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Histopathological Changes in the Liver and Spleen of Albino Mouse, *Mus musculus* Linnaeus, 1758 Experimentally Infected with the Hydatid Cyst of *Echinococcus granulosus* (Batsch, 1786) and Its Treatments with Oxfendazole

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Abstract: The present study was conducted as an attempt to chemically treat secondary hydatid cyst disease in the Albino mouse *Mus musculus* strain Balb/c. Oxfendazole (OFZ) was used at a concentration of 30 mg/kg, and the histopathological changes in the liver and spleen were examined. The efficiency of the drug was observed compared to the positive untreated control group. However, some histopathological changes were noticed in liver and spleen of the OFZ-treated mice.

Keywords: *Echinococcus granulosus*, Oxfendazole, Liver, Spleen, Histopathological changes

Introduction

Hydatid cyst infection is one of the hazards to human health, due to the economic losses which it causes to farm animals infected with hydatid cysts, and the accompanying deficiency of proteins and vitamins, as well as the production of milk, meat and wool for sheep and cows. In addition, it may result in delayed growth and fertility, and damage to organs affected by the disease such as the liver and lungs (Gangadhar et al., 2012).

Hydatid cyst disease in Iraq is due to *Echinococcus granulosus* (Batsch, 1786). Because of the importance of hydatid cyst disease from human health and veterinary point of view, it has been adopted experimentally to infect mice, as they are similar model to humans in order to study the growth and development of the parasite and its role by clarifying the relationship between the parasite and the host (Fotiadis et al., 1999). Recently, Sadek (2022) investigated the efficacy of oxfendazole, praziquantel and albendazole in treatment of albino mice, experimentally infected with hydatid cysts.

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Materials and Methods

Mus musculus strain, Balb/c, were used in the current study, which were purchased from the National Center for Drug Control and Research in Baghdad. In addition, some individuals were obtained from laboratory-bred mice breeding.

White mice were intraperitoneally (I/P) injected with a challenge dose of 2000 live protoscolices (Wangoo et al., 1989), as well as I/P injections in positive untreated control group. The positive control group (10 mice) was given the same amount of the challenge dose, and the negative control group (10 mice) was injected with 0.2 ml/mice of physiological saline.

Oxfendazole (OFZ) (Synanthic®, Fort, Dodge, Mexico) was used at a concentration of 30 mg/kg body weight, equivalent to 0.04 mg/ml (Gavidia et al., 2010).

An attempt to treat secondary hydatidosis in mice, experimentally infected with protoscolices, by injecting through their intraperitoneal cavity with orally OFZ which was used in a suspension at a concentration of 5%.

Groups of mice were dosed one week after giving them a challenge dose of 0.25 ml of the drug by oral administration as one dose per week for three months. The group of negative control mice was dosed with the same volume of distilled water (Rafiei et al., 2009).

Medical syringes with a volume of 1 ml were used for this purpose, and needles of 21 degrees with a modification of the tip of the pointed needle and converting it to the tip of a scepter, by cutting off the sharp needle tip and replacing it with a tip made of thermal silicone to prevent injury or scratching of the mouth during the administration of the drug.

Parameters of the Study

Examination of Secondary Hydatid Cysts in Mice

Internal organs (liver and spleen) were visually examined after killing and dissecting mice, in order to determine the sizes, numbers and locations of developing secondary hydatid cysts four months after infection and treatment of groups of mice treated with the drug as well as the control group after the same period. The developing hydatid cysts were accurately separated and their diameters and weights were measured to the nearest 1 mm and gram, respectively. In the case of hydatid cysts that developed in the form of assemblies with a correlation between their cysts, their weights and diameters were measured as a single mass (Pérez-Serrano et al., 1997). Because of the difficulty of separating some hydatid cysts from each other's, their weight, number and diameter of the cysts formed for each of those gathering were determined. As for the calcified and bruised cysts, the fibrous cover was torn off by scissors and a sample of its internal contents was taken and placed on a glass slide with a drop of eosin colored drop, and its contents were examined. Casado et al. (2001) equation was followed to determine the relative efficacy of treatment by calculating the percentage reduction in the number of hydatid cysts as:

(Average number of cysts in the control group- average number of cysts in the treatment group)/ Average number of cysts in the control group x 100.

Preparation of Histological Sections

Histological sections of the resected organs (liver and spleen) of mice were prepared according to the method of Bancroft & Stevens (1982) according to the following steps:-

Fixation: Tissues were cut with a volume of 1 mm^3 taken from animal organs (liver, spleen) and placed in Bouin's solution for a whole day and then placed in ethanol alcohol at a concentration of 70%.

Dehydration: Atomization was conducted and the organ tissues were passed in water with increasing concentrations of ethyl alcohol: 50%, 60%, 70%, 80%, 90% and 100% for 60 minutes for each concentration.

Clearing: Xylene was used. The liver and spleen pieces were placed in a glass container containing xylene for two hours, to obtain the best dilution, and they were placed in a second glass container containing xylene for another hour.

Infiltration: Saturation of the tissues with paraffin wax was done at a melting point of 58 °C by using three glass containers in which the liver and spleen pieces were placed for 60 minutes in each bowl in an electric oven at a temperature of 56 °C. The saturated tissues with paraffin wax at a melting point of 58 °C by using three glass containers in which the liver and spleen pieces were placed for 60 minutes in each bowl in an electric oven at a temperature of 56 °C.

Embedding: The tissues were transferred into L-shaped wax iron molds after the base of the molds had been frozen and left in a cool place to freeze. It was separated from the mold and kept in a cool place until it is cut.

Trimming and Sectioning: The wax molds containing pieces of tissue were cut and after trimming and fixing them in a rotary microtome device, they were cut for the purpose of obtaining slices with a thickness of 4-5 micrometers with a few drops of distilled water, then the slices were placed on the slides at 40 °C hotplate to brush and flatten the sections.

Dewaxing: The glass slides were placed in the electric oven to melt the wax from the tissue pieces at 60 °C for 20 minutes, and the paraffin wax was completely removed from the tissue pieces to prepare them for the dyeing process.

Staining: To stain the glass slides, the slides were passed in a series of descending alcohols: 100%, 90%, 85%, 70% and 50% for two minutes in each concentration, until they were blue in color. Then, they were stained with eosin stain to dye the cytoplasm in a reddish pink color for 15-30 seconds, then dipped in water and taken out and transferred to an ascending series of ethyl alcohol at concentrations of 50%, 60%, 70%, 80%, 90% and 100% for two minutes in each concentration, then transferred to xylene for the purpose of purification for 10 minutes.

Downloading Distyrene Plasticizer Xylene (DPX): The DPX was used to load the glass slides and put them on a hot plate for 60 minutes to dry, then the glass slides were covered with cover slip.

Examination and Photography: The tissue sections were microscopically examined by using Olympus optical microscope. After examining them, those sections were photographed by using Olympus compound microscope equipped with a BX45 camera.

Results

Histological Changes in the Liver and Spleen

The histological sections of the liver of normal mice, are consisting of lobules. Each lobule is mediated by a branch of the hepatic vein called the central vein, which occupies each lobule of hepatocytes. The lobules are arranged in the form of radial cords, so that spaces are observed between them, which are called hepatic sinusoids (Plate 1). In the positive control condition, scattering and irregularity of hepatocytes and fibrosis were observed (Plate 2). When using OFZ, histological sections of the treated mice's liver showed hepatocyte hypertrophy, central vein expansion, blood congestion, and nuclei lysis of some hepatocytes (Plate 3).

