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# Effect of pH concentration on hydatid cyst protoscolices infectivity: In vitro and in vivo study

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***Abstract:*** *Hydatid disease is an endemic disease in certain areas of the world. It is a widespread chronic zoonotic disease caused by helminthic larval stage of tapeworm Echinococcus granulosus.*
*To study firstly the effect pH on the viability of protoscolices of sheep hydatid cyst in vitro. Secondly, to see the effect of the pH treated protoscolices on macrophage viability and its effect on secondary hydatidoasis in .vivo*
*Protoscolices were isolated from sheep hydatid cysts and exposed to three pH concentrations (4, 7, and 9) for (6, 24, and 72) hours. Then four mice groups were injected intraperitoneally (I.P.) with protoscolices treated these concentrations after 72 hrs plus hydatid cyst fluid as a positive control group. Then peritoneal macrophages are isolated after 6 weeks to see its viability. After three months the mice group are killed and dissected for the protoscolices infectivity assessment.*
*The results showed that the higher pH concentrations (9) and low pH (4) decrease significantly the viability of protoscolices in comparison with the hydatid cyst fluid positive control group. This decrease in viability has direct influence on the macrophage viability and protoscolices infectivity in vivo.the protoscolices viability and increase the macrophage viability which decrease the number of formed cysts and its diameters in vivo.*

***Keywords:*** *Protoscolices. pH. Cyst. Hydatid .Macrophage. Viability.*

## I. Introduction

Hydatid disease caused by the larval stage of tapeworm Echinococcus granulosus is usually seen in the regions with sheep and dogs. This parasite usually settles and forms cysts in liver and lung with a frequency of 60% and 20-30% respectively. The involvement of other organs may occur in case of dissemination of scoleces by the blood (1) .This disease remains a significant public health problem in endemic areas such as Turkey, the Middle East, South America, and Australia. As an endemic disease, it causes social and economic losses for countries. (2)

The definