A trial treatment of Hydatidosis in white mice by Immunization and Chemicals

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Abstract. The present study was conducted as atrial to treat secondary hydatidosis in white mice of species Mus musculus. (Balb/c strain), experimentally infected with secondary hydatid cysts of sheep origin. The immunization process was carried by using CF/PS antigen which consist hydatid cyst fluid (CF) antigen, and protoscolices (PS) antigen mixed together and by using two concentrations 7.5, 15 mg/ml. Then derivatives of Benzimidazole, Mebendazole (M) and drugs Albendazole (A) were used 10 and 40 μm/gm body weight respectively. The mixture of drugs was used one week after challenged dose. Criteria taken into consideration to define the activity of the immunization and chemotherapy in this study were reduction in the numeral of cysts, the coefficient of split cells in spleen and boon marrow, and measurement cellular and humoral immune response. The results of the present consider demonstrated those portray the viability about immunization also chemotherapy all the while over decay those numeral of growing cyst, toward that perspective stately both humoral and cell-mediated immunity. Those outcomes indicated raise those safe reactions that replicated diminish numeral Also breadth for hydatid growth. Those goal that viability of immunotherapeutic state over reversal those developing from growing hydatid cysts to mice.

Keywords. Hydatid cyst, Treatment, Albendazole, Mebendazole

1. Introduction

Cystic Echinococcosis (CE) produced by the metacestode (larval) phase of Echinococcus granulosus is still an essential public health concern in several countries of the world so hydatid cyst remnants a important public health hazard in endemic regions in Iraq .CE is worldwide distributed , endemic in Iraq [1]. The reality of medical procedure is as yet the main line fix of patients with hydatidosis and may related complexities or patient might be carefully denied for various causes, consequently it is huge to refresh the mending techniques like immunization with self-antigens of cystic liquid and proto scolices [2], also utilizing of different compound exacerbates might aid in the medication for patients, for example, Albendazole and Mebendazole, were utilized by various researchers or different chemicals that need an influence on the patients part [3].

2. Materials and Methods

Fertile hydatid cysts, size (6-9 cm) lodged in livers of sheep slaughtered the fluid containing viability protoscolices used according [5, 6] .The viability of protoscolices was 97% (Figure-1).

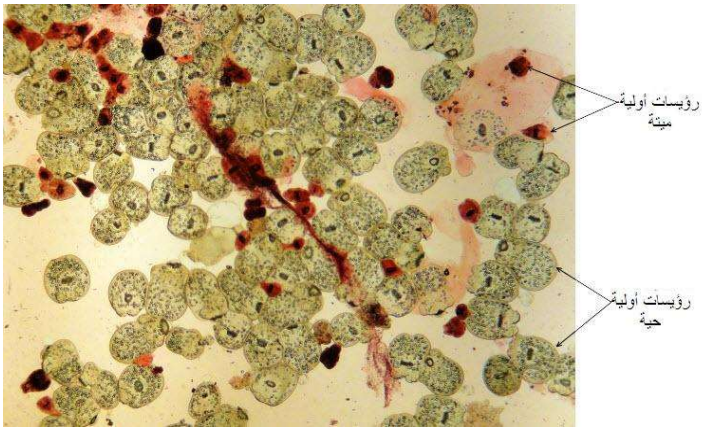

Figure 1. Microscopic appearance showing the viability of the protoscolices (10 X).

3. Preparations of Antigen

a. Cystic Fluid Antigens (CFAgs): the hydatid growth fluid separated by utilizing Millipore filter paper (0. 22 μm) two times (30mg/ml) and saved solidified clinched alongside -20 °C until utilize (6).

b. Protoscolices antigens (PSAgs): (50mg/ml) as stated by those method of **[7, 8]**. Ninety days post-challenged infection all animal groups sacrificed to detect the reduction percentages growth of hydatid cysts in internal organs as (Heath,1976)

$$\textbf{Reduction\%} = \frac{Mean\ No.of\ cysts\ in\ positive\ group - Mean\ No.of\ cysts\ in\ immunized}{Mean\ No.of\ cysts\ in\ positive\ group} x100$$

c. Therapeutic Medications Preparation: Albendazole (A) furthermore Mebendazole(M), were buying starting with nearby drug store similarly as pills, which readied as stated by those method of (9).

4. Experimental Design

Sixty-five mice were divided subjectively under four aggregations similarly as following:

a. First Group: (n=15) injected CFAgs (50μg/g) toward day 0, 7 times then get booster dose half of the primary dose (25 μg/g).

b. Second Group: (n=15) inoculated as for to start with assembly yet for PSAgs. Together first and second groups injected for a blending about CF/PS Ags (50μg/g/ b. W. mice) at day 0, later 7 days get booster dose half of the to primary dosage (25μg/g).

c. Negative Group: (n=10) injected 0. 1 ml S/C for sterile PBS. During day 21 after immunization sacrificed 5 animals from all immunized group (first, second also third) and negative control group, gathered those blood specimens to immunity articulation. In day 28 every last one of immunized animals tested by (2000 protoscolices) I/P.

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

e. Treated Group (CF/PS group): toward day 7 post-challenge, those remaining from inoculated animals (first and second groups) administered 0. 25 ml of Albendazole (10μg/g equal7. 5mg/ml) Furthermore Mendazole (40μg/g equal 25mg/ml), orally, on one occasion dose/5 days / three months [10].

5. Study Parameters

a. Titration about Antibodies (Indirect Haemagglutination Test) took after a method of [11] should measure those humoral immune response (volumetrictiter from claiming antibodies) in the serum.

b. Examination of secondary hydatid cyst in mice [12].

c. Measurements Hypersensitivity test (skin test): done according to [13].

d. Mitotic index [14].

6. Results

Determined alongside table (1): diminished number and diameter of cysts significantly in mice of dealt with aggregation (concentration 7. 5 and 15 mg/ml) at (p≤ 0. 01); and the diminish rate were (3. 36% and 8. 45%), respectively, in comparison of control positive group (0. 00).

Table 1. The reduction percentages of hydatid cyst growth and their diameters in treated group in comparison of positive control group.

Groups	Mean number and diameter of cysts ± SD			
	Nos of cysts	Reduction %	Diameter	Reduction %
7.5 mg / ml	4.8±0.90 b	82.73	2.30 ±0.20 a	3.36
15 mg / ml	3±0.60 b	89.20	2.18 ±0.10 a	8.4
Positive control	27.8±3.80 a	0.00	2.38 ±0.08 a	0

Different small letters denotes to significant differences between groups on (P≤ 0.01).

Table 2 the spread of translucent growths cysts in differing inward organs of immunized-treated and positive control groups; significantly in liver, gut wall and mesentery, peritoneal cavity, lungs, diaphragm and spleen in treated mice at concentration 7.5 mg/ml the cysts showed up additional in liver, peritoneal cavity, at extents 1.7,1.5 and 1.6, separately, in concentration of 15 mg/ml was identified in liver, peritoneal cavity and mean number of cysts was 1.9, 0.8, individually, in correlation of positive group (8 and 5), individually.

Table 2. A mean numbers of secondary Hydatid cysts and their scattering in diverse internal organs of treated mice in comparison of positive group.

Groups	**The mean number of cysts ± SE**		
	7.5mg/ ml	**15mg/ml**	**Positive control**
liver	1.7±0.0300 b	1.9±0.1200b	8±1.1500a
Peritoneal cavity	1.9± 0.0200 b	0.8±0.1100c	5±0.2200a
Gut wall and mesentry	1.6± 0.500 b	0.0±0,000c	12.7±1.0100a
Diaphragm	0.0 ±0.000b	0.2±0.0010	0.0±0.000a
Spleen	0.0±0.000b	0.1±0.0005b	2±0.0400a
lung	0.0±0.000b	0.0±0.000a	0.1±0.0005a

Different small letters denotes to significant differences between groups on (P≤ 0.01).

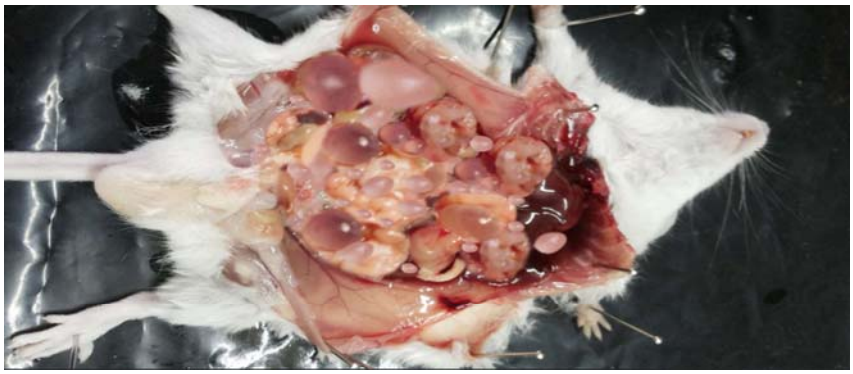

Figure 2. The positive control mice shows the number, size and location of the hydatid cyst.

