Histopathological Changes of Experimental Hydatidosis in Liver and Spleen of Albino Mice: Age and Sex Effect

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Summary:

Background: A number of investigators have carried out experimental infections of hydatidosis, using albino mice as an experimental animal model, but there was disagreement on the effect of strain, sex and age of this model.

Materials and Methods: Two hundred and forty mice (120 males and 120 females) were injected intraperitoneally with a single dose of 2000 protoscolices (PSCs) /mouse at four ages (3-4, 7-8, 10 and 20 weeks). Each age group consisted of 60 mice (30 males and 30 females); in which 15 animals of each sex were the treated group, while the other 15 animals were a control group (injected with normal saline). Five animals from each age and sex were sacrificed at one, two and four month post-injection, and their livers and spleens were collected for histopathological examination.

Results: the highest numbers of cysts and the most severe histopathological changes in livers and spleens of mice were observed at the age 3-4 weeks in both sexes. These changes increased with time post-infection. The changes became more severe in males than females in the age 7-8 weeks, but at the age 20 weeks, the females showed the most severe histopathological changes, while the age 10 weeks showed the lowest changes in both sexes.

Conclusion: The age and the sex of the host can modify the course of infection with hydatidosis, and the present results suggest that, mice at the ages 3-4 (in both sexes) and 7-8 weeks old (males) produce the most severe pathological changes in these organs. The more resistant age to hydatidosis was observed at 10 weeks old in both sexes.

Keywords: Histopathological Changes, hydatidosis, liver and spleen, albino mice, effect of ages and sexes.

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Introduction:

Echinococcosis or hydatidosis (HD) is one of the oldest diseases known to man. The causative agent is *Echinococcus granulosus*[1]. The life cycle of *E. granulosus* involves a definitive and an intermediate host. Hydatid cysts are classified as primary or secondary. The primary cysts, which are the commoner variety, are formed as a result of direct infection with hydatid embryos in the organs. Hence, a rupture of a primary cyst (e.g. during surgery) can result in dissemination and formation of secondary cysts in the pleural or peritoneal cavity. The secondary cysts grow slowly [2]. The technique for establishing secondary hydatid cyst experimentally via inoculation of laboratory animals (especially rodents) with *Echinococcus* protoscolices (PSCs) has been investigated by many workers. A number of investigators have carried out experimental infections of HD. Some used eggs; others used protoscolices of *E. granulosus* as the infective agent [3, 4]. For the most common experimental animal, the laboratory albino mouse, there was a disagreement on the effect of sex and age of the host. Schwabe *et al.* [5] found that younger mice were more susceptible to infection with *E. granulosus*, and it was a more severe infection. Kammerer and Perez-Esandi [6] in their experimental study used 4-5 weeks old mice. Juma [7] used 3-5 weeks old mice. Al-Salami [8] used 3 weeks old mice. Hashemitabar *et al.* [9] used 6weeks old mice. Al-Nasiri [10] used 4-7 weeks old mice. They used these ages without mentioning the reason for choosing such ages, except Al-Nasiri [10], who found that 5 weeks old mice is a suitable age for experimental infection of HD.

The gender of the host may or may not affect the development of larval stage of *E. granulosus*. Gemmell [11] found that the prevalence of *E. granulosus* at all ages studied was higher in female than male sheep. Frayha *et al.* [12] found that female mice harbour fewer and smaller cysts than males, while Al-Saegh [13] reported that female mice were apparently more susceptible to secondary hydatid disease than males but the difference was statistically not significant. In experimental infection of HD, the workers used different sexes of white mice without mentioning the effect of sex on their work [6, 7, 8, 10, and 14]. This study was established to shed light on the relationship between the age and sex of mice and the pathological changes that consequently occur in liver and spleen, since these organs have a role in the immune status of the host, and they are a target for the infection.

Materials and Methods:

Collection of Hydatid Cysts and Protoscolices: Protoscolices were obtained from fertile hydatid cysts recovered from the liver of naturally infected sheep that were slaughtered at Al-Shua'la city abattoir. The cyst surface was disinfected with 70%

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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 a 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.

Infection and Age and sex of host: One hundred twenty male and 120 female mice were injected intraperitoneally with a single dose (2000 PSCs/mouse) at four ages (3-4, 7-8, 10 and 20 weeks). Each age group consisted of 60 mice (30 males and 30 females); in which 15 animals of each sex were the treated group, while the other 15 animals were a control group (injected with normal saline). Five animals from each age and sex were sacrificed at one, two and four month post-injection, and their liver and spleen were collected for histopathological preparation.

Histopathological preparation and examination: Specimens of liver, spleen and cyst were fixed in 10% formalin for 48 hours, and then subjected to washing, dehydration, clearing, impregnation, embedding, sectioning (5 µm thick sections) and staining (haematoxylin and eosin)[15].

Results:

The highest number of cysts present in mice was observed in 3-4 weeks old males (18 cysts, 13 of which were in the liver) one month post-infection followed by the age group 7-8 weeks, in which the males showed 16 cysts at the same time post-infection. The same observations were true in the second and fourth month post-infection. For females, the age group 3-4 weeks recorded the highest numbers of cysts in liver (6, 2 and 3 cysts) at one two and four months post-infection, while in the age 7-8 weeks no cyst was developed at all months post-injection. At the age 20 weeks, females established more cysts than males, especially four months post-infection (7 *vs.* 1 cyst). Similarly, females showed more cysts than males two months post infection at age 10 weeks (Table-1). In the spleen no cysts developed at any ages or sexes.

Table 1: Number and diameter of cysts present in mice at different ages and sexes infected with 2000 PSCs/mouse of Echinococcus granulosus one, two and four months post-intraperitoneal infection.

Age and Sex of mice		Cyst Information								
		One Month			Two Months			Four Months		
		Total No. of Cysts	No. of cysts in liver	Diameter (mm) Mean ± S.E.	Total No. of Cysts	No. of cysts in liver	Diameter (mm) Mean ± S.E.	Total No. of Cysts	No. of cysts in liver	Diameter (mm) Mean ± S.E.
3-4 weeks	♂	18	13L	1.0 ± 0.9	12	12L	1.0 ± 1.3	12	7L	1.7 ± 1.0
	♀	12	6L	1.0 ± 0.9	2	2L	1.5 ± 2.1	5	3L	2.1 ± 2.0
7-8 weeks	♂	16	16L	1.0 ± 0.8	9	6L	2.0 ±1.9	11	11L	2.0 ± 1.8
	♀	0	N.D.	N.D.	0	N.D.	N.D.	0	N.D.	N.D.
10 weeks	♂	0	N.D.	N.D.	1	N.D.	3.0	0	N.D.	N.D.
	♀	0	N.D.	N.D.	4	1L	3.1 ± 2.0	0	N.D.	N.D.
20 weeks	♂	1	1L	4.0	5	1L	5.2 ± 4.5	1	1L	2.0
	♀	1	1L	1.0	9	1L	2.0 ± 1.0	7	2L	2.0 ± 1.5

L: Liver; N.D.: Not Detected.

Histopathological Changes in Liver: Mice infected at age 3-4 weeks showed slight pathological changes in liver tissue in both sexes one month post-infection, and these changes increased with time. They were represented by massive focal or spotty necrosis (Figure 1A) with infiltration of inflammatory cells (lymphocytes), some present as aggregation of lymphoid tissue (Figure 1B). There was hydropic degeneration, with moderate or severe cell swelling (ballooning degeneration) or ischemic hydropic degeneration (Figure 1C), and degeneration with fatty changes also was seen. Also there was dilatation or widening of the sinusoids with congestion and hemorrhage in the blood vessels (Figure 1D). The cysts, which were present at this age, had a well developed fibrous layer, laminated layer and germinal layer without protoscolices present even after 4 months post-infection (Figure 2 A and B). Livers of 7-8 weeks old mice showed moderate histopathological changes. These changes were represented by inflammatory cell infiltration, increase of lymphoid tissues, mild necrosis, sinusoid dilation, increase in biliary ducts (hyperplasia) (Figure 3), and severe hydropic degeneration. Also there was accumulation of glycoprotein in hepatocytes (Figure 4). These changes were more sever in male mice of this age. The highest number of hydatid cysts present in the liver was observed in

males of this age group. Some of these cysts were only degenerative cysts, while other were developed and had three layers: fibrous, laminated and germinal, without protoscolices (Figure 5). The fibrous layer contained giant cells and macrophages. Some liver cysts showed calcification adjacent to the liver parenchyma (Figure 6). In mice aged 10 weeks old, although these mice approximately had no cysts developed at all months post-infection in both sexes, yet the liver tissue of some of them showed the same mild pathological changes mentioned before. Some livers showed granulomatous changes, sinusoid dilatation, chronic inflammatory cells and focal or spotty necrosis (Figure 7). The last age group (20 weeks), showed the same pathological changes in the liver, and they were observed in both sexes especially in female mice. Histopathological Changes in Spleen: In mice aged 3-4 weeks old, the pathological changes in the spleen were similar in both sexes, which were increased with time post-infection, especially at the second and fourth month. These changes were widening in white pulp or hyperplasia and reduction in red pulp, presence of megakaryocytes, and congestion in blood vessels with presence of hemosidrin pigment (Figures 8, 9 and 10). The other age groups showed the same pathological changes, which were increased with time of infection, especially in male mice aged 7-8 weeks old. Mild or no pathological changes were seen in female mice aged 7-8 weeks old and male mice aged 10 weeks old. The other groups showed the same pathological changes.

Discussion:

Since the susceptibility of animals to infectious agents is modified greatly by age and sex [14], four age intervals were selected in the present work: 3-4 weeks old, which was sexually and immunologically immature, 7-8 weeks old, which was sexually mature and immunologically immature, 10 weeks old, which was sexually and immunologically mature and 20 weeks old, which represented aged animals [17]. The results showed that the more susceptible ages to secondary hydatid disease were mice at the ages 3-4 weeks old in both sexes and 7-8 weeks old males, which had the highest number of cysts. The susceptibility then increased in females at age 20 weeks, but it did not reach the level attained by younger ages (3-4 and 7-8 weeks). The same results were obtained from the histopathological changes that occured in livers and spleens of infected mice in the same ages and sexes. These findings agreed with the finding of Schwabe *et al.* [5], who found that white mice seven weeks old or younger at the time of inoculation were highly susceptible to initial infection with PSCs intaperitoneally. Also, they found that 10 weeks old mice or older were relatively resistant to an initial infection. Dobson [18] reported that following a single oral infection with *Nematospiroides dubius* larvae, both immature and mature male Swiss mice harbored more worms than females, and age resistance to this parasite was observed in both male and female mice. Al-Saegh [13] recorded that the unweaned and weaned, but less than two months' old mice were apparently more susceptible to infection, but the difference was not significant. The latter finding is in good agreement with the finding of the present study. This may be due to natural increase in the immune response with age. This seems to hold true with mice aged 10 weeks in spite of sex difference. Also these findings can be attributed to the effect of sex hormones on susceptibility of animals to hydatid disease. In this regard, Nakanishi, *et al*. [19] suggested that male sex hormone, testosterone, but not female sex hormone has a regulatory role in the susceptibility and cellular response of BALB/c mice to infection with *Brugia pahangi*. In experimental hydatidosis Frayha, *et al.* [12] found that female mice harbored fewer and smaller cysts than males. This was indicative that male hosts were more susceptible to infection with hydatid disease. Moreover, the course of infection was modified by treatment with exogenous hormones, and testosterone seemed to increase the susceptibility of animals of both sexes, while estradiol tended to decrease susceptibility [11]. The reasons behind females becoming more susceptible than males in older ages (20 weeks) and produce more pathological changes may be due to the reduction in sex hormones at this age, in which reproduction started to cease [16]. On the other hand Dudzinski and Mykytowycz [18] found that male rabbits were less susceptible to helminth infection than females. 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 [21]. Rogan [22] and Macintyre *et al.* [23] 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 [24]. So the increase in size of the white pulp is related to an increase in defense cells and cell proliferation. This finding corresponds with the results of Ali-Khan [25]. On the other hand the increase in number of megakaryocytes, which produce platelets that have antibody receptors, paralleled the increase in antibodies [26]. In conclusions, the age and the sex of the host can modify the course of infection with hydatidosis and the histopathological changes that are produced in liver and spleens in albino mice. The present results suggested those mice at the ages 3-4 (in both sexes) and 7-8 weeks old (males) produce the most severe pathological changes in these organs. Reverse results were observed in females at the age 7-8 weeks old. The more resistant age to hydatidosis was observed at 10 weeks old in both sexes. These results can be attributed to the maturity of the immune system and to the effect of sex hormones on the course of hydatid disease.

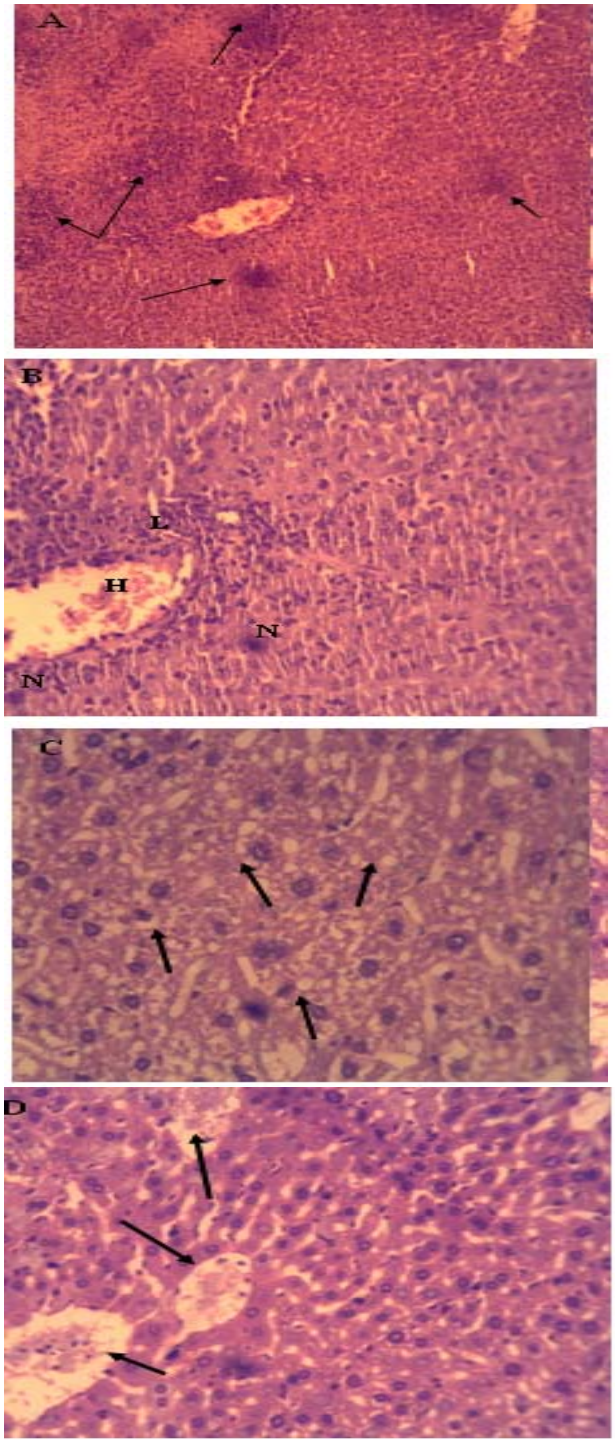

Figure (1): Section in liver of 3-4 weeks old male mouse 4 months post intraperitoneal injection with 2000 protoscoleces. A-Massive necrosis or spotted necrosis (arrows) (E&H stain, 10X). B-Necrosis (N), hemorrhage (H), lymphoid tissues (L) (E&H stain, 10X). C-Hydropic and fatty degeneration (arrows) (E&H stain, 10X). D-Blood vessel congestion (arrows) (E&H stain, 10X).

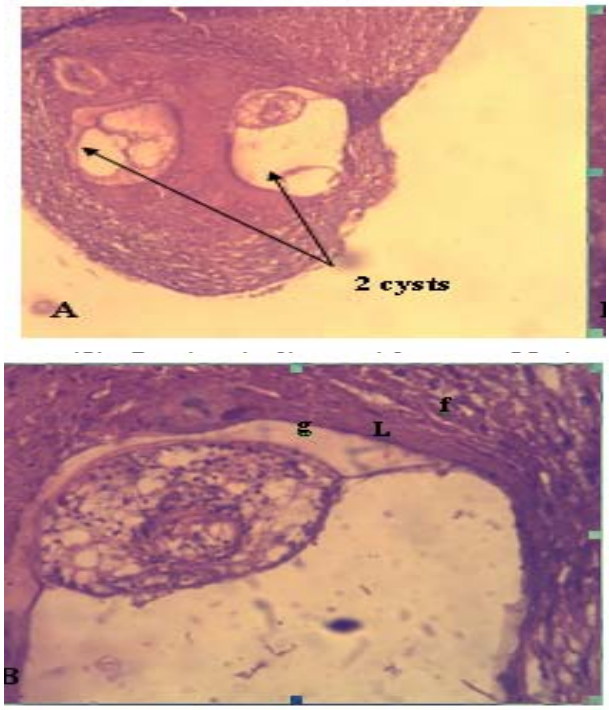

Figure (2): Section in liver with cyst of 3-4 weeks old male mouse 4 months post intraperitoneal injection with 2000 protoscoleces. (A) (E&H stain, 4X), (B) Part of A magnified cyst showing germinal layer (g), laminated layer (L), fibrous layer (f) (E&H stain, 10X).

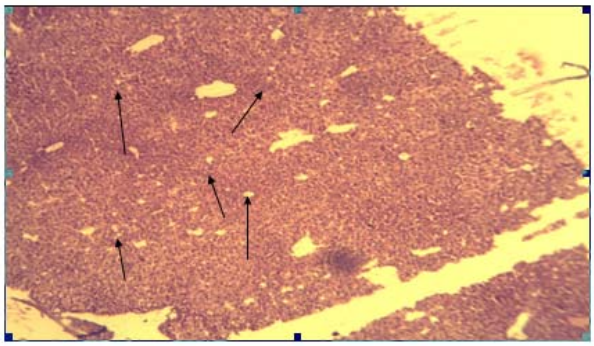

Figure (3): Section in liver of 7-8 weeks old male mouse 4 months post intraperitoneal injection with 2000 protoscoleces showing increase in biliary ducts (arrows) (E&H stain, 4X).

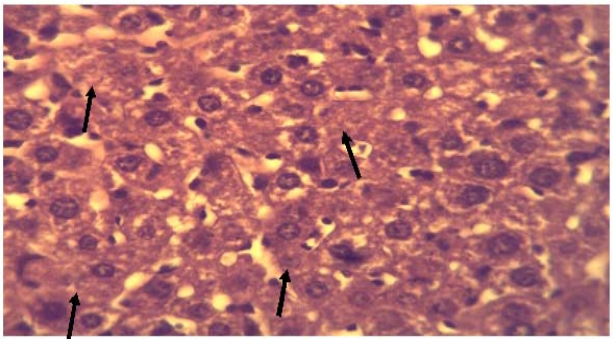

Figure (4): Section in liver of 7-8 weeks old male mouse 4 months post intraperitoneal injection with 2000 protoscoleces showing glycoprotein granules in hepatocytes (arrows) (E&H stain, 40X).

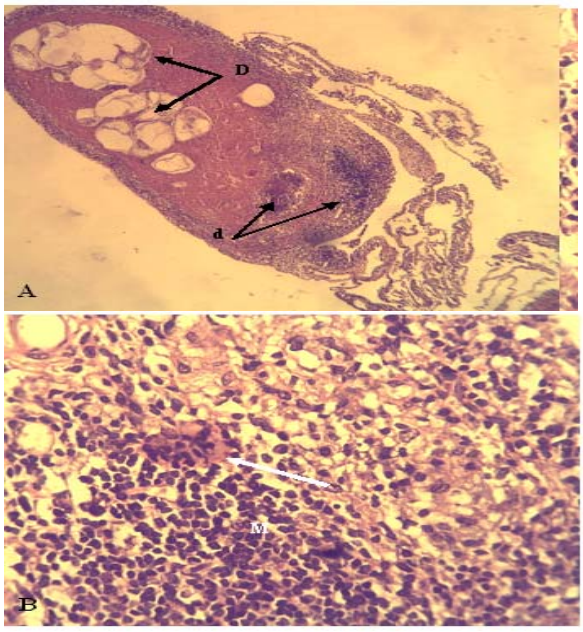

Figure (5): Sections in liver with two hydatid cysts of 7-8 weeks old male mouse 4 months post intraperitoneal injection with 2000 protoscoleces showing: (A) Section at low magnifications, developing cysts(D) and degenerating cysts (d) (E&H stain, 4x10). (B) Giant cells (small arrow) and macrophage cells (M) in fibrous layer of the cyst (E&H stain, 20x10).

