Experimental Trial with a Heat-Shocked Protoscolex Extract as a Vaccine Candidate for Protection Against Hydatid Disease

Kist Hidatik Hastalığına Karşı Koruma İçin Aşı Adayı Olarak Isıl Şoklanmış Protoskoleks Ekstrakt İle Deneysel Çalışma

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ABSTRACT

Objective: Cystic echinococcosis is distributed worldwide and is an important public health challenge in many countries. The present study was an experimental trial to use hydatid antigens derived from viable protoscoleces cultivated at 37 and 45°C for 4 h as a vaccine candidate for protection against hydatid infection.
Methods: Balb/c mice were immunized with hydatid antigens extracted from protoscoleces exposed to 37 and 45°C as well as partially purified hydatid antigens containing 30, 60, and 90 µg of heat shock protein 70 administered with or without an adjuvant.
Results: Crude antigens from protoscoleces exposed to 37°C conferred non-significant immunity with protection and reduction rates that ranged from 0% to 25% and 77.69% to 98.38%, respectively. In mice receiving crude antigens from protoscoleces exposed to 45°C, the protection and reduction rates ranged from 0% to 66.66% and 94.62% to 98.92%, respectively. The purified antigen from protoscoleces exposed to 45°C conferred significant immunity with absolute protection observed in mice immunized with 60 and 90 µg of the antigen combined with the adjuvant. Immunological parameters (anti-hydatid antibody titer and lymphocyte transformation %) showed a negative correlation with the number of cysts. The assessment of renal and liver functions showed non-significant differences ($p>0.05$) in comparison with the liver and renal functions of non-immunized mice of the negative control group.
Conclusion: Purified hydatid antigens containing heat shock protein 70 confer high levels of protection against hydatid infection in mice (*Turkiye Parazitol Derg 2016; 40: 1-8*).

Keywords: Heat shock protein, Protoscoleces, Hydatid disease.

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ÖZ

Amaç: Kistik ekinokokkoz tüm dünyada yayılmaktadır ve birçok ülkede görülen önemli bir halk sağlığı problemidir. Bu çalışma hidatik kist enfeksiyonuna karşı bir aşı adayı olarak, 37 ve 45°C'de 4 saatte üretilen canlı protoskolekslerden türemiş hidatik antijenler kullanılarak yapılan deneysel bir çalışmadır.
Yöntemler: Balb/c fareler, bir adjuvanla ya da adjuvansız uygulanan ısıl şoklanmış 30, 60 ve 90 µg protein 70 içeren kısmen purifiye hidatik kist antijenlerinin yanı sıra, 37 ve 45°C'ye maruz kalmış protoskolekslerden elde edilen hidatik antijenlerle immünize edildiler.
Bulgular: 37°C'ye maruz kalmış protoskolekslerden elde edilen ham antijenlerle, koruma ile anlamlı olmayan immünite ve sırasıyla %0 ile %25 ve %77,69 ile %98,38 arasında değişen azalma oranları ortaya konuldu. 45°C'ye maruz kalmış protoskolekslerden elde edilen ham antijenler verilen farelerde, koruma ve azalma oranları sırasıyla %0 ile %66,66 ve %94,62 ile %98,92 arasında değişiklik gösterdi. 45°C'ye maruz kalan protoskolekslerden elde edilen purifiye (saflaştırılmış) antijen, adjuvan ile kombine 60 ve 90 µg antijenle immunize edilmiş farelerde gözlenen mutlak koruma ile birlikte anlamlı bir immünite ortaya koymuştur. İmmünolojik parametreler (anti-hidatik kist antikor titresi ve lenfosit transformasyon %) kist sayısı ile negatif bir korelasyon göstermiştir. Negatif kontrol grubundaki immünize olmamış farelerin karaciğer ve böbrek fonksiyonları ile kıyaslandığında, böbrek ve karaciğer fonksiyonlarının değerlendirilmesinde anlamlı olmayan farklılıklar bulunmuştur ($p>0,05$).
Sonuç: Isıl şoklanmış protein 70 içeren purifiye hidatik antijenler, farelerde hidatik kist enfeksiyonuna karşı koruma sağlamaktadırlar. (*Turkiye Parazitol Derg 2016; 40: 1-8*)

Anahtar Kelimeler: Isıl şoklanmış protein, Protoskoleks, Hidatik kist hastalığı.

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INTRODUCTION

Cystic echinococcosis is distributed worldwide and is an important public health challenge in many countries (1-6). In Iraq, the disease is regarded as one of the most important public health and socioeconomic problems (7-9).

In endemic areas, serious steps should be taken toward to prevent and control the disease, including the designing of studies focusing on the development of vaccine candidates derived from parasite tissues. In Iraq, many studies have been conducted in this field, most of which have tried to use attenuated protoscoleces (10-11). Other investigators have tried to use excretory/secretory antigens or antigens derived from hydatid fluid as vaccine candidates for protection against hydatid disease (12).

Recently, heat shock proteins, which are a family of proteins expressed in all living cells when exposed to stressful conditions such as high temperature, irradiation, and infection, have attracted the attention of immunologists (13-17). These proteins have been found to function as molecular chaperones, preventing the stress-induced aggregation of partially denatured proteins and promoting their return to the native conformation (18-19). Currently, there is a great deal of interest in developing vaccines using heat shock protein fusion proteins that would generate powerful immune responses in the absence of adjuvants (4, 16, 20). This research was conducted to study the immunogenicity of hydatid antigens derived from heat-shocked protoscoleces and the possibility of using such antigens as vaccine candidates for providing immunity against hydatid infection.

METHODS

In vitro cultivation of protoscoleces for the expression of heat shock protein 70

The procedure described by Martinez et al. (21) was followed. Briefly, protoscoleces were aseptically collected from the infected livers of sheep slaughtered in a slaughterhouse in Erbil. The viability of the protoscoleces was assessed prior to cultivation by methylene blue exclusion and flame cell biting. Only those batches of protoscoleces with 95%-99% viability were subjected to cultivation. Two batches of 25000 protoscoleces per 3 mL of medium 199 (HIMEDIA, India) were separately incubated at 37 and 45°C for 4 h. At the end of the incubation, the medium was removed and the precipitated heat-shocked protoscoleces were washed three times with cold PBS (0.15 M; pH 7.2) and stored at −70°C until further use.

A protoscolex extract solution was prepared as described by Seyyedi et al. (22). Heat-shocked (37 or 45°C) protoscoleces were thawed and washed three times with cold PBS (0.15 M; pH 7.2). Protoscoleces in three volumes of homogenizing buffer [10 mM EDTA, 2 mM phenylmethylsulfonyl fluoride (Sigma), 0.15 M NaCL, and 50 mM Tris; pH 7.5] containing 0.5% (v/v) Triton X-100 were freeze-thawed in three cycles of 10 min in liquid nitrogen followed by 10 min in a water bath at 37°C; they were then homogenized by a homogenizer in an ice bath, and finally, the homogenized protoscoleces were disintegrated by a sonicator. The homogenized and sonicated protoscoleces were then centrifuged at 5000g for 30 min at 4°C. The supernatant (preparation 1, 37°C, 1, 45°C) was then aliquoted and stored at −70°C until further use as a crude vaccine preparation.

Partial purification of hydatid antigens containing heat shock protein 70 by column chromatography

A Sephadex G-150 column (1.5×45 cm; Pharmacia Fine Chemicals) was prepared as described by Hotzhaner (23). After the gel settled, the column was eluted with 4-fold PBS at pH 7.4. The protoscolex extract solution (crude preparation 1, 45°C) was run through the column and eluted with PBS (pH 7.4) at 4°C. Fractions of 3 mL of eluted fractions were collected in sterile cupped plastic tubes. The optical density of each fraction was recorded by a digital spectrophotometer at a wavelength of 280 nm. The fractions were then aliquoted, labeled, and stored at −70°C until further use.

Detection of heat shock protein 70 in the column chromatograph, eluted fractions

An indirect hemagglutination test (IHT) as described by other workers (24) was performed for the detection of heat shock protein 70 in the eluted fractions. The eluted fractions that gave absorbance at 280 nm UV spectrophotometer were adsorbed on sheep red blood cells as a source of heat shock protein 70 antigens and were detected by monoclonal anti-heat shock protein 70 (Sigma, USA). The fractions that gave positive reactions for heat shock protein 70 were used as purified hydatid antigens containing heat shock protein 70 (preparation 2, 45°C). The protein content in the extract solutions was determined by the method described by Lowry et al. (25).

Immunization protocol

Balb/c mice, males and females aged 6–8 weeks with body weight ranging between 18.5 and 27.5 g, were used for the immunization experiments. The immunization protocol was conducted as described by others (26). Briefly, in experiment 1, three groups each of 12 mice were subcutaneously injected with 0.1 mL of normal saline (0.85%) containing 30, 60, or 90 µg of preparation 1 (37°C)mixed with 0.1 mL of Freund's complete adjuvant (FCA) for the first immunization. The second immunization was conducted after 4 weeks with the same preparation, with the only difference being that the FCA adjuvant was replaced by Freund's incomplete adjuvant (FIA). Further, three groups of 12 mice each were subcutaneously injected with 0.2 mL of normal saline containing 30, 60, or 90 µg of preparation 1 (37°C) without the adjuvant for both immunization protocols.

In experiments 2 and 3, the protocol above is repeated with the preparations 1 (45°C) and 2 (45°C), respectively.

Control groups

1. Adjuvant control: A group of 12 mice were subcutaneously injected with 0.1 mL normal saline mixed with 0.1 mL of FCA for the first immunization. The second immunization was conducted after 4 weeks with same preparation, with the only difference being that FCA was replaced by FIA.

2. Positive control: A group of 12 mice were subcutaneously injected with 0.2 mL of normal saline for both immunization protocols. After 4 weeks, the mice were intraperitoneally challenged with 2000 protoscoleces.

3. Negative control: A group of 12 mice were subcutaneously injected with 0.2 mL of normal saline for both immunization protocols.

Four weeks after the last immunization, blood samples were collected by cardiac puncture from 4-5 mice of each group; then, all groups, except the negative control group, were intraperitoneally injected with 0.2 mL of normal saline containing 2000 protoscoleces as described by Dematteis et al. (27). All mice were killed 90 days after the administration of the challenge dose. The internal organs were examined for secondary hydatid cysts. The site and number of cysts were determined, and the size of each cyst was measured using a ruler. Infected organs and cysts were fixed in formal saline (10%) for histopathological examinations.

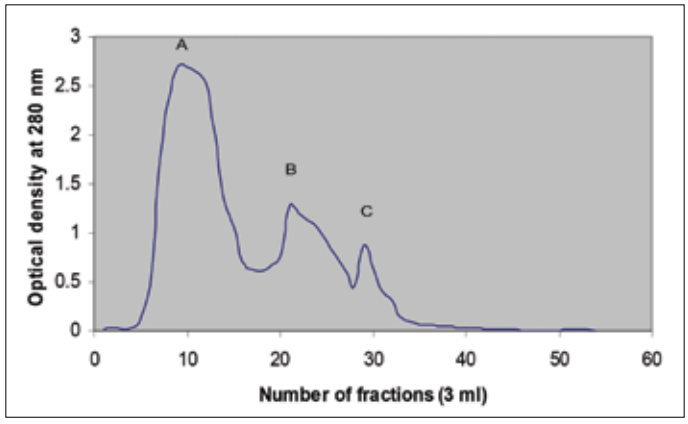

Figure 1. Elution pattern of extracts of 45°C treated protoscoleces, passed through Sephadex G-150 column (1.5x45 cm).

Immunological parameters

A- The lymphocyte transformation test (LTT) was performed as described by Shubber et al. (28).

B-IHT was performed for the detection of anti-hydatid antibodies in the sera of mice. The procedure described by Parija and Ananthakrishnan (24) was followed.

Assessment of liver and renal functions

Commercial kits from Biolabo Reagents (France) were used for the determination of liver-related enzyme, alanine aminotransaminase, and aspartate aminotransaminase activities in the sera of experimental mice. Renal function was assessed by the estimation of blood urea levels using a commercial kit provided by bioMerieux (France).

Statistical analysis

Student's t-test was used for the comparison of the number and size of cysts between the positive control and immunized groups. The correlation coefficient (r) was calculated between immunological parameters and number of cysts. Reduction and protection rates were calculated as described by Piacenza et al. (29). P≤0.05 was considered to be statistically significant.

RESULTS

Partial purification of heat shock protein 70

The chromatography pattern of hydatid antigens derived from protoscoleces exposed to 45°C is shown in Figure 1, in which three protein peaks (A, B, and C) are observed. Peaks A (frac-

Table 1. Reduction rate of secondary hydatid cysts, protection rate, number and size of cysts in mice immunized with hydatid antigens derived from protoscoleces exposed to 37 C° for 4 hours.

Experimental groups	No. of mice	No. of infected mice	No. of cysts (Mean±SD)	Mean of cyst size±SD(mm)	Protection (%)	Reduction of cysts (%)
Immunized with 30 µg plus adjuvant	4	4	83 (20.75±15.33) (p<0.05)	0.77±0.8 (p<0.01)	0	77.69
Immunized with 60 µg plus adjuvant	4	3	10 (2.5±1.91) (p<0.05)	0.67±1.2 NS	25	97.3
Immunized with 90 µg plus adjuvant	4	3	24 (6±6.37) (p<0.05)	0.86±0.84 (p<0.01)	25	93.54
Immunized with 30 µg	6	6	9 (1.5±0.54) (p<0.05)	0.1±0 (p<0.01)	0	98.38
Immunized with 60 µg	7	6	16 (4±2.29) (p<0.05)	0.28±0.3 NS	14.28	95.69
Immunized with 90 µg	5	4	11 (2.2±1.92) (p<0.05)	0.62±0.7 (p<0.05)	20	98.03
Immunized with adjuvant	6	6	153 (25.5±12.75)	0.43±0.48	0	72.5
Positive control	4	4	372 (93±4.05)	0.42±0.5	0	

NS: Non- significant (P>0.05)

tions 6-18), B (fractions 19-28), and C (fractions 29-32) were collected from Sephadex G-150. All these fractions were examined by IHT for heat shock protein 70 using monoclonal anti-heat shock protein 70. Heat shock protein 70 was detected in peaks A and B in fractions 8 and 20-25.

Immunization study

Mice receiving hydatid antigens derived from protoscoleces exposed to 37°C (preparation 1) showed a relatively minimum resistance to infection with protection and reduction rates ranging from 0% to 25% and 77.69% to 98.38%, respectively. The size of secondary hydatid cysts showed no significant difference ($p>0.05$) in mice receiving 60 µg of the antigen with the adjuvant and 90 µg of the antigen without the adjuvant. In the other groups receiving preparation 1 (37°C), the size of developed cysts was significantly reduced ($p<0.05$, $p<0.01$) respectively than that developed in the positive control group and in the mice immunized with the adjuvant alone (Table 1).

Mice immunized with preparation 1 (45°C) showed relatively moderate resistance to infection with protection and reduction rates ranging from 0% to 66.66% and 94.62% to 98.92%, respectively. The number of developed cysts was significantly ($p<0.05$) less than that developed in the positive control group and mice immunized with the adjuvant alone. The size of developed cysts was variable and showed significant difference in mice receiving 30 and 90 µg of the antigen with the adjuvant ($p<0.01$ and $p<0.05$, respectively) and 60 µg of the antigen without the adjuvant ($p<0.01$) in comparison with the size of cysts developed in the positive control group (Table 2).

In the other experimental groups immunized with preparation 2 (45°C), the immunization protocol conferred higher protection and reduction rates, with absolute protection observed in mice immunized with 60 and 90 µg of the antigen with the adjuvant. The size of developed cysts was also significantly reduced ($p<0.01$) in all mice receiving preparation 2 (45°C) in comparison with that developed in the positive control group and mice immunized with the adjuvant alone (Table 3).

Liver and renal function assessment is shown in Tables 4, 5, and 6. Non-significant differences ($p>0.05$) in biochemical parameters were observed between the immunized mice and negative control group.

The results of immunological parameters are shown in Table 7. There was a negative correlation between number of cysts and both immunological parameters, LTT ($r=-0.0695$) and anti-hydatid antibody titers by IHT ($r=0.1042$).

DISCUSSION

Because the intermediate hosts for *Echinococcus granulosus* including humans show little or no natural resistance to infection with hydatid disease, many attempts have been made to stimulate the immune responses in murine models (13, 26, 30-32) and sheep (33, 34) to develop immunity against hydatid infection. In this study, we found that the subcutaneous injection of mice with

Table 2. Reduction rate of secondary hydatid cysts, protection rate, number and size of cysts in mice immunized with hydatid antigens derived from protoscoleces exposed to 45 C° for 4 hours.

Experimental groups	No. of mice	No. of infected mice	No. of cysts (Mean±SD)	Mean of cyst size±SD(mm)	Protection (%)	Reduction of cysts (%)
Immunized with 30 µg plus adjuvant	6	6	30 (5.0±3.3) (p<0.05)	0.26±0.19 (p< 0.01)	0	94.62
Immunized with 60 µg plus adjuvant	6	4	12 (2.0±3.09) (p<0.05)	0.65±0.64 NS	33.33	97.84
Immunized with 90 µg plus adjuvant	4	2	4 (1±1.41) (p<0.05)	0.28±0.17 (p<0.05)	50	98.92
Immunized with 30 µg	6	3	25 (4.16±7.86) (p<0.05)	0.41±0.44 NS	50	95.52
Immunized with 60 µg	6	4	20 (3.33±3.72) (p<0.05)	0.21±0.15 (p<0.01)	33.33	96.41
Immunized with 90 µg	6	2	7 (1.16±2. 4) (p<0.05)	0.4±0.42 NS	66.66	98.75
Immunized with adjuvant	6	6	153 (25.5±12.75)	0.43±0.48	0	72.5
Positive control	4	4	372 (93±40.05)	0.42±0.5	0	

NS: Non- significant (P > 0.05)

Table 3. Reduction rate of secondary hydatid cysts, protection rate, nuber and size of cysts in mice immunized with partially purified hydatid antigens, containing heat shock protein 70, derived from protoscoleces exposed to 45 C° for 4hours.

Experimental groups	No. of mice	No. of infected mice	No. of cysts (Mean±SD)	Mean of cyst size±SD(mm)	Protection (%)	Reduction of cysts(%)
Immunized with 30 µg plus adjuvant	5	1	3 (0.6±1.34) (p<0.05)	0.1±0 (p<0.01)	80	99.35
Immunized with 60 µg plus adjuvant	6	0	0 (p<0.05)	0 (p<0.01)	100	100
Immunized with 90 µg plus adjuvant	6	0	0 (p<0.05)	0 (p<0.01)	100	100
Immunized with 30 µg	5	4	11 (2.2±2.16) (p<0.05)	0.15±0.07 (p<0.01)	20	97.63
Immunized with 60 µg	6	2	6 (1.0±1.55) (p<0.05)	0.15±0.08 (p<0.01)	66.66	98.92
Immunized with 90 µg	4	3	10 (2.5±1.73) (p<0.05)	0.17±0.1 (p<0.01)	25	97.31
Immunized with adjuvant	6	6	153 (25.5±12.75)	0.43±0.48	0	72.5
Positive control	4	4	372 (93±40.05)	0.42±0.5	0	

Table 4. Biochemical parameters in the sera of mice immunized with hydatid antigens derived from protoscoleces exposed to 37 C° for 4 hours.

Experimental groups	ALT activity (IU/ml)	AST activity (IU/ml)	Blood urea (mg/dl)
Immunized with 30 µg plus adjuvant	38.23±15.1 NS	67.21±19.5 NS	40.47±5.19 NS
Immunized with 60 µg plus adjuvant	41.55±0.49 NS	79.97±6.89 NS	40.47±5.19 NS
Immunized with 90 µg plus adjuvant	25.49±8.4 NS	74.73±28.6 NS	28.86±8.28 NS
Immunized with 30 µg	40.16±23.2 NS	76.29±13.1 NS	33.5±10.14 NS
Immunized with 60 µg	22.35±1.97 NS	45.75±4.9 NS	33.9±13.34 NS
Immunized with 90 µg	40.86±2.47 NS	78.22±4.9 NS	25.99±3.06 NS
Immunized with adjuvant	46.79±0.49	83.63±1.23	33.54±7.22
Negative control	39.89±5.2	83.28±8.3	31.2±2.6
NS: Non- significant (P > 0.05)			

hydatid antigens derived from protoscoleces exposed to 37°C, which is the normal physiological temperature of the hosts, conferred no significant protection in mice challenged with 2000 viable protoscoleces. This might be due to the fact that the protoscolex extract solution contains a mixture of T-independent and -dependent antigens that can stimulate the host immune system to produce high levels of both protective and non-protective immunoglobulins (35, 36). Baz et al. (37) have shown that protoscolex somatic antigens induce the production of all classes and subclasses of immunoglobulins, except IgG3, in CD4+-depleted mice. On the other hand, Dematteis et al. (27) have shown that the sera of mice experimentally infected with viable

Table 5. Biochemical parameters in the sera of mice immunized with hydatid antigens derived from protoscoleces exposed to 45 C° for 4 hours.

Experimental groups	ALT activity (IU/ml)	AST activity (IU/ml)	Blood urea (mg/dl)
Immunized with 30 µg plus adjuvant	51.21±0.98 NS	92.88±25.2 NS	34.22±4.7 NS
Immunized with 60 µg plus adjuvant	49.93±6.4 NS	84.86±10.9 NS	35.34±12.7 NS
Immunized with 90 µg plus adjuvant	39.98±7.2 NS	81.36±0.5 NS	37.44±8.3 NS
Immunized with 30 µg	39.43±1.9 NS	84.68±4.7 NS	39.3±20.5 NS
Immunized with 60 µg	47.83±1.5 NS	68.1±4.9 NS	47.79±25.5 NS
Immunized with 90 µg	33.69±10.6 NS	79.1±2.7 NS	38.62±11.5 NS
Immunized with adjuvant	46.79±0.49	83.63±1.23	33.54±7.22
Negative control	39.89±5.2	83.28±8.3	31.2±2.6
NS: Non- significant (P>0.05)			

Table 6. Biochemical parameters in the sera of mice immunized with hydatid antigens derived from protoscoleces exposed to 45 C° for 4 hours.

Experimental groups	ALT activity (IU/ml)	AST activity (IU/ml)	Blood urea (mg/dl)
Immunized with 30 µg plus adjuvant	46.82±10.3 NS	86.1±2.2 NS	30.0±10.4 NS
Immunized with 60 µg plus adjuvant	38.58±14.5 NS	86.42±13.5 NS	28.65±6.1 NS
Immunized with 90 µg plus adjuvant	27.93±0.99 NS	76.47±1.5 NS	33.2±3.9 NS
Immunized with 30 µg	41.38±10.1 NS	81.71±6.9 NS	34.38±6.75 NS
Immunized with 60 µg	35.42±5.3 NS	81.19±3.2 NS	32.42±0.7 NS
Immunized with 90 µg	55.68±2.2 NS	71.1±15.5 NS	32.7±1.4 NS
Immunized with adjuvant	46.79±0.49	83.63±1.23	33.54±7.22
Negative control	39.89±5.2	83.28±8.3	31.2±2.6
NS: Non- significant (P>0.05)			

protoscoleces contain significant levels of immunoglobulins specific to the carbohydrate epitopes of protoscolex somatic antigens that play a key role in the activation of the non-protective TH2 arm of the immune response. Therefore, in spite of the production of anti-hydatid antibodies and the relatively high percentages of lymphocyte transformation in mice immunized with preparation 1 (37°C), the animals showed low protection rates because these immune elements may be non- protective.

This study revealed that mice immunized with 60 and 90 µg of purified heat-shocked protoscolex antigens combined with the adjuvant showed a 100% protection rate. Such high rates of protection and reduction in these groups may be explained by the fact that protoscolex cells, like other eukaryotic cells, are responding to elevated temperature by increasing the synthesis of various proteins including heat shock proteins (38, 39). The most striking example of this is *Escherichia coli* heat shock proteins, which account for 1.6% of the total cell protein under normal growth conditions and can accumulate to 15% of the total protein after heat shock (38). The efficacy of hydatid antigens derived from protoscoleces exposed to heat shock is related to the synthesis of various proteins and heat shock proteins. These proteins have been shown to induce the synthesis of pro-inflammatory cytokines such as TNF alpha, interleukin-1, interleukin-6, and interleukin-12; the release of nitric oxide and C-C chemokines by macrophages, monocytes, and dendritic

Table 7. Lymphocyte transformation test and anti-hydatid antibody titer by indirect hemagglutination test results in experimental mice

	Experimental groups	LTT percent	IHT titer
Immunized with preparation 1,37°C (crude)	30 µg with adjuvant	15.035	4
	60 µg with adjuvant	16.88	8
	90 µg with adjuvant	11.57	8
	30 µg without adjuvant	19.75	8
	60 µg without adjuvant	12.43	8
	90 µg without adjuvant	8.97	8
Immunized with preparation 1,45°C (crude)	30 µg with adjuvant	21.05	16
	60 µg with adjuvant	14.69	8
	90 µg with adjuvant	9.76	8
	30 µg without adjuvant	24.61	16
	60 µg without adjuvant	23.83	4
	90 µg without adjuvant	10.99	4
Immunized with preparation 2,45°C (purified)	30 µg with adjuvant	24.51	8
	60 µg with adjuvant	25.71	4
	90 µg with adjuvant	18.39	4
	30 µg without adjuvant	24.14	8
	60 µg without adjuvant	24.96	4
	90 µg without adjuvant	4.41	4
Immunized with adjuvant alone		4.9	Negative
Negative control		4.75	Negative

NS: Non- significant (P > 0.05)

cells (40); and the maturation of dendritic cells through the induction of the upregulation of MHC I, MHC II, CD86, and CD40 (18). Another important role of heat shock protein 70 is that it acts as a CD40 ligand and binds to CD40 on dendritic cells and macrophages, inducing the synthesis of interleukin-12, which stimulates the development of the TH1 subset of lymphocytes and enhances TH1, CD8+ TC, and natural killer cells to produce gamma interferon. Gamma interferon stimulates the protective mechanisms of the immune system by inducing class switching into IgG1 and IgG3; upregulating MHC I, MHC II, and co-stimulatory molecules on antigen-presenting cells; and enhancing the microbicidal activity of phagocytes (41, 42).

Mice receiving the purified hydatid antigen containing heat shock protein 70 showed more resistance than those immunized with crude heat-shocked protoscolex antigens, which may be due to the presence of many immunomodulators such as T-independent antigens and major specific hydatid antigens such as antigen B (120–160 KDa), which is an important immunomodulator and potent activator of the non-protective TH2 arm of the immune response (43). High levels of this antigen, because of its relatively large size, were removed during gel filtration, and only those hydatid antigens, including antigen 5, with a molecular weight of approximately 70 KDa were eluted in same fractions with heat shock protein 70.

This study suggests that incubation of viable protoscoleces in 45°C for 4 h induces the overexpression of various antigens including heat shock proteins, heat shock protein 70 in particular, which can be used as a vaccine candidate for protection against hydatid infection in mice. Designing experiments to study the efficacy of antigens prepared from heat-shocked protoscoleces in the protection of other hosts such as sheep against challenge infection with viable eggs or onchospheres of *E. granulosus* is recommended.

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