Histopathological Changes of Hydatidosis in the Liver and Spleen of Albino Mice: Dose Effect Changes

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Abstract:

Background: A number of investigators have carried out experimental infections of hydatidosis or cystic echinococcosis, using albino mice as an experimental animal model, but there has been disagreement about the effect of the parasite infective dose on the outcome of infection. Studies, which have been carried out in Iraq and other places used protoscolices (PSCs) to produce cystic echinococcosis, but the inoculation dose showed variations.

Objectives: This study was established to shed light on the relationship between the number of injected PSCs and the pathological changes that consequently occur in the liver and spleen, as these organs have a role in the immune status of the host, and they are a target for such an infection.

Materials and Methods: Ninety male mice aged 7-8 weeks were injected intraperitoneally with 6 doses (250, 500, 1000, 1500, 2000, 2500) of PSC/mouse. Fifteen mice were injected for each dose. All doses were paralleled by one control group of 15 mice injected with normal, saline. Five animals were sacrificed at one, two and four month post-injection. Their livers and spleens were collected for histopathological examination.

Results: Microscopical examination of livers and spleens of animals injected with doses: 250, 500, 1000, 1500, 2000 and 2500 PSCs/ mouse showed severe pathological changes which increased with dose and period post-infection.

Conclusion: Both liver and spleen were affected in mice injected with different doses of PSCs, even if it did not show a well developed cyst. These effects were greater as the doses and post-infection period increased, especially in the four months post- infection.

Key words: Histopathological changes, hydatidosis, liver and spleen, albino mice, dose effect.

Introduction

Hydatid disease, also known as echinococcosis or hydatidosis, is caused by infection with the larva (metacestode) of tapeworms of the genus *Echinococcus* [1]. *Echinococcus* species require two hosts for the completion of the life cycle. The definitive hosts are carnivores harboring the mature tapeworms in the intestine, while metacestodes develop in the liver and other viscera of intermediate hosts of *Echinococcus* (e.g. humans, sheep, cattle…etc.) [2].

The development of the larval stage of *E. granulosus* to form a hydatid cyst is slow and variable. It depends on a number of factors; including species, strain of parasite and host, and the degree of infection [3]. Other factors such as age, sex and immunological status of host have also been implicated [4]. A number of investigators have carried out experimental infections of cystic echinococcosis, using albino mice as an experimental animal model, but there has been disagreement about the effect of the parasite infective dose on the outcome of infection.

These studies, which have been carried out in Iraq and other places, used protoscolices (PSCs) as the causative agent of cystic echinococcosis, but their inoculation doses were variable. Kammerer and Perez-Esandi [5] injected 1000 PSCs/mouse. Juma [6] used 1500 PSCs/mouse. Al-Salami [7] and Hashemitabar *et al.* [8] used a challenge dose of 2000 PSCs/mouse. Al-Kaisy [9] used 3000 PSCs/mouse. Finally, Al-Nasiri [10] used different doses: 625, 1000 and 2000 PSCs/mouse, and she found that 625 PSCs/mouse was a sufficient dose to establish an experimental infection.

This study was established to shed light on the relationship between the number of injected PSCs and the pathological changes that consequently occur in the liver and spleen, since these organs have a role in the immune status of the host and are a target for such an infection.

Materials & Methods

i. ***Collection of Hydatid Cysts and Protoscolices***: Protoscolices (PSCs) were obtained from fertile hydatid cysts recovered from the livers of naturally infected sheep that were slaughtered at Al-Shua'la City abattoir. The cyst surface was disinfected with 70% ethanol in the presence of a burner flame, while the cyst fluid was aspirated with a disposable 20ml syringe with a 19-gauge needle under aseptic conditions to collect PSCs. To insure maximum collection of PSCs, the cyst was flushed 3-4 times with sterile normal saline containing 200 I.U. /ml penicillin and 1 mg/ml streptomycin. Protoscolices were collected in sterile centrifuge tubes and washed 3 times with sterile normal saline.

ii. ***Infection and Doses***: Ninety male mice aged 7-8 weeks were injected intraperitoneally with 6 doses of PSCs (250, 500, 1000, 1500, 2000, 2500 PSCs/mouse), 15 mice for each dose, and all doses were paralleled by one control group of 15 mice which were injected with normal saline (the total number was 105 mice). Five

animals were sacrificed at one, two and four month post-injection. Their livers and spleens were collected for histopathological preparation.

iii. ***Histopathological Preparation and Examination***: Specimens of mice livers, spleens and the newly developed hydatid cysts were fixed in 10% formalin for 48 hours. They were then subjected to washing, dehydration, clearing, infiltration, embedding, sectioning (5 µm thick sections) and staining with haematoxylin and eosin [11].

Results

Histopathological Changes in the Liver:

Microscopical examination of mice livers that were injected with either low doses of 250, 500, or high doses of 2000 and 2500 PSCs/ mouse showed severe pathological changes, which increased with post-infection period. These changes were congestion of blood vessels, dilatation in blood sinusoids, focal degeneration and necrosis; in addition to increase in central vein size (Figure 1). Also there was infiltration of inflammatory cells and increase in Kupffer cells (Figure 2). Mice infected with the doses 1000 and 1500 PSCs/ mouse showed moderate pathological changes.

The liver of mice injected with 2000 and 2500 PSCs/ mouse (higher doses) showed the most severe changes, especially congestion of blood vessels. There was simple fibrosis surrounding the biliary duct and portal vein (portal area). These ducts were surrounded by inflammatory cells and fibrosis with collagen deposition. An abnormal mitosis in inflammatory cells was observed around the fibrosis surrounding the biliary duct and portal vein plus the above changes (Figure 3). The hydatid cysts were present in some livers of infected animals were well developed, containing germinal layer, laminated layer and the beginning of the adventitious layer or fibrous capsule, but there were no protoscolices even after four months post infection (Figure 4).

Histopathological Changes in Spleen:

The most severe histopathological changes were observed in spleens at the second and fourth month post-infection, especially in animals infected with the high doses (2000 and 2500 PSCs/mouse). These changes were hyperplasia or widening of the white pulp and reduction in the red pulp (Figure 5A), presence of megakaryocytes (Figure 5B), and congestion in blood vessels with presence of hemosedrin pigment (Figure 5C). Some mice showed focal areas of degeneration in the spleen and necrotic tissue around the cyst (Figure 6).

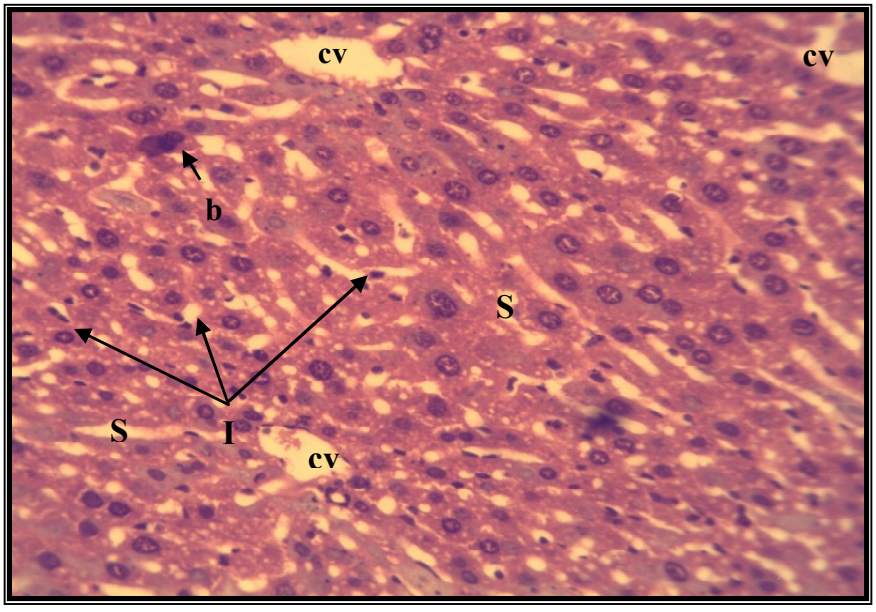

Figure (1): Section in liver of mouse intraperitoneally injected with 250 PSCs after 2 months showing mild focal degenerative cells (d), with mild inflammatory cells (I), and slight increase in central vein size (cv), dilatation in sinusoid (S) (H&E stain, 10X).

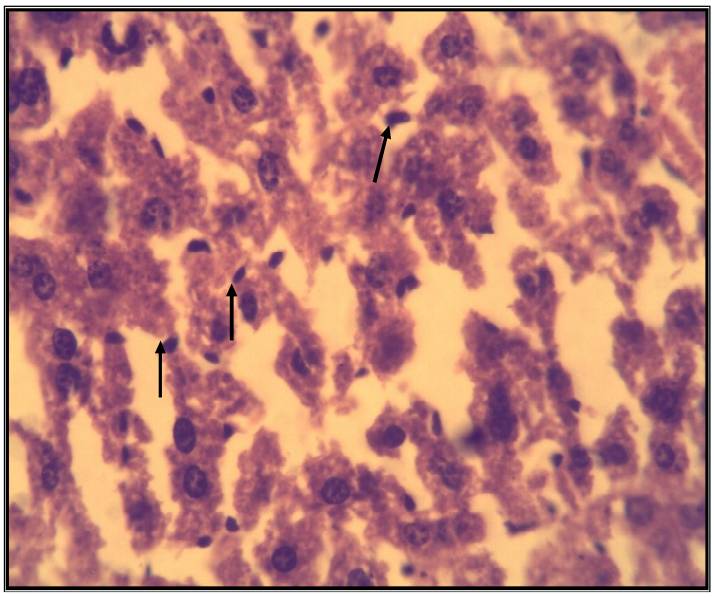

Figure (2): Kupffer cells (arrows) in section of liver of mouse intraperitonealy infected with 500 PSCs after 2 months (H&E stain, 40 X).

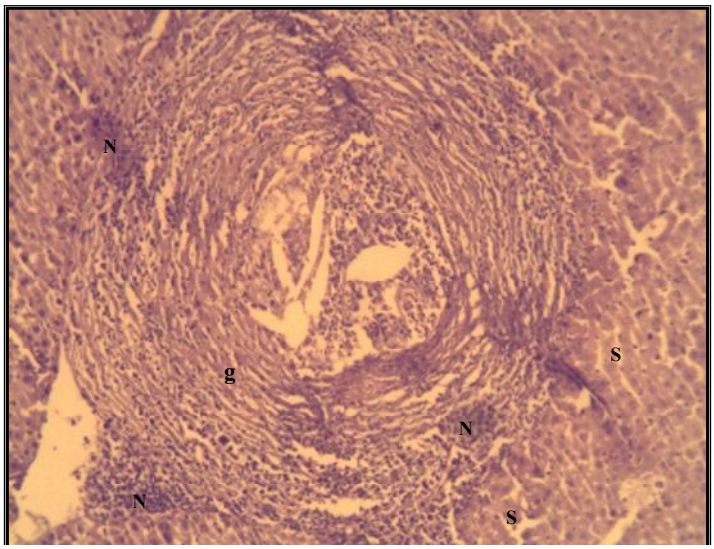

Figure (3): Section in liver of a mouse intraperitonealy infected with 2000 PSCs/mouse after 2 months showing granulomatous changes (g), sinusoids dilatation (s), and focal necrosis (N) (H&E stain, 10 X).

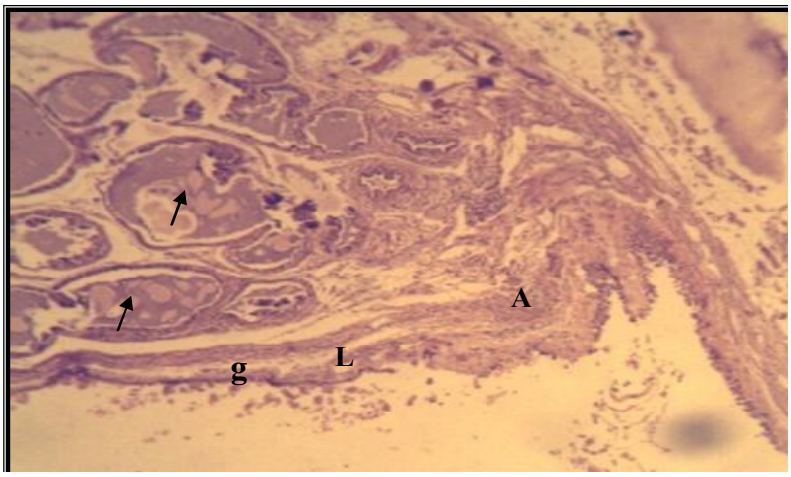

Figure (4): A well developed hydatid cyst in the liver of a mouse intraperitonealy infected with 500 PSCs after 4 months containing germinal layer (g), laminated layer (L) and the beginning of the adventitious layer or fibrous capsule (A), but there are no protoscolices and contain dead hydatid cysts (arrows) (H&E stain, 4 X).

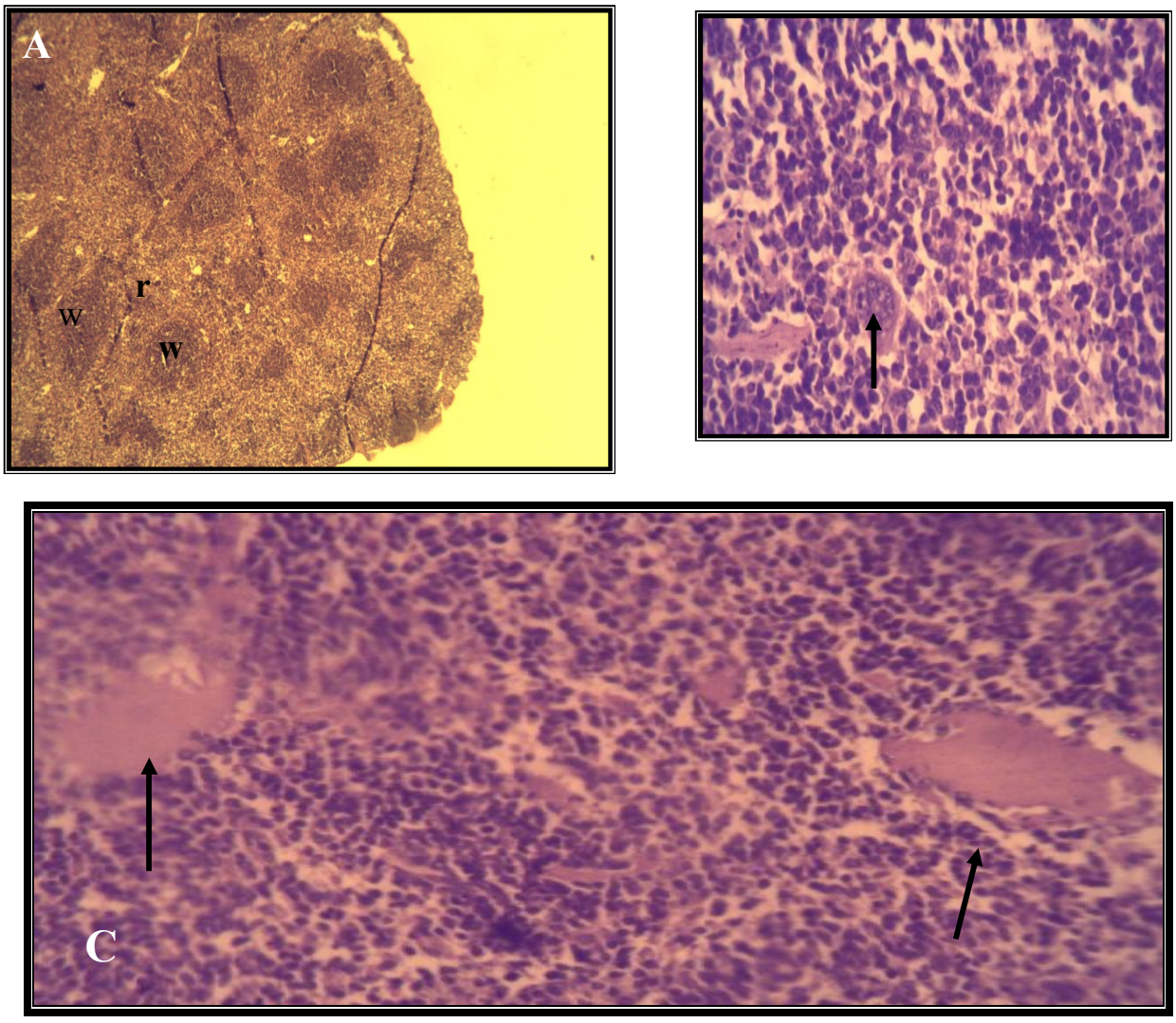

Figure (5): Section of the spleen of a mouse intraperitoneally injected with 2500 PSCs/mouse: **(A)** widening in white pulp (w) and reduction in red pulp (r) (H&E stain, 4X), **(B)** megakaryocyte (arrows) (H&E stain, 40X) and **(C)** hemosidrin pigment and congestion in blood vessels (arrows) (H&E stain, 10 X).

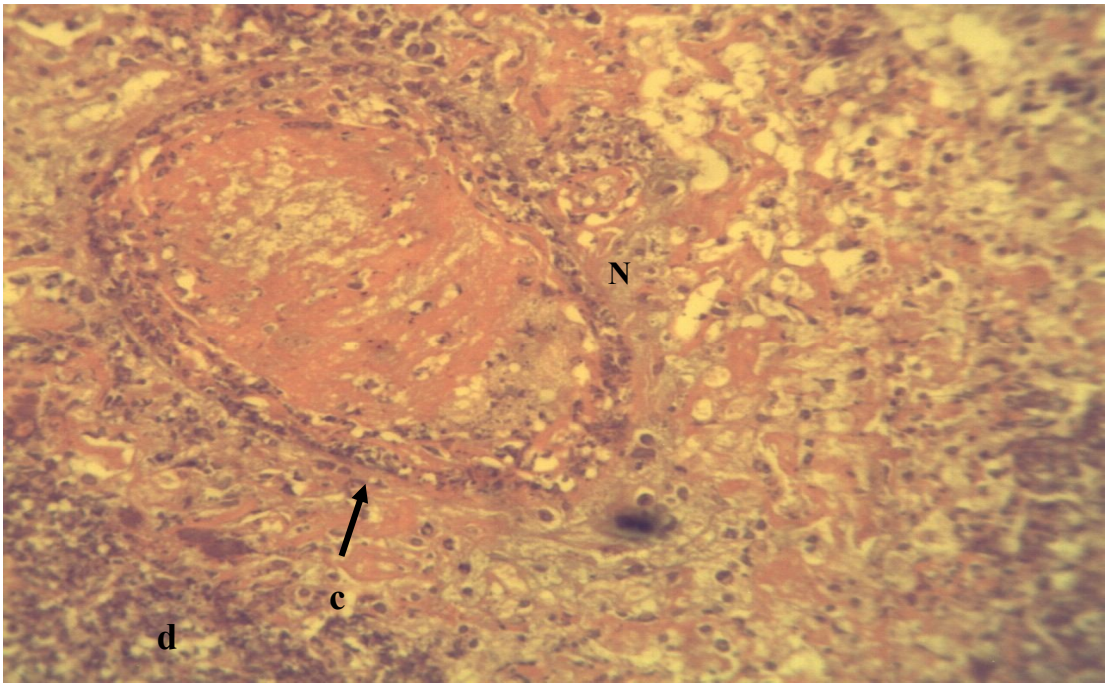

Figure (6): Section in spleen with cyst in male mouse 7-8 weeks old injected with 1000 PSCs/ mouse, four months post-infection, showing cyst (c), degeneration tissue (d), and focal necrotic tissue (n). (H&E stain, 40 X).

Discussion

In this study, the histological changes of liver of mice injected with different doses of PSCs were dependent on dose, as well as the post-infection period. The most severe changes were recorded in livers of animals injected with 2500 PSCs/mouse compared with a more subtle liver changes in mice injected with 250 and 500 PSCs/mouse. It is generally accepted that the liver is the essential body organ responsible for detoxifying and withdrawal of many injurious substances in the body [12] and the hepatic cells account for the formation of much of the blood proteins, but not gamma globulins [13]. The histopathological changes may be due to the connection between the immune status of the host and liver status. Thus, it is quite reasonable to detect great pathological consequences in this organ in mice subjected to the effects of *E. granulosus* PSCs or their antigens. Similar studies done by Guo *et al.* [14], Hanada *et al.* [15], Fayed *et al.* [16], Rashed *et al.* [17] and Al- Kaisy [9] showed the same pathological changes. The infiltration of the portal areas by lymphocytes and increase in Kupffer cells indicates an inflammatory reaction in liver tissue and have a role in defense mechanisms [18].

The histopathological findings in the spleen may be related directly to the function of the spleen as a secondary lymphoid organ in which the recognition and presentation of non-self antigens occurs [19]. Rogan [20] and Macintyre *et al.* [21] also observed that the PSCs were non-specific mitogens that lead to an increase in the multiplication of T and B lymphocytes, although this effect was not related to cyst development [22]. So the increase in size of the white pulp is related to an increase in defense cells and cell proliferation. This finding agrees with the results of Ali-Khan [23]. On the other hand the increase in number of megakaryocytes, which produce plateletes that have antibody receptors, paralleled the increase in antibodies [24].

Finally it can be concluded that both liver and spleen were affected in mice injected with different doses of PSCs, even in mice that showed no developing cysts or infection (these may be because of some PSCs fail to develop and die, being killed by the inflammatory reaction, and act as antigens that stimulate and influence the inflammatory reaction or the cyst need more time to become established). Large effects observed in the livers and spleens of mice injected with low doses (250 and 500 PSCs/mouse). These effects increased as the dose increased, especially in high doses (2000 and 2500 PSCs/mouse) and the period post-infection increased, especially at four months post-infection.

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