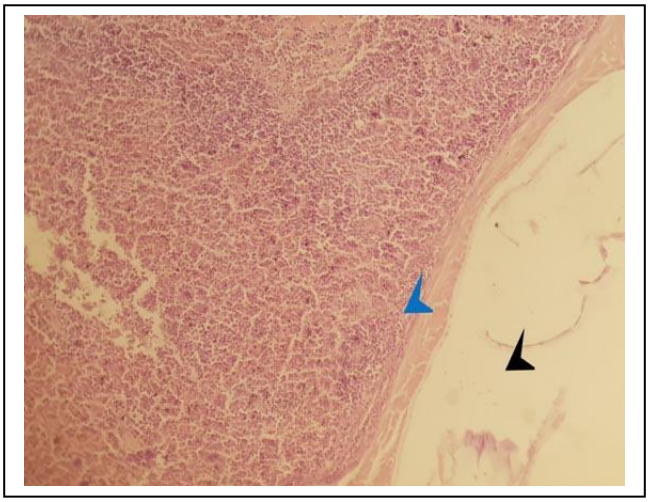

Figure 6. A section of positive control spleen of mice shows hydatid cyst (black arrow), surrounded by massive inflammatory infiltrate (blue arrow) H&E 125×

The histological examination of the liver-infected HC and treated with 50mg/kg BW/day of mebendazole shows the presence of mild vacuolation of the centrilobular area compared to the infected without treatment group (Figure 7), while the lungs show mild pulmonary vascular congestion, and emphysema (Figure 8), and also in spleen shows normal white pulp, and normal red pulp (Figure 9).

The histological examination of the infected liver with HC and treatment with hot aqueous extract of *P. pelagicus* at the concentration (8g/kg BW/day) for 2 weeks shows vacuolation of hepatocytes in the centrilobular area (Figure 10), while in the lungs show intensive peri-bronchiolar inflammation, and pulmonary vascular congestion (Figure 11). Whereas appearing in the spleen deposition of amyloid-like material in the white pulp, extramedullary hematopoiesis (Figure 12).

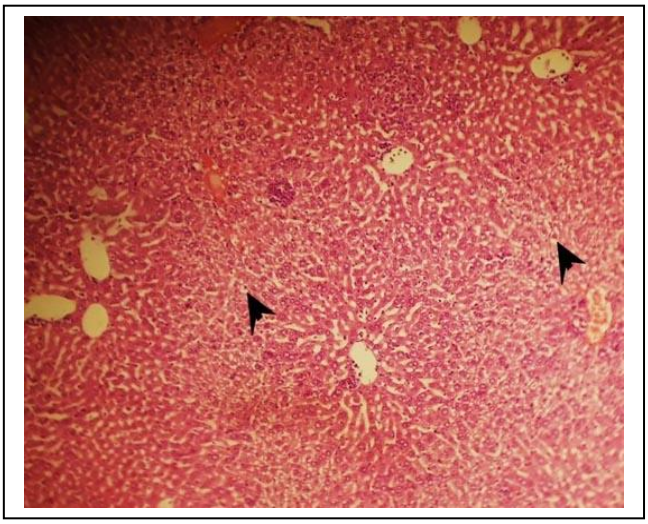

Figure 7. A section of liver treat with 50 mg/kg of mebendazole shows mild vacoulation of the centri-lobular (blackarrow) H&E 125×

