EXPERIMENTAL STUDY ON SOME PROTECTIVE METODS AGAINST HYDATID CYST INFECTION IN MICE

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SUMMARY

Experimental study albion mice as a host was conducted in on attempt to inactivate the larval stage of Echinococcus granulosus by formalin treatment . Animals were divided into three groups. The first group was inoculated subcutaneously with alive ovine hydatid protoscolices , whereas the second group was inoculated similarly with 0.75% formalin- treated protoscolices. The rhird group was remained as negative control . a comparison was held between the three groups from haematological , immunological and histopathological points of view. The haematological picture revealed lymphopenia, eosinophilia and neutrophilia in both treated groups of mice. Infected

mice in groups 1 and 2 responded subcutaneous injection of the antigen by production of antibodies detected by indirect haemagglutination test. Similar responses did not occur in noninfected control mice. Pathologically, results showed that the cellular were concentrated at the site of inoculation and at the draining lymph nodes without spreading to other organs . The formalinized protoscolices failed to induce development or formation of any cysts at the site of inoculation , in comparison with live protoscolices . The challenge of both treated groups at 4 weeks post primary infection showed that 80% of the infected and vaccinated animals resisted infection .

INTRODUCTION

Hydatid disease in Iraq is one of the most important endemic diseases (1,2) . Sheep and cattle are susceptible to infection and the hydatid cysts are noticed primarily on the liver . lungs , disposal and other organs of slaughtered animals . faulty disposal of infected organs may lead to recycling of Echinococcus granulosus in stray dogs (3).

Marshal et. al. (4) craig (5) studied the serum antibody response in sheep infected with E. granulosus hydatid cysts following parental

immunization with hydatid cyst fluid . The authors found that 60% of the infected animals responded to intramuscular injection of the antigen by rapid production of antibodies which were detected by indirect haemagglutination (1H) test whereas the similar responses did not occur in any of the non infected controls . Moreover , the 1Htechnique was the most suitable one for detection of Hydatid antibodies (4) . On the other hand , the studies of Ali- Khan (7) and lightowlers et. al. (6) showed that the infection with hydatid cysts is accompanied by substantial lymphopenia and neutrophilia with a reversal in the ratio of lymphocytes to neutrophils . Al- kannany (8) observed lymhopenia , nentrophilia and eosinophilia when the hydatid disease was reproduced experimentally in mice .

Histopathological changes in different infected organs were studied and discussed by many authors (8,12) . The refractory or partially susceptible or abort the larval cyst mass by mobilizing a massive inflammatory reaction against the pathogen ; in contrast . hypersusceptible hosts show an insignificant tissue reaction to larval cyst mass (10,13) .

The objective of the present study was to conduct a preliminary trial for production of formalinized hydatid cyst vaccine by using formalin for the inactivation of ovine hydatid cyst protoscolices .

MATERIALS AND METHODS

Ainmals :

Fifty albino mice were divided into there groups . The first group of twenty mice was inoculated with 1250 olive protoscolices subcutaneously , whereas the second group of the same number was inoculated by the same route and dose with formalinized protoscoleices . The third group of 10 mice remained as a negative control .

Protoscolices :

Protoscolices were collected from ovine hepatic hydatid cystes according to the procedure of AL-Kannany (8) . The viability of the protoscolices was approved by 0.1% aqueous eosin . The protoscolices were counted and a concentration of 1250 cells per 0,1 ml of hydatid fluid was prepared .

Formalin treatment :

The protoscolices were treated with 0,75% formalin as a final concentration at 26 ˚C for 48 hours .

The treated cells were washed five times with phosphate buffer at pH 7.2 to get rid of formalin .

Sampling :

Blood films and sera samples were collected from the heart of 15 mice in groups 1 and 2, and from 5 mice from the negative control at 4 weeks post infection . Thereafter , these mice were sacrificed and examined macroscopically . pieces of liver , spleen , axillary and brachial lymph nodes, thymus, heart, muscles and skin were collected and fixed in carnoy's solution for microscopical examination .

Differential leucocytic count (DLC) :

The method of Schalm et. al. (14) was carried out for determination of DLC in blood films .

Indirect hemagglutination (1H) test :

1H test was performed according to techniques slightly modified from those described by Marshall et. al. (4) , using glutaraldehyde – treated mice red blood cells sensitized by direct contact with sheep hydatid cyst fluid .

Histopathological examination :

The carnoy's fixed tissue samples were processed by the histokinate paraffin sections were prepared at the

thickness of 4-6 . Harris – hematoxylin and eosin stain was used (15) .

Challenge test :

At 4 weeks post infection , 5 mice from each group were challenged subcutaneously with 1250 protoscoleices . The animals were necropsied 4 weeks later and examined macroscopically .

Statistical analysis :

Data were statistically analyzed by analysis of variance.

The accepted level of significance was at $P < 0.05$.

RESULTS

Differential leucocytic count :

Table 1 indicate lymphopenia , eosinophilia and neutrophilia in both treated groups of mice . These changes were more significant ($P< 0.01$) in the first group .

Indirect hemagglutination test :

The results of 1H antibody titer to immumization with .

Alive protoscolices (group 1) (titer 1049 . 1 which is amear of 15 mice) and immumizatiation with formalinized protoscolices (group 2) (titer 559.5 which is amean of 15 mice) , High titers of 1H antibodies has been induced by the both treated

groups being highest in the first group which received alive protoscolices . Similar responses did not occur in any of 5 noninfected controls .

Pathological findings :

Mice inoculated with alive protoscolices :

The site of inoculation at 4 weeks post infection appeared as soft palpable subcutaneous lump without any discrete margin . There was enlargment of axillary and bracheal lymph nodes . cysts proliferation were restricted to small foci in the fibroblastic corona . The cyst foci were vascular and lacked laminated layer . The foci were encircled lightly by mature and immature neutrophils . Several inflammatory foci consisting of lymphocytes and eosinophils were scattered throughout the corona . A number of blood capillaries in the connective tissue around the corona showed lymphocytic cuffs . The muscular layer adjacent to the corona showed myositis and interstitial edema (Fig.1) . Histological examination of axillary and bracheal lymph nodes showed depletion of lymphocytes and replacement by plasma cells . The medulla and subcapsular sinuses showed plasmacytosis and histiocytosis (Fig.2) .

Mice inoculated with formalinized protoscolices :

There was no cyst formation at the site of inoculation . The subcutaneous response was evaluated macroscopically and by palpation . Histological examination of the subcutaneous tissues revealed the presence of a granulomatous lesions consisted of connective tissue infiltrated by inflammatory cells and showed degenerative changes . Microscopically , axillary and brachial lymph nodes revealed lymphocytic depletion and increasing in capillary sinuses .

No histopathologic alteration were abserved in other organs examined in all the other groups of animals .

Challenge test :

Results of the challenge for the three groups showed that one mouse from groups 1 and 2 four mice from group 3 exhibited a cysts in the site of inoculation .

DISCUSSION

The results showed that the immunological heamatological and pathological reactions were some what the same when albino mice were inoculated either with alive or formalinized protoscolices of E. granulosus , and the animals were stand against the challenge one month post primary infection .

The results of blood investigation indicated that their was lymphopenia in both infected groups of mice except that the lymhopenia was much clear in the group which infected with alive protoscolices . this may be due to the depletion of T-lymphocytes in the axillary and bracheal lymph nodes . The results indicated too that there were eosinophilia and neuerophilia which are in accordance with the results of AL- kannany (8) . but not with those of Ali- khan (7) who did not observe any neutrophilia although he stated that the hydatid disease is always accompanied by sustantial lymphopenia and neutrophilia .

In 1986 Marshall et. al. (4) applied the 1H technique for detection the immune response against the hydatid disease . the same technique with slight modification was applied in this study and the results showd a good level of immunity in both treated groups of animals . The titer in case of alive protoscolices was 1049 , while in case of formalinized one was 559.5. It has been stated stated that a titer less than 559.5 is quite enough for protection against infection with the hydatid disease (4,16) .

The pathoiogical examination showed that the cellular reactions were concentrated at the site of inoculation

and at the draining lymph nodes only without wide spreading into other organs which were examined . In the group which was inoculated with alive protoscolices the formation of the cyst was clear in the second group ,the formalinized protoscolices did not succeed in forming any cyst . This obsrvation suggested the presence of an antigens in host responsible for resistance to establishment of protoscolices . The cellular and histological reactions appeared in this study were more or less in agreement with the findings of other workers (8-13) .

Further study is in progress to manifest the other parameters must be adopted to confirm this . Thereafter this trial should be applied in ovine and bovine species .

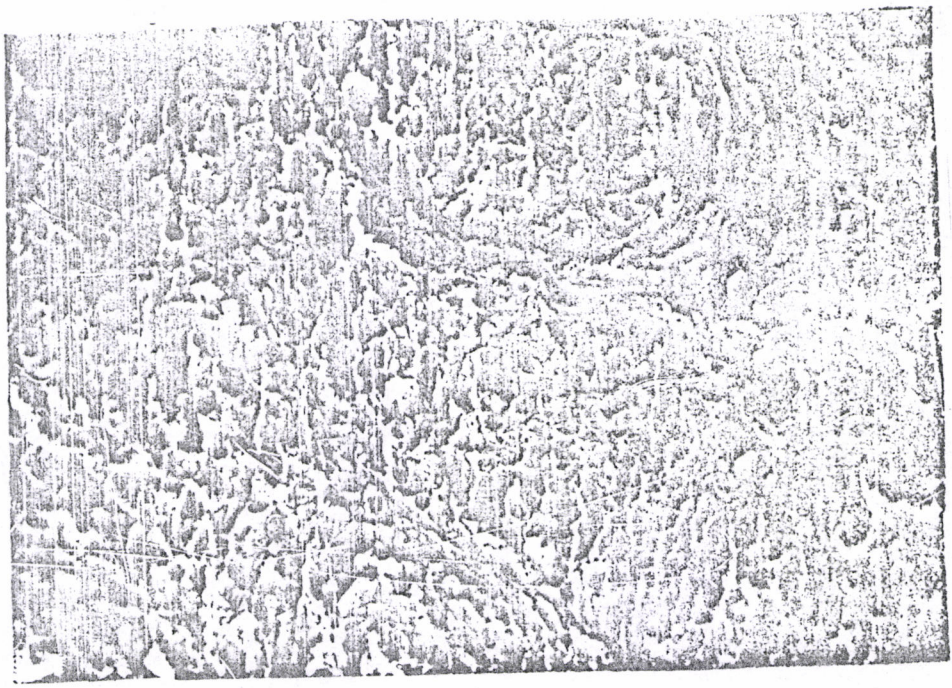

Figure (2) . Section of the axillary lymph node . Note the depletion of germinal centers and the infilt-tration of plasma histiocytes H & E x 250 .

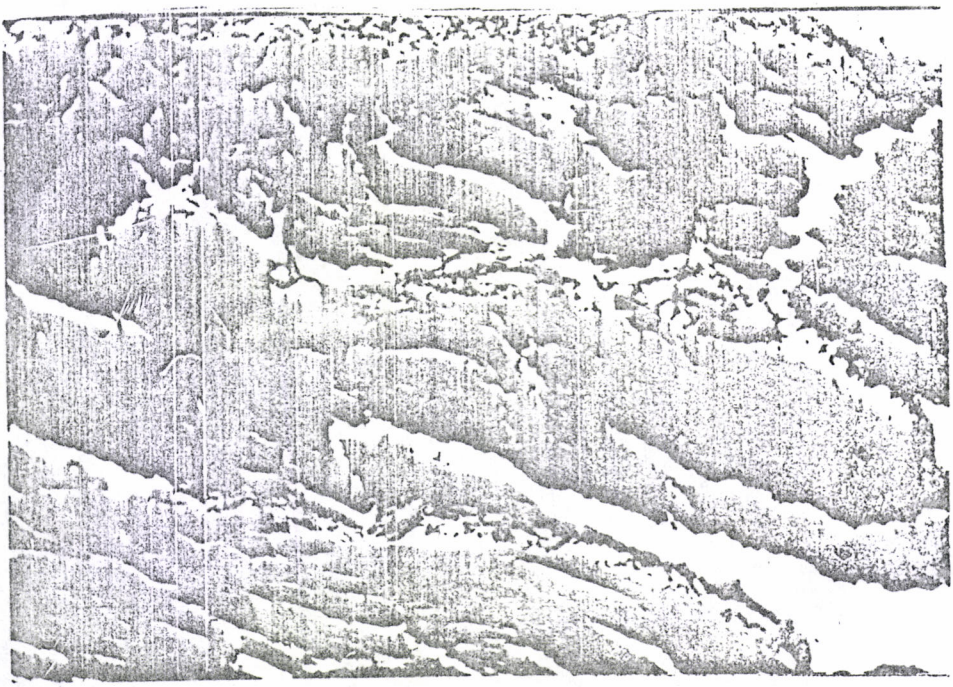

Figure (1). Section of the subcutaneous tissue revealing myositis and interstitial edema . H & E x 250.

Table 1 : Differential leucocytic count in mice inoculated by alive and formalinized protoscolices .

Cells	No. of cells %		
	Control * Untreated	a live ** protoscoleices	formalinized ** protoscoleices
Lymphocytes	85 ∓ 0.6	60∓0.2 ##	71∓0.5 #
Eosinophils	2 ∓ 0.5	19∓0.3 ##	9∓0.7 #
Neutrophils	11∓0.7	18∓0.1 ##	16∓0.6 #
Monocytes	3 ∓ 0.9	2 ∓ 0.9	3 ∓ 0.5
Basophils	1 ∓ 0.9	1 ∓ 0.8	1 ∓ 0.9

*Mean of 5 mice ** Mean of 15 mice ∓ SD
# p < 0.05 ## P < 0.01

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دراسة تجريبية حول بعض طرق التمنيع ضد الإصابة بالأكياس الصدرية في الفئران

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الخلاصة

اعتمدت هذه الدراسة التجريبية محاولة لأبطال فاعلية الدور اليرقي لطفيلي المشوكات الحبيبية Echinococcus granulosusi بواسطة المعاملة بالفورمالين وتم استخدام الفئران البيضاء كمضيف . قسمت الحيوانات التجريبية الى ثلاثة مجاميع – حقنت المجموعة الأولى بالرؤوس الأولية المأخوذة من الأغنام وحقنت المجموعة الثانية برؤوس أولية معاملة بمادة الفورمالين بنسبة 0,75% ، بينما استعملت المجموعة الثالثة من الحيوانات كمجموعة سيطرة سالبة .أجريت المقارنة بين المجاميع الثلاثة من الناحية الدموية والمناعية والنسيجية .أظهرت الصورة الدموية لفئران المجموعة الأولى والثانية وجود نقص في الخلايا اللمفية وزيادة في كل من الخلايا الحمضات والعدلات مقارنة مع المجموعة الثالثة غير المعاملة ، كذلك بينت هذه الدراسة بأن فئران المجموعتين المعاملتين أظهرت استجابة مناعية بعد حقن الرؤويسات الأولية تحت الجلد حيث تم قياس تلك الاستجابة باستخدام اختيار التلازن الدموي غير المباشر ، بينما لم تظهر فئران المجموعة الثالثة أي استجابة لهذا الاختبار .اما من الناحية المرضية ، فأن النتائج بينت بأن الاستجابة الخلوية تركزت عند موقع الحقن وكذلك في العقد اللمفية المرابطة لموقع الحقن ولم يسجل أي انتشار للآفات في الأعضاء الأخرى ، لقد فشلت الرؤويسات الأولية المعاملة بالفورمالين من إنبات أي كيس عدري في موقع الحقن مقارنة مع الرؤويسات الأولية الحية .
إن اختبار التحدي لفئران المجموعة الأولى والثانية وبعد أربعة أسابيع من الحقن الأولي أظهر 80 % من هذه الفئران قد قاومت الخمج .