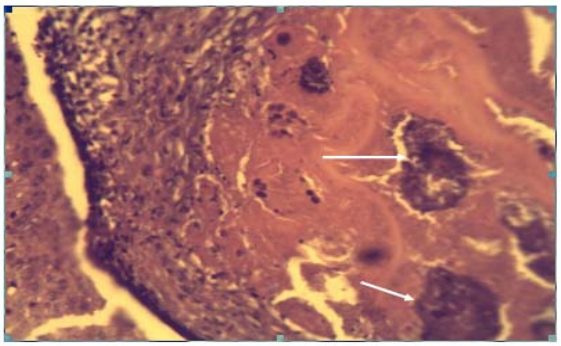

Figure (6): Section in liver with cyst of 7-8 weeks old male mouse 2 months post intraperitoneal injection with 2000 protoscoleces showing calcification adjacent to liver tissue (arrows) (E&H stain, 20x10).

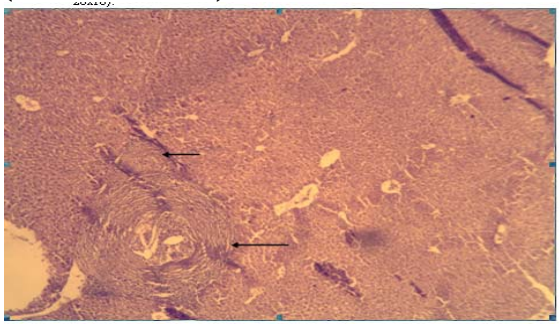

Figure (7): Section in liver of mouse 10 weeks old male mouse 2 months post intraperitoneal injection with 2000 protoscoleces showing: two granulomatous changes in liver (arrows) (H&E stain, 4 × 10).

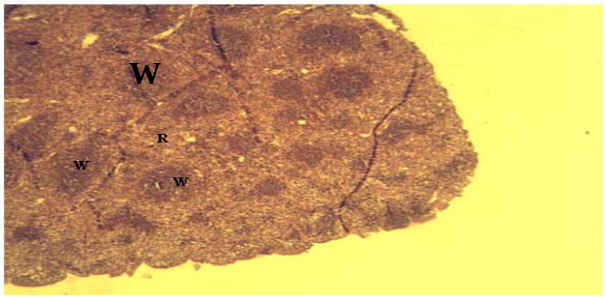

Figure (8): Section in spleen of mice aged 3-4 weeks old male mouse 4 months post intraperitoneal injection with 2000 protoscoleces showing widening in white pulp (W) reduction in red pulp (R) (H&E stain, 4×10).

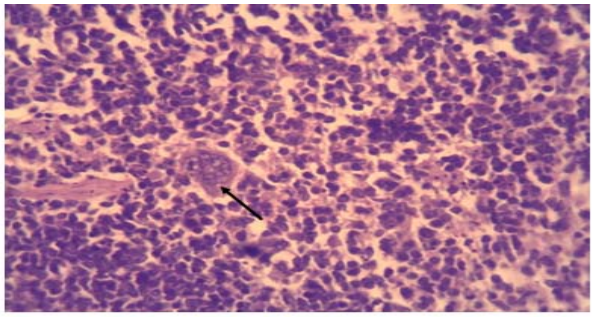

Figure (9): Section in spleen of mice aged 3-4 weeks old male mouse 4 months post intraperitoneal injection with 2000 protoscoleces showing megakaryocytic (arrow) (H&E stain, 20×10).

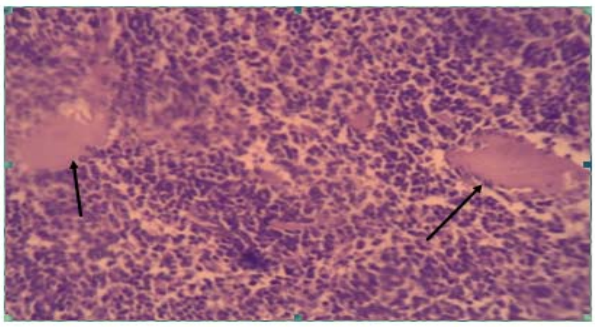

Figure (10): Section in spleen of mice aged 3-4 weeks old male mouse 4 months post intraperitoneal injection with 2000 protoscoleces showing hemosidrin pigment and congestion in blood vessels(arrows) (H&E stain, 20×10).

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