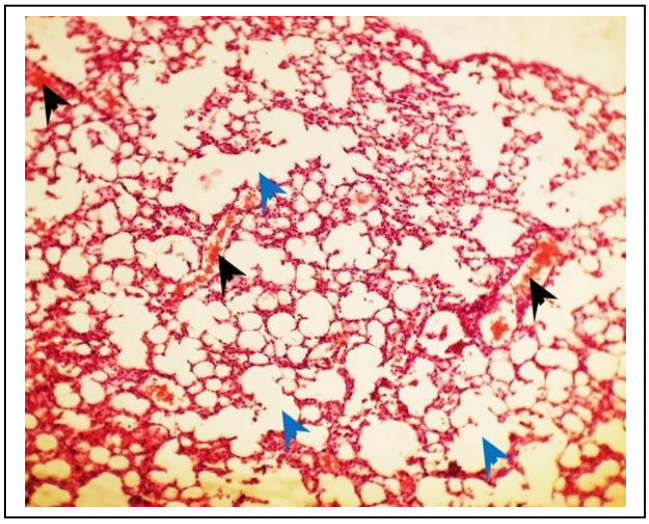

Figure 8. A section of lung treat with 50 mg/kg of mebendazole shows, mild pulmonary vascular congestion (black arrow), emphysema (blue arrow) H&E 125×

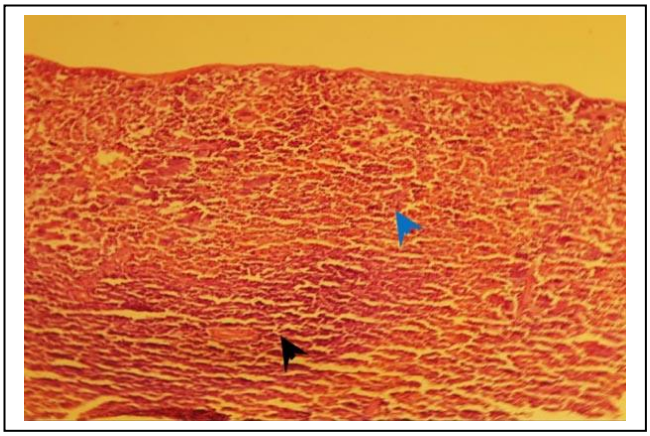

Figure 9. A section of spleen treat with 50 mg/kg of mebendazoleshows normal white pulp (black arrow), normal red pulp (blue arrow) H&E 125×

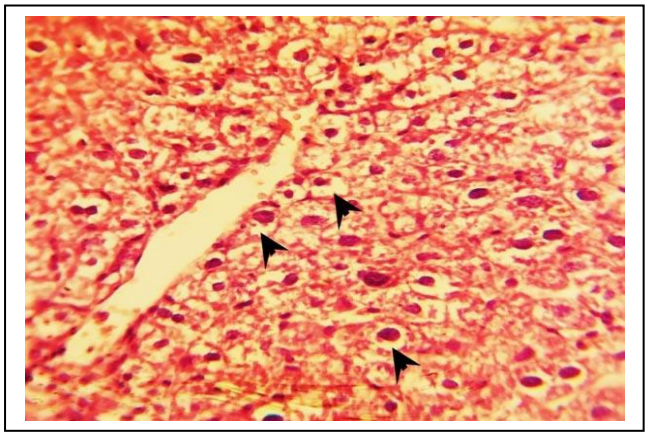

Figure 10. A section of liver treat with 8g/kg of *P. pelagicus*shows marked vacuolation of hepatocytes in the centri-lobular area (black arrow) H&E 500×

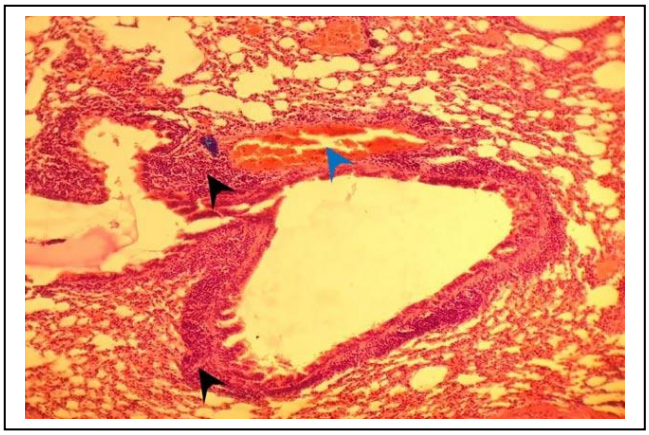

Figure 11. A section of lung treat with 8 g/kg of *P. pelagicus* shows intensive peri-bronchiolar inflammation (black arrow), pulmonary vascular congestion (bluearrow) H&E 125×

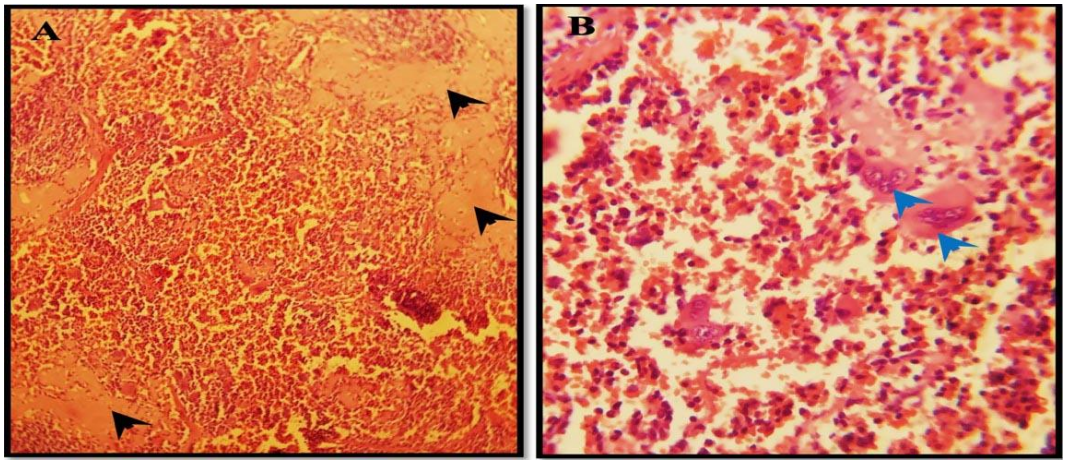

Figure 12. A section of spleen treat with 8g/kg of *P. pelagicus*shows deposition of amyloid like material in the white pulp (black arrow), extramedullary hematopoiesis. H&E **A**) 125× **B**) 500×

Histological changes in HC-infected mice treated with 16 g/kg BW /day of *P. pelagicus* (Blue crab**)** showed vacuolation of hepatocytes in the centrilobular area (Figure 13), while in lungs showed mild peri-bronchiolar inflammation, perivascular inflammation, and pulmonary vascular congestion (Figure 14) As for the spleen, the same changes were observed in the previous concentration, where no change was observed in the two concentrations on the spleen as shown in the previous section (Figure 12).

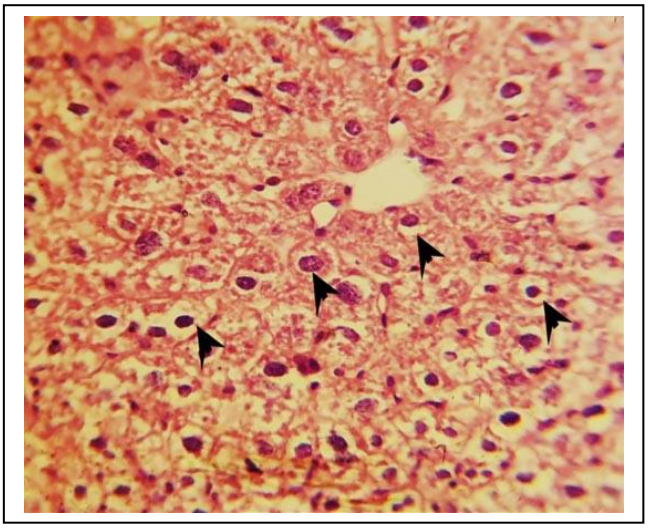

Figure 13. A section of liver treat with 16 g/kg of *P. pelagicus* shows marked vacuolation of hepatocytes in the centri- lobular area (black arrow) H&E 500×

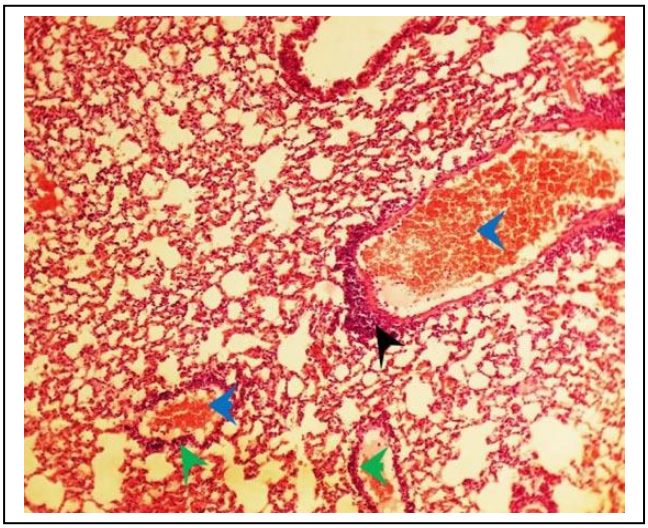

Figure 14. A section of lung treat with 16 g/kg of *P. pelagicus*shows mild peri-bronchiolar inflammation (black arrow), perivascular inflammation (green arrow) pulmonary vascular congestion (blue arrow) H&E 125×

4. Discussion

Hydatid cysts are considered an important zoonotic disease throughout the world. Although surgery is the most effective treatment, chemotherapy is the preferred method where the surgeon is not available. The results of the current study confirmed, from a macroscopic, the presence of a number of hydatid cysts in the liver, spleen, and lungs in the control-positive infected group which was consistent with the study of Mustafa, Azzal (12). This study showed that the organ most affected by hydatid cysts in laboratory mice is the liver, which is consistent with the study (12) due to the presence of liver parenchymal cells from which the parasite feeds. Also study on infected spleen showed that splenomegaly might be due to attributed to the proliferation of lymphocytes as a result of their division, the effect of activation, and their secretion of monokinis, and this is a natural result, as the spleen is the largest lymphoid organ in the production of antibodies (13, 14).

Histologically, the efficacy of mebendazole and crustaceans extract in the treatment of hydatid cysts in mice was investigated; the results showed that histological changes in liver and lung sections were shown in the untreated positive control group noted massive areas of inflammation around the hydatid cyst this is because the hydatid cyst is a foreign body that stimulates the immune reaction of the host tissues, which leads to surrounding it with a fibrous capsule infiltrated with inflammatory cells, as well as exposing the cells adjacent to its site to degeneration and morphological changes (15). These changes that occurred in the tissues of these organs may be due to a decrease in the host's immunity (16) or to the fact that the positive control groups did not receive activation or stimulation of their immune system, which led to the emergence of the histological changes (17).

The group treated with *P. pelagicus* (crustacean extract) showed deposition of amyloid-like material in the white pulp in the spleen; this result agrees with the (12) result of distorted antibodies, amyloid substances appear in the case of chronic infection in parasites, which leads to the deposition of immune substances in the spleen (Al-Nazal,2005). Vascular congestion had been seen in infected lungs, and this corresponded with the study of Inam Falah (18), and also in infected liver showed marked vacuolation of hepatocytes in the centrilobular area, that is mean mild infection; this may be because one of the main components of the crustacean extract is amino acids, which have a role in tissue repair.

While the group treated with mebendazole, a significant improvement was observed in the tissues of the liver, spleen, and lungs, and this is consistent with what was mentioned by Al-Dabagh (19), where the result of this study was attributed to the fact that the drug can interfere with the feeding of the parasite when the microtubules disappear, and this leads to a halt in the process of absorbing nutrients or transferring secretions, and then the death of the parasite.

Authors' Contribution

Study concept and design: E. S.
Acquisition of data: E. S.
Analysis and interpretation of data: H. A. M.
Drafting of the manuscript: N. K. T.
Critical revision of the manuscript for important intellectual content: N. K. T.
Statistical analysis: E. S.
Administrative, technical, and material support: E. S.

Ethics

The research of the present study on laboratory animals has been carried out and approved according to the rules set by the University of Basra Ethics Committee.

Conflict of Interest

The authors declare that they have no conflict of interest.

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