As for the normal shape of the spleen tissue (negative control), white pulp, lymphatic follicle, germinal center, marginal band and red pulp were observed (Plate 4). When compared with changes that occurred in the positive control, rupture of the splenic parenchyma tissue, necrosis of dead cells and expansion of the white pulp were observed (Plate 5). As for the changes in the spleen tissue in OFZ-treated mice, a slight necrosis of the parenchyma tissue and a clear emergence of the white pulp at the expense of the pulp were observed (Plate 6).

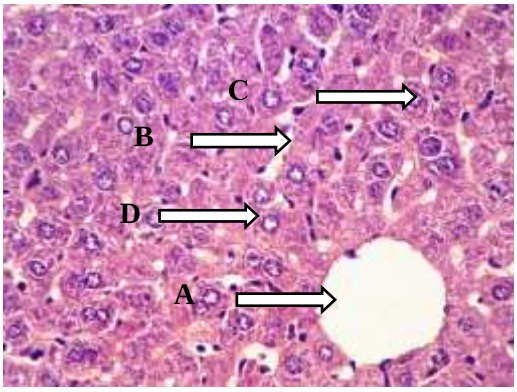

Plate 1: Negative control shows the architecture of liver tissue around the central vein (A), hepatic cords (B), hepatic sinusoids (C) and Kupffer cells (D). H&E, 250X.

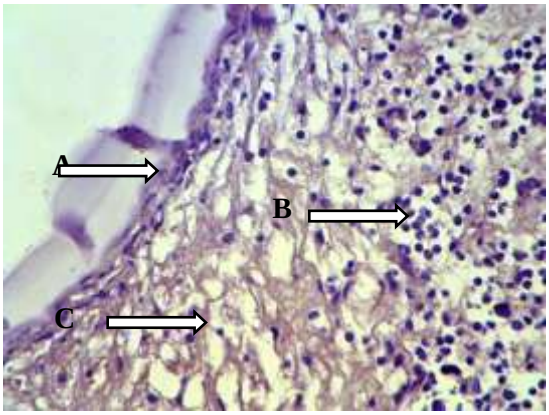

Plate 2: Positive control shows the presence of scattering hydatid cyst wall (A), irregularity of hepatocytes (B) and fibrosis (C). H&E, 400X.

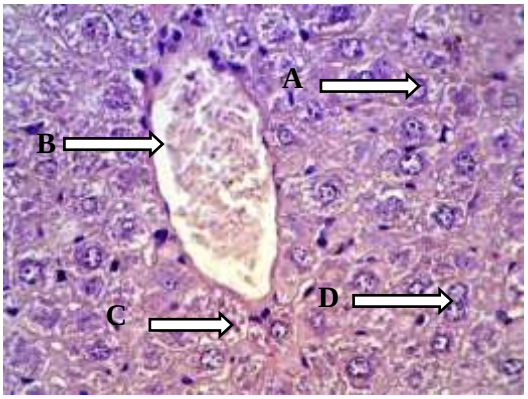

Plate 3: Treatment with OFZ shows hepatocyte hypertrophy (A), central vein dilation (B), blood congestion (C) and nucleus degeneration of some hepatocytes (D). H&E, 250X.

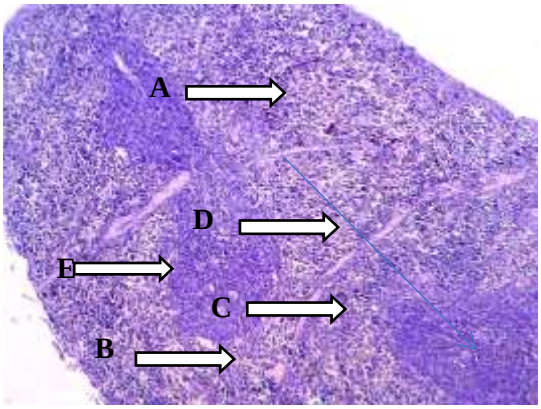

Plate 4: Negative control of normal shape of spleen tissue shows white pulp (A), lymphatic follicle (B), germinal center (C), marginal zone (D) and red pulp (E). H&E, 100X.

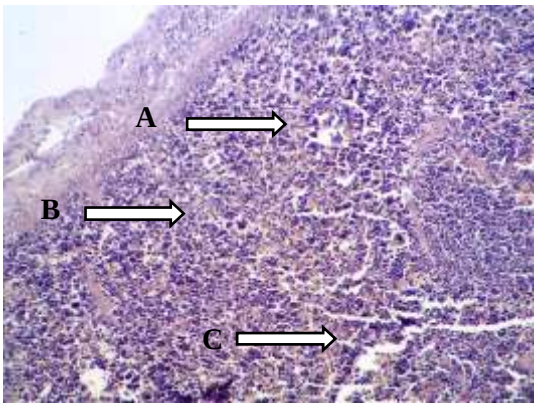

Plate 5: Positive control shows the presence of a rupture in the splenic parenchyma tissue (A), necrosis of dead cells (B) and expansion of the white pulp (C). H&E, 250X.

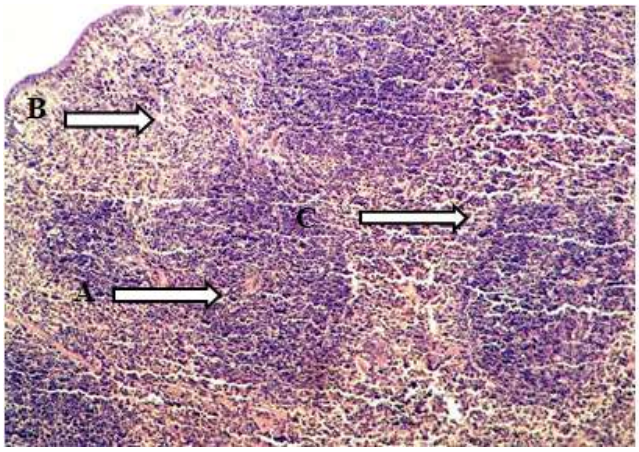

Plate 6: OFZ treatment shows simple necrosis in the parenchymal tissue of the spleen (A) and clear protrusion of the white pulp (B) at the expense of the red pulp (C). H&E, 250X.

Discussion

In intermediate hosts, the disease mostly remains asymptomatic and is usually detected at post mortem inspection. The hydatid cysts grow slowly and take several years to cause symptoms. The cysts are primarily located in liver and lungs but could be found in many other organs such as spleen, heart and kidneys (WHO/OIE, 2001). The infiltration of inflammatory lymphocytes at the site of injury due to the release of toxic substances in the liver tissue by the parasite leads to activation of eosinophil formation as the size of the hydatid cyst increases, as well as an increase in the number of macrophages that remove the host tissues affected by infection (Pollaco et al., 1978).

The parasite evades the immune system of its host by blocking the surface of its antigens and interfering with this antigen-presenting mechanism in addition to the

granulocyte as an attempt to defend the host against infection through the production of cytokines IL-8 and IL-12 (Vuitton, 2003).

The histopathological changes in the liver of mice treated with OFZ showed hepatocyte hypertrophy, central vein expansion, blood congestion and the nucleus degeneration of some hepatocytes (Plate 3) compared with the changes that occurred in the positive control, represented by the occurrence of scattering and irregularity of hepatocytes and the occurrence of fibrosis, which indicates the role of the drug in reducing the damage that occurred, by using of OFZ and Albendazole in combination form gave better results because of restoration of apoptosis pathway (Hussein et al., 2021). As for the changes in the spleen tissue of the mice treated with OFZ, it was noted that the damage was less, represented by simple necrosis of the parenchyma tissue and a clear emergence of white pulp at the expense of the red pulp (Plate 6) compared to the positive control. Sadek & Al-Taie (2022) also confirmed that OFZ gave the best treatment of laboratory animals infected with hydatid cysts.

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