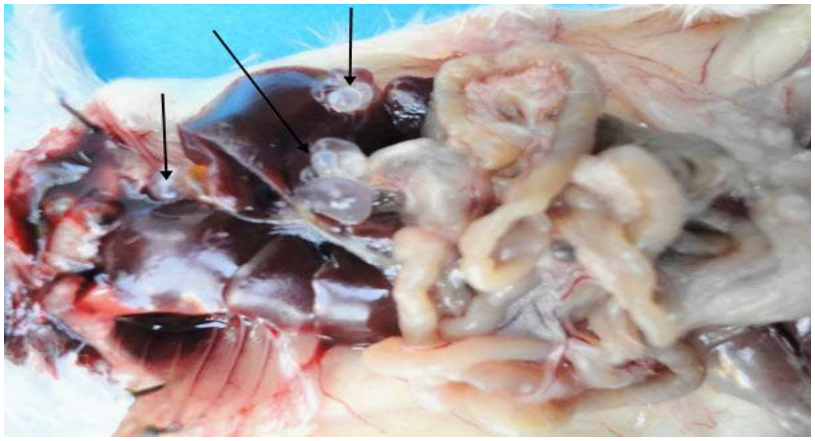

Figure 3. The hydatid cyst of the abdominal cavity in mice treatment at 7.5 mg/ml

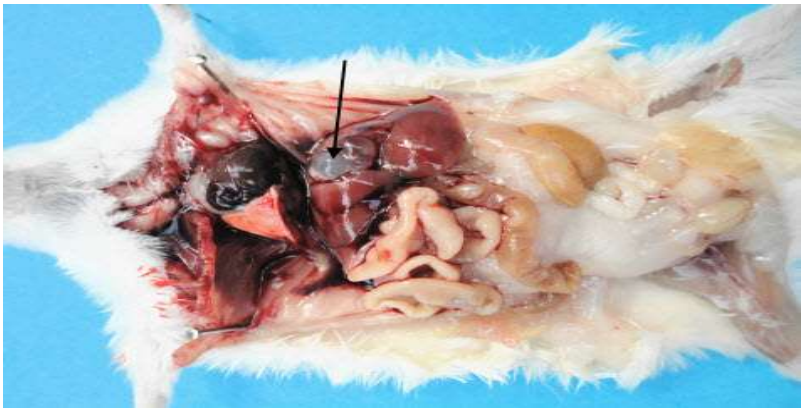

Figure 3. The hydatid cyst of the abdominal cavity in mice treatment at 15 mg/ml

6.1. Delayed-Type Hypersensitivity:

An upper significant mean thickness of footpad performed (P≤0.01) at 24 hours (21 day, 7.5 and 15 mg/ml) (0.50±0.07, 0.36±0.06), separately, where diminished (0.10±0.60, 0.10±0.60) at 48 hours post-immunization when in correlation of the negative control mice (Table-4, 5).

Table 3. Mean of footpad thickness dring 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*	0.50±0.07* B	0.36±0.06a B	0.00±0.00a A	-----
30	0.24±0.11b A	0.13±0.0.46a AB	0.00±0.00c A	0.47±0.06a B
60	0.23±0.08b B	0.33±0.07a A	0.00±0.00c A	0.16±0.14a AB
90	0.01±0.18b B	0.27±0.11b B	0.00±0.00b A	0.63±0.13a A
120	0.26±0.10b A	0.32±0.116b B	0.00±0.00c A	0.69±0.13a A

Table 4. Mean of footpad thickness during 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±0.60a B	0.10±0.06a B	0.00±0.00a A	-----
30	0.20±0.04a A	0.19±0.0.05a A	0.00±0.00b A	0.26±0.03a B
60	0.17±0.08b B	0.18±0.05a A	0.00±0.00c A	0.29±0.03a AB
90	0.00±0.00b B	0.10±0.001b B	0.00±0.00b A	0.33±0.06a A
120	0.25±0.09b A	0.09±0.06c B	0.00±0.00c A	0.36±0.01a A

*Only immunized mice.

