

**Effect of attenuated protoscolices of hydatid cyst worm
(*Echinococcus granulosus*) on lymphocytes viability *in vivo*
تأثير الرؤيسات الاولية الموهنة لدودة الاكياس المائية (*Echinococcus*
granulosus) في عيوشية الخلايا للمفاوية داخل الجسم الحي**

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Abstract

Hydatidosis is a widespread chronic zoonotic parasitic disease caused by helminthic larval stage of tapeworm *Echinococcus granulosus*.. In last year's many methods are used to activate and modulate the response of immune system of the host in order to evade the invasion of the parasite. The present investigation was aimed to find out the effect of attenuated protoscolices on the viability of lymphocytes which play very important role in the immune response against many parasites and the reflect of this attenuation on hydatid cyst protoscolices infectivity *in vivo*. Four groups of white male BALB/c mice were experimentally infected with helium-neon laser attenuated protoscolices that exposed to helium –neon laser for (10,30,50,100) minutes respectively. The results showed increase in lymphocyte viability ($p<0.01$), especially among mice that exposed to laser for 50 minutes (77.00 ± 1.42) in comparison to control group(50.66 ± 3.23),this increase in lymphocyte viability causes statistically ($p<0.01$) decrease in protoscolices infectivity (both cyst numbers and diameters)in comparison with positive control group which cause decrease in lymphocyte viability.

Keywords: *Echinococcus granulosus*, Protoscolices. Attenuation. Lymphocytes, Viability, Laser.

الخلاصة

يعد داء الاكياس المائية من الامراض الانتقالية المشتركة المزمنة الناتجة عن الخمج ببرقة الدودة الشريطية المشوكة الحبيبية *Echinococcus granulosus* , وخلال السنوات الاخيرة فقد استعملت العديد من الطرائق وذلك لتفعيل وتعديل استجابة الجهاز المناعي من اجل الحد من انتشار هذا الطفيلي. تهدف الدراسة الحالية لمعرفة تأثير الرؤيسات الاولية المضعفة في عيوشية الخلايا للمفاوية والتي تلعب دورا مهما في الاستجابة المناعية للعديد من الطفيليات وتأثير ذلك في خمجية الرؤيسات الاولية المضعفة داخل الجسم الحي . فقد استعملت اربع مجاميع من ذكور الفئران البيض للخمج التجريبي بالرؤيسات الاولية الموهنة بأشعة هليوم – نيون للاكياس المائية وبمدد تعريض (10 , 30 , 50 , 100) دقيقة , اظهرت النتائج واعتمادا على وقت التعريض بأن مجموعة الرؤيسات المعرضة ل 50 دقيقة اشعة هليوم – نيون زيادة في عيوشة الخلايا للمفاوية ($p<0.01$) وبلغت (77.00 ± 1.42) مقارنة مع مجموعة السيطرة غير المضعفة (50.66 ± 3.23) , وهذه الزيادة في عيوشة الخلايا للمفاوية ينجم عنها انخفاض معنوي ($p<0.01$) في استضادية الرؤيسات الاولية من خلال الانخفاض المعنوي في حجم الاكياس المائية واحجامها مقارنة بمجموعة السيطرة الموجبة والتي يحصل فيها انخفاض في عيوشة الخلايا للمفاوية.

Introduction

Cystic echinococcus (CE) is a widely endemic helminthic disease caused by infection with metacestodes (larval stage) of the tapeworm *Echinococcus granulosus* that cycles between canines particularly dogs as definitive hosts and various herbivores as intermediate hosts (1). The disease is characterized by the growth in the host internal organs, mostly liver and lung, of steadily fluid – filled, unilocular cysts surrounded by a two layered hydatid cyst wall, inner nucleated germinal layer, where protoscoleces bud, and an outer acellular laminated layer surrounded by a host

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fibrous capsules (2,3). It constitutes a serious public health problems in various parts of the worlds. The annual incidence rates of diagnosed human cases / 100,000 inhabitants vary widely(4).

Hydatid cyst secretes major immunodominant antigens which are thought to be responsible for immunomodulatory activities promoting its survival within a mammalian host (5). These activities have extraordinary abilities to control host immune rejection mechanisms through alternation or suppression of the functional lymphocytes by secreting substance from the cyst wall which interfere locally with immunocompetent cells which facilitate long term survival of the parasite(6).

Lymphocytes play a vary important role against parasitic infections especially *E. granulosus* whether this role is pathogenic or protective in the immune response and they accordingly on antigenic stimulation elaborates a majority of soluble molecules that mediate interaction between cells called interleukins from T. lymphocytes or immunoglobulines called from B. lymphocytes (7). The aim of this study is to find out the effect of attenuated protoscolices on the viability of lymphocytes which play very important role in the immune response against many parasites and the reflect of this attenuation on hydatid cyst protoscolices infectivity *in vivo*.

Materials and Methods:

Five groups of white BALB/c mice were used for experimental infections (each group consist of 12- 16 mouse, their age 10-12 weeks, weight 22-24 gm). Protoscolices were isolated from cysts in sterile conditions according to (8) method, and their numbers were fixed to 2000 protoscolices / 1 ml of sterile phosphate Buffer Saline (P.B.S) with pH(7.2) and their viability were tested according to(9) method using eosin stain (viability must be more than 98%). The inbred males BALB/c mice groups were injected as follow: Four groups of mice were inoculated intraperitoneally(I.P.) with four protoscolices groups which exposed to helium- neon laser for (10, 30, 50 and 100 minutes) at the wave length 632.8nm with power 10 nm. The fifth group was inoculated I.P. with non exposed 2000 protoscolices/ 1ml of sterile hydrated cyst fluid used as a positive control group. After 4 days the four mice groups inoculated with a live non attenuated 1000 protoscolices / 1 ml P.B.S. as challenge dose. After 4,6 and 8 weeks T. lymphocytes were separated according to (10) method and their viability were tested by dye exclusion (0.2%) trypan blue stain (11). Then one hundred cells were counted and percentage of lymphocyte viability was calculated using haemocytometer under compound microscope. After 25 weeks all mice groups were killed and dissected under dissected microscope and the infectivity of protoscolices was investigated by recording cyst numbers and their diameters micrometer.

Statistical Analysis: The data were analyzed by one-way ANOVA. Using SPSS program (version 10) and Excel application. The results are expressed by the means \pm standard deviation (SD). Results were considered, Non significant at $p > 0.05$, significant at $p < 0.05$, highly significant at $p < 0.01$.

Results:

Four weeks following the incubation of attenuated protoscolices, the results showed increase in lymphocyte viability ($p < 0.01$) especially among mice which were exposed to the laser for minutes (77.00 ± 1.42), while the least exposure for 10 minutes showed no significant ($p > 0.05$) difference in lymphocytes viability is decrease during 100 minutes exposure. This significant increase was continued during the 6th week of exposure in comparsion with the positive control group, restarted to decreased in 100 minutes exposure (Table 1).

Similarly after 8 weeks, attenuated protoscolices with helium – neon laser demonstrated significant increase ($p < 0.01$) in lymphocyte viability for 50 minutes exposure which were (88.66 ± 2.43) in comparison with positive control group (37.00 ± 1.33). This increase in lymphocyte viability

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causes statistically significant ($p < 0.01$) decrease in protoscolices infectivity (both cyst number and diameters) in comparison with positive control group among 50 minutes exposure (Table 2).

Therefore, attenuated protoscolices with helium –neon laser on the viability of lymphocytes especially for specific period of exposure and started to decrease when the time of exposure is prolonged.

Discussion:

The principal finding of this study is that helium-neon laser attenuated protoscolices were able to stimulate the T. lymphocytes against the experimental hydatidosis in mice infected with freshly isolated protoscolices of *E. granulosus* especially at higher exposure time 50 minutes.

No studies were done previously about the effect of attenuated protoscolices by helium –neon laser on lymphocytes viability. The alive attenuated protoscolices have immunosuppressive effect on T. cell viability. It has been found that the *E. granulosus* causes severely depression to T. cells in later stages of infection *in vitro* (12). The higher dose of protoscolices increase the number of suppressor cells accompanied by decrease in the number of T. helper cells. Furthermore infection with higher dose of this parasite may result in non- specific suppression of T. cell activity (13). Because of radiation, the attenuated protoscolices were unable to overcome the immune system of the host (14) and considered highly immunogenic to stimulate and activates the immune system against the challenge dose (15). The results of this study agree with (16) who conclude that attenuated protoscolices with different laser rays give partial and absolute immunity against experimental hydatidosis depending on time of exposure, The inoculation of attenuated protoscolices intraperitoneally sensitized immunocompetency T. cytotoxic which causes the destruction of the challenge dose (17). The protoscolices which exposed to U.V. for a prolong time will lost its immunogenic activity, while for short time exposure will give the host absolute immunity (18). Immunity of BALB/c mice to injected protoscolices induced both Th1 and Th2 type response (19).

In conclusion, the results indicate that attenuated protoscolices stimulate the immune system causing increment of lymphocytes viabilities especially at 50 minutes helium- neon exposure which let protoscolices to develop and grow.

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Table 1. The effect of protoscolices *Echinococcus granulosus* exposed to helium- neon laser on lymphocytes viability *in vivo*.

Time of exposure Minutes	Lymphocytes viability: Mean \pm SD		
	After 4 weeks	After 6 weeks	After 8 Weeks
H.C.F(+ control)	50.66 \pm 3.23	44.33 \pm 1.12	37.00 \pm 1.33
10	55.00 \pm 1.42*	60. \pm 2.87**	69.00 \pm 3.65**
30	61.33 \pm 1.52**	60.33 \pm 1.32**	66.33 \pm 2.23**
50	77.00 \pm 1.42***	79.33 \pm 3.21***	88.66 \pm 2.43***
100	59.00 \pm 2.32*	70.66 \pm 5.63***	70.00 \pm 2.12***

Data are expressed as Mean \pm S.D.

* NS P>0.05

**S P<0.05

*** HS P<0.01

Table 2. Effect of attenuated *E. granulosus* protoscolices with helium- neon laser on the numbers and diameters of cysts after 25 weeks.

Time of exposure Minutes	Cysts numbers			Cysts diameters(mm)		
	Mean	\pm	S.D	Mean	\pm	S.D
(control)	16.13	\pm	2.00	4.213	\pm	1.792
10	16.88	\pm	1.16*	4.088	\pm	0.380*
30	10.25	\pm	3.44**	2..813	\pm	0.577*
50	4.13	\pm	3.33***	1.275	\pm	3.64***
100	4.63	\pm	1.43***	1.875	\pm	0.420***

Data are expressed as Mean \pm S.D.

* NS P>0.05

** S P<0.05

*** HS P<0.01