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Study the Histopathology of Immuno-Therapytrail in Mice Infected with Hydatidcysts

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Abstract: *The study was trail to treated secondary hydatid cysts in white micepost-infection with Echinococcosis of sheep origin, by immunization with a mixture of CFAgs/PSAgs prepared from haydatid Cyst Fluid antigens (CFAgs) and Proto scolices antigens (PSAgs) respectively, two concentrations (7.5 &15 mg/ml) in addition to two derivatives of benzimidazole; albendazole (A) and mebendazole (M) 10 and 40µg/gm body weight, respectively, one week after challenged dose with protoscolices.* ***Objective****: to determine the efficacy of immunization and chemotherapy simultaneously in reduction the number of growing cysts, then measured both humoral and cell-mediated immunity.* ***Results:*** *elevation the immune responses that reflected decrease number and diameter of hydatid cysts.* ***Conclusions:*** *efficacy of immunotherapeutic state in regression the growing of hydatid cysts in mice.*

Keywords: *Echinococcosis (Hydatid cysts), therapy and immunization.*

1. INTRODUCTION

Echinococcus organisms are very complex multi cellular pathogens, highly immunogenic, stimulating proinflammatory cellular responses, significantly antibody production, T cells and other cell-mediated responses in human and intermediate hosts (**Yin *et al*.; 2014**). The fact of surgery is still the first line treatment of patients with hydatidos is and may attendant difficulties or patient may be surgically ineligible for several reasons, so it is important to update the therapeutic methods like immunization with self antigens of cystic fluid and proto scolices (**Al-Qaoud*et al*.;2008; Eknfer, 2014**),and using of different chemical compounds may help in the treatment of patients, such as Albendazole, Mebendazole, were used by many researchers or other chemicals that have an impact on the patients' part (**Ul-Bari *et al*;2011; El-harti*et al*; 2014; Gavara*et al*; 2015).**

The present experiment was treated the infected mice with Echinococcos is by immunization with antigens derived mainly from the cystic fluid and protoscolices antigens mixedwith two drugs Albendazole and Mebendazole then estimated the humoral and cell-mediated immune responses in infected mice with secondary hydatid cysts growth and development.

2. MATERIALS AND METHODS

a. Cystic Fluid Antigens (CFAgs): filtered the hydatid cyst fluid by using Millipore filter paper (0.22µm) twice (30mg/ml) and kept frozen in -20 °C until use.

b. Protoscolices antigens (PSAgs): (50mg/ml) according to method of **(Migeuze*et al.*, 1996).**

c. Therapeutic Drugs Preparation; Albendazole(A) and **Mebendazole**(**M**)**,**were buying from local pharmacy as pills, which prepared according to (**Anadol*et al;*2001; Eckert &Deplazes, 2004).**

d. Indirect Heamagglutination: according to (Herbert, 1978).

2.1. Experimental Design

Sixty-five mice were divided randomly into four groups as following:

a. First Group: (n=15)injected S/C with CFAgs(30mg/ml) (50µg/g) at day 0, 7 days then get booster dose half of the first dose (25 µg/g).

b. Second Group: (n=15) immunized as in first group but with PSAgs.

Both first and second groups injected with a mixture of CF/PSAgs(50µg/g/ B.W. of mice) at day 0, later 7 days get booster dose half of the first dose (25µg/g).

c. Negative Group: (n=10) injected 0.1 ml S/C of sterile PBS.

- At day 21 post immunization sacrificed 5 animals from each immunized group (first, second and third) and negative control group, collected the blood samples for immunity statement.
- At day 28 all the immunized animals challenged by (2000protoscolices) I/P.

d. Positive Group: (n=10) infected with (2000 proto scolices) I/P.

e. Treated group: At day 7 post-challenge, the remaining from immunized animals (first and second groups) administered 0.25ml of Albendazole (10µg/g) and Mendazole 40µg/g, orally, once dose/5 days / three months (**Rafie*et al.*;2009**).

2.2. Parameters of Study Including

a. Measurements of footpad thickness (skin test): done according to **(Ali-Khan, 1978).**

b. Titration of Antibodies (Indirect Haemagglutination Test) followed a method of **Vatankhah*et al.*, (2004)** to measure the humoral immune response (volumetrictiter of antibodies) in the serum.

c. Post-treatment all the animals of treated group were sacrificed for pathological examination.

d. Statistical Analysis:

Use as analysis of unidirectional (One-way anova) and the analysis of bi-directional in the analysis of the humoral immune response. To analyze the data statistically Use the statistical ready **SPSS (2008**), and to study the moral differences between the averages of Use Dunkin polynomial test (**Duncan, 1955**).

3. RESULTS

In table (1): decreased number and diameter of cysts significantly in mice of treated group (concentration 7.5 and 15 mg/ml) on ($p \leq 0.01$);and the reduction percentage were (3.36% and 8.45%), respectively, compared with control positive group (0.00).

Table1. *Decreased number of growing cysts and their diameters in treated group as compared with positive control group.*

Groups	**Average number and diameter of cysts ± SD**			
	NOs of cysts	**Reduction %**	**Diameter**	**Reduction %**
7.5 mg / ml	4.80 ± 0.90 b	82.73	2.30 ± 0.20 a	3.36
15 mg / ml	3.00 ± 0.60 b	89.20	2.18 ± 0.10 a	8.4
Positive control	27.80 ± 3.80 a	0.00	2.38 ± 0.08 a	0.00

❖ *Different small letters refers to significant differences between groups on ($P \leq 0.01$).*

Table (2); the spread of transparent cysts in different internal organs of immunized-treated and positive control groups; significantly in liver, peritoneal cavity, gut wall and mesentery, diaphragm, spleen, lungs, in treated mice concentration 7.5 mg/ml the cysts appeared more in liver, peritoneal cavity, at rates1.7,1.5 & 1.6, respectively, in concentration of 15 mg/ml was observed in liver, peritoneal cavity and average number of cysts was 1.9, 0.8, respectively, compared with positive group (8 and 5), respectively (Figure-1).

Table2. *Average numbers of secondary Hydatid cysts and their distribution in different internal organs of treated mice as compared with positive group*.

Groups	The average number of cysts ± SE					
	Liver	Peritoneal cavity	Gut wall and mesentery	Diaphragm	Spleen	Lungs
7.5mg/ ml	1.7 ± 0.0300 b	1.5 ±0.0200 b	1.6 ± 0.0500 b	0.0 ± 0.0000 a	0.0 ± 0.0000 a	0.0 ± 0.0000 a
15 mg /ml	1.9±0.1200 b	0.8±0.1100 c	0.0 ± 0.0000 a	0.2 ± 0.0010 a	0.1 ± 0.0005 b	0.0 ± 0.0000 a
Positive control	8 ± 1.1500 a	5 ± 0.2200 a	12.7 ± 1.0100 a	0.0 ± 0.0000 a	2.0 ± 0.0400 a	0.1 ± 0.0005 a

❖ *Different small numbers refers to significant differences between groups on (P≤ 0.01).*

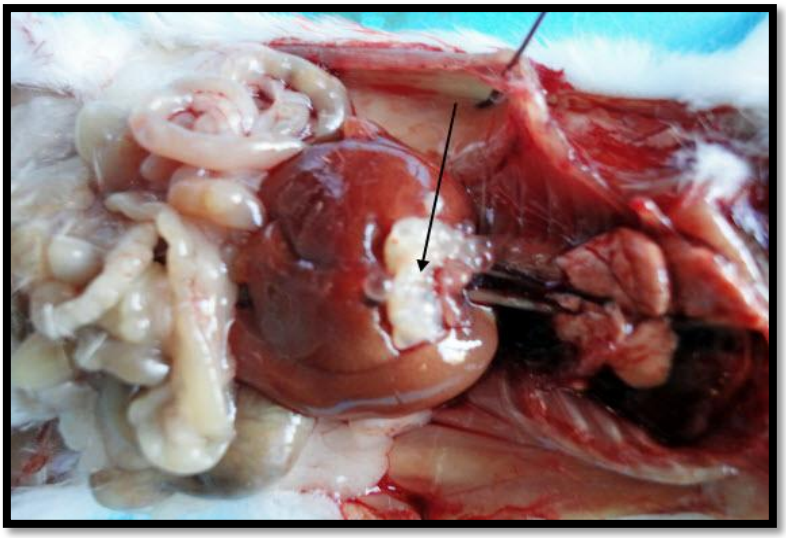

Figure1. *liver of immunized animal showed the decreasing number and diameter of hydatid cysts.*

3.1. Results of Immune Response

a. Delayed-Type Hypersensitivity: in Tables (3&4) a higher significant mean thickness of footpad appeared (P≤0.01) at 24 hours (21 day, 7.5 and 15 mg/ml) (0.50±0.07, 0.63±0.06), respectively, where decreased (0.10±0.06, 0.10±0.06) at 48 hours post-immunization when compared with the negative control mice.

Table3. *Mean of footpad thickness at24 hours of immunized, treated and control groups.*

groups / days	foot-pad thickness Mean±SD			
	7.5 mg/ml	15 mg/ml	Negative control	Positive control
21*	0.50±0.07[a] A	0.63±0.06[a] A	0.00±0.00[b] A	----
30	0.24±0.11[b] B	0.46±0.13[a] AB	0.00±0.00[c] A	0.47±0.06[a] B
60	0.23±0.08[b] B	0.33±0.07[b] B	0.00±0.00[c] A	0.61±0.14[a] A
90	0.18±0.01[b] B	0.27±0.11[b] B	0.00±0.00[c] A	0.63±0.13[a] A
120	0.26±0.10[b] B	0.32±0.11[b] B	0.00±0.00[c] A	0.69±0.13[a] A

Table4. *Mean of footpad thickness at 48 hour of immunized, treated and control groups.*

groups / days	Mean of foot-pad thickness Mean±SD			
	7.5 mg/ml	15 mg/ml	Negative control	Positive control
21*	0.10±0.06[a] B	0.10±0.06[a] A	0.00±0.00[b] A	----
30	0.20±0.04[b] A	0.19±0.05[a] A	0.00±0.00[c] A	0.26±0.03[a] B
60	0.17±0.08[b] B	0.18±0.06[b] A	0.00±0.00[c] A	0.29±0.03[a] AB
90	0.00±0.00[b] B	0.10±0.001[b] B	0.00±0.00[c] A	0.33±0.06[a] A
120	0.25±0.09[b] A	0.09±0.06[b] B	0.00±0.00[c] A	0.36±0.01[a] A

** Only mice immunized*

Lowercase letters indicate various significant differences between the groups horizontally on probability. (P≤ 0.01)

Uppercase letters indicate various significant differences between the periods vertically on the possibility (P≤ 0.01).

b. Indirect Haemaglutination Test: Table (5) shows high significant titer of antibodies in (15 mg/ml) (115.20±12.80 and 204.80±31.35, respectively) at day 21 and 120post-immunization on (p≤ 0.01) compared with the negative control mice (0.00 ±0.00).

Table5. *Antibody titers in immunized, treated and control groups at 21 and 120 days*

Groups	Antibody titers Mean ± SD	
	21 day	120 day
7.5 mg/ml	115.20±12.80 [a] B	140.80 ±20.90[a] B
15 mg/ml	204.80 ±31.35[a] A	230.40 ±17.07[a] A
Negative control	0.00 ±0.00[a] C	0.00 ±0.00[a] C
Positive control	—	47.60 ±8.51 D

**Only immunized mice.*

Uppercase letters indicate various significant differences between the groups on the possibility of vertically. (P≤ 0.01)

Lowercase letters indicate a similar lack of significant differences between periods horizontally.

4. HISTOPATHOLOGY

a. 7.5 mg/ml:

- **Liver:** The regenerative nodules of hepatocytes characterized by disorganization of hepatic architecture, more acidophilic besides presence of bile pigment diffused or within macrophages. Focal necrotic areas in hepatic parenchyma infiltrated with inflammatory cells (Figure-2). Thickening in portal areas due to severe infiltration of mononuclear cells diffusely and around congested blood vessels(Figure-3).
- **Spleen:** fibro-proliferation of capsule and trabecular, infiltration of histiocytes mainly macrophages and lymphocytes within red pulp (Figure-4).

b. 15mg/ml:

- **Liver:** There were multiple necrotic areas; eosinophilic, granular and infiltrated with mononuclear cells like lymphocytes and macrophages and haylinized areas of regressed parasitic cysts(Figure-5). Severe congestion and great dilated and filled with blood of veins and sinusoidal blood capillaries with edematous fluid also noted besides few inflammatory cells in their lumen.

c. Positive control group: in liver severevacuolar degenerative and necrotic changes of hepatocytes, congestion of central veins and sinusoidal blood capillaries. Congestion of portal veins with perivascular infiltration of mononuclear cells also diffusely infiltrated in stroma also presence of bile pigment. Multiple small focal aggregations of mononuclear cells were noted in liver parenchyma with presence of apoptic bodies(Figure-6)and(Figure-7) appeared the great thickening of splenic fjbrous capsule and hyperplasia of lymphoreticular tissue .

d. Negative control group: No significant lesions recorded.

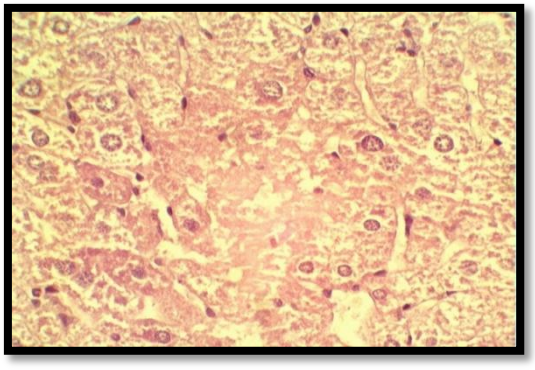

Figure2. *Liver of immunized and treated mouse (7.5mg/ml); appeared with small focal necrotic area of regressed cyst with few lymphocytes. (H&E stain, 40X).*

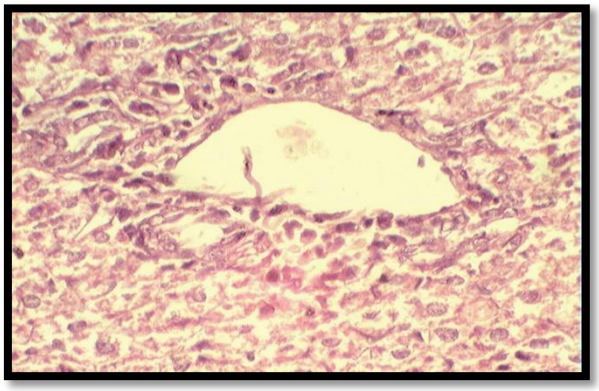

Figure3. *portal region of liver (15 µg/ml); periportal vein (dilated) infiltration of mononuclear cells and periductule in portal area.(H&E stain, 40X).*

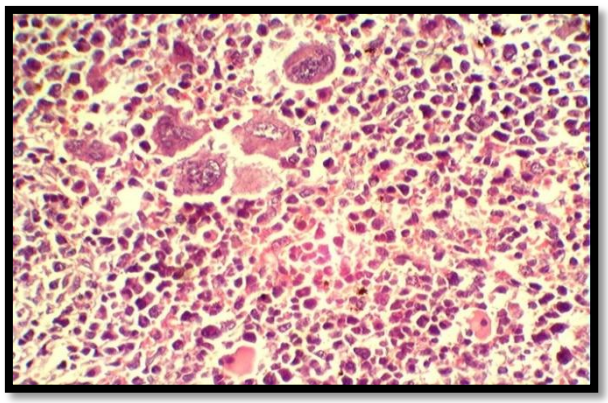

Figure4. *Spleen of positive goup; heavy infiltration of inflammatory cells (lymphocytes, macrophages and plasma cells) proliferation of megakaryocytes also. (H&E stain, 40X).*

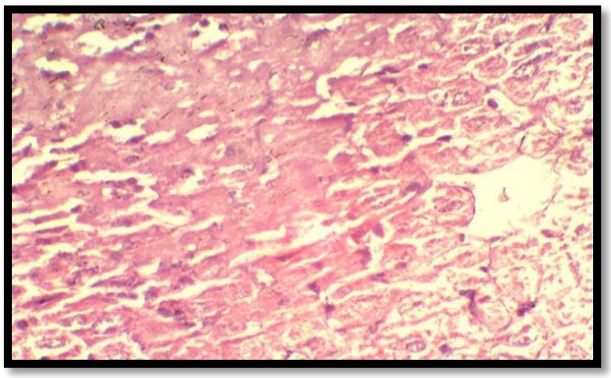

Figure5. *Liver of immunized and treated mouse (15 µg/ml); appeared with eosinophilicnecrotic area and haylinized represented un developing parasitic cyst.(H&E stain, 40X).*

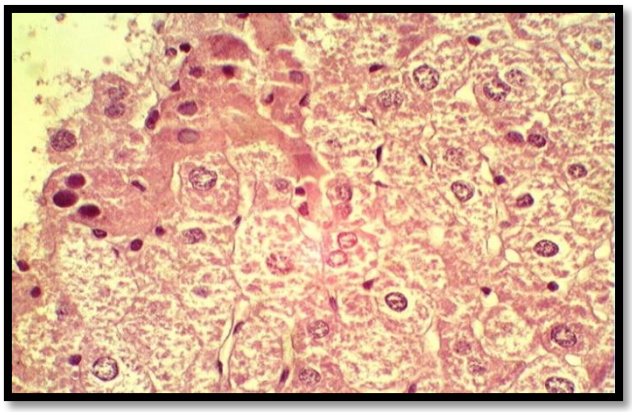

Figure6. *Apoptotic bodies and necrotic hepatocytes (lost their nuclei) in liver parenchyma of positive group. (H&E stain, 40X).*

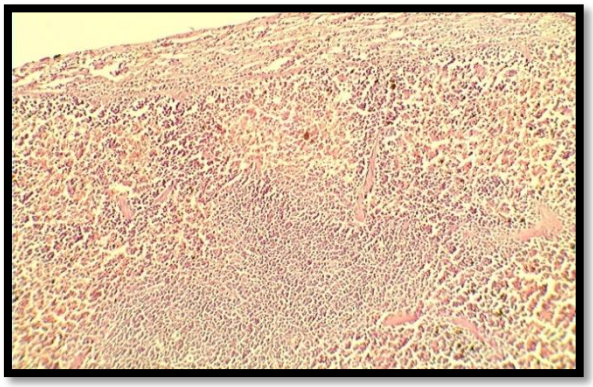

Figure7. *Spleen of positive group showed proliferation of fibrous connective tissue in capsule and subcapsular with moderate lympho-reticular hyperplasia in white pulp extened to red pulp..(H&E stain, 40X).*

5. DISCUSSION

Many series of studies tested the validity of many chemicals Anthihlmintic for the treatment of Hydatidosisand more commonly used benzimidazoel compounds which gave promising signs to control the disease. It has proven many of the studies the effectiveness of Albendazole and real estate Mebendazole against disease in humans **(Chirieki, 2002).**

Cystic fluid and protoscolices antigens were used as vaccines against *Echinococcus* **(Hashemitabar*et al*., 2007)** and demonstrated the importance of these antigens in reduction the growth of cysts also produced a high level of antibodies post-immunized **(Yossifi*et al*., 2010). (Lightowlers*et al*., 2003; Eknfer,2014)**improved the effectiveness use of several antigens (mixture) than if you used a single antigens, so the present study was improved reduction of developing cysts post immunotherapy with a mixture of CFAgs/ PSAgs a combination of Albendazole and Mebendazole **(Pérez-Serrano *et al*., 1997)**that it is necessary to give the drug shortly after the challenge dose because the impact of the killer primary appears best the free layer laminate.the reasons for the success of the drug Albendazole in control the disease haydatidos is that it is impenetrable for the cyst as sufficient levels of the metabolic compounds as Albendazole Sulfoxide which leads to the reduction of the numbers of primary and lower pressure inside the cyst, and this facilitates the process of suction watery fluid from the sac and remove it surgically so it is preferred giving before the surgery **(WHO, 1996).**

The high reduction percentage of cysts diameter were noticed post-treatment and immunizationas compared with the control positive group, **(Rafiei*et al*., 2009).**The resistant of host-immune responses by some haydatide cysts was due to the possession of different growth rates according to their own characteristics and spreadcysts in different organs of mice from control positive group and treated mice and a higher average number of cysts has scored in each liver, wall gut and mesentery and these results were identical to the findings of the **(Alnasery, 2006)** .

The high rate of pad-thickness in immunized mice (7.5 and 15 mg/ml, at day 21) compared with mice control negative that agreed with **Alnasery (2006)**, where observed high rate of delayed hypersensitivity reactions in mice immunized by primates initial antigen, which indicates the role of the antigens used in stimulating cellular immune response. The lower rate of delayed hypersensitivity reactions in mice treated concentration of 7.5 and 15 mg/ml compared with control positive group could be attributed to efficient therapeutic method used in the current study (immunization and chemicals) in the reduction the numbers of secondary haydatid cysts .

The antibody titers increased in current study at 21 day post-immunization. **Delves *et al*., (2006)** noted that the first exposure to the antigen stimulate the primary immune response represented by IgA, so the levels of IgG is low in positive animals, in spite of growth of the parasite and the formation of cysts which ensures constant stimulation parasitic antigens and possibility of a link antigens with antibodies and immune complexes are appearing in the serum of patients **(Rogan & Craig, 1997). Sadjjadii (2009)**also revealed the formation of immune complexes, which cannot be easily diagnosed by tests of immunity.

The second exposure to the antigen (booster dose) augmented secondary immune response, characterized by a high level of IgG**(Delves *et al.*2006 ,**that in consistent with the increase antibody titer in treated mice .

El-On (2003)had another idea may resulted from ruptured of hidden cysts as a result of therapy followed by liberation new antigens on the production of antibodies, and antibody levels may remain elevated for several years after successful treatment. the host's response to infection is neutralized through the influence of material in those therapeutic response and vice versa **(Alnasry, 2006).**

The infiltration of mononuclear cells mainly lymphocytes and macrophages in hepatic parenchyma and spleen post-immunization and treated groups reflected the stimulation of cell mediated immunity against parasitic infestation and small granulomatous lesions which replaced the regressed hyadatid cyst **(Al-Dehami, 2005)**comparing with affected animals in positive control group which showed areas of implanted hyadtid cyst besides lymphoid hyperplasia of white pulp in spleen and thickening of portal areas due to inflammatory cells infiltration diffusely or around congested blood vessels apoptosis also noted.

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