Uppercase letters show numerous significant differences between the periods vertically on the probability (P≤ 0.01).

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

6.2. Indirect Haemaglutination Test:

Table (5) displays high significant titer of antibodies in (15 mg/ml) (115.2012.80 and 204.8031.35, separately) during day 21 and 120 post-immunization on (p≤ 0.01) in comparison of the negative control mice (0.00 0.00).

Table 5. Antibody titers in immunized, treated and control groups at 21 and 120 days

Groups	**Antibody titers Mean ± SD**	
	21*day	**120day**
7.5mg/ml	115.20± 12.80a B	140.80±20.90a B
15mg/ml	204.80±31.35a A	230.40±17.07a A
Negative control	0.00±0.00a C	0.00±.00a C
Positive control	-----	47.60 ±8.51

*Only immunized mice.

Uppercase letters show numerous significant differences between the groups on the probability of vertically (P≤ 0.01). Lowercase letters indicate a similar lack of significant differences between periods horizontally.

Table 6. Percentage Mitotic index of spleen and bone marrow in mice after 21 days of immunization and compared with the rate in the negative control.

Groups	**Percentage of Mitotic index ± SD**		
	Immun, 7.5mg/ml	**Immun.15mg/ml**	**Negative control**
Spleen	34.50±3.40a	37.0±4.70a	27.60±0.50b
Boon marrow	27.55±1.93a	27.55±1.93a	21.85±1.85b

Different small letters denotes to significant differences between groups on (P≤ 0.01).

7. Discussions

Many researches were interested about the preparation of specific vaccines aimed to stimulate the specific immune responses (humoral and cellular immunity) against the growth of used a mixture of HCF and protoscoleces antigens conducted the immunostimulatory effects of these antigens in decrease growth of hydatid cysts post-challenge as (15) who expressed the activity of different (mixture) antigens

in induction the immune response as comparing with using single antigens which occurred in the present study were the animal groups immunized with HCF or PS Ags separately it was not efficient in reduction the growth of hydatid cysts as the mixture of them were appeared more effective in reduction the growth and induce strong immune response in prevent the parasitic infection(16) who recorded the efficacy of stimulating immune response post immunization with antigens in preventing the growth of cysts . The high decrease ratio of cysts diameter were observed post-treatment and immunization in comparison of the control positive group, (17).

The resistant of host-immune responses perusing certain hydatid cysts might have been owed of the proprietorship from claiming different growth proportions as stated by their particular aspects and spread cysts in distinctive organs for mice from control positive group treated mice and a higher mean number from claiming cysts need scored previously, each liver, wall gut and mesentery, furthermore these results were matching of the discoveries of the (18). Those secondary rate of pad-thickness in immunized mice (7. 5 also 15 mg/ml, in day 21) in examination from claiming mice control negative that suitably for (19), the place distinguished helter skelter rate of late hypersensitivity responses to mice immunized by primates initial antigen, that indicates the part of the antigens utilized within fortifying cell division safe reaction. The lower percentage of late hypersensitivity responses in mice treated concentration about 7. 5 and 15 mg/ml compared with control positive group might make distinguished should strong restorative strategy utilized within those present consider (immunization furthermore chemicals) in the diminishment those numbers from secondary hydatid cysts.

The antibody titers improved in recent study at 21 day post-immunization. There was a signficant rise in mitotic index both the spleen and the bone marrow in the mice, in comparison of the negative control. This increase is explained by the fact that the primary vision antigen contains adsorptive components that possess the capacity of the T and B lymphocyte (20). Early diagnosis of the disease by distinguishing antibodies in the serum of patients is an important step in the treatment of the disease, and there are several methods for identifying antibodies in the serum of patients infected hydatidosis, including the test of indirect blood circulation (IHA), which is a valuable method and has a high efficiency in the serological diagnosis of cyst disease (21).This test was used after 21 days of immunization to measure the ability of the CF/PS antigen to stimulate the immunoglobulin response, as the results showed a high level of antibody in the mice with concentrations of 7.5 and 15 mg/ml in comparison of the negative control mice, and this result was agreed with (22) where the ability of the cystic fluid antigen and the primary vision antigen to stimulate the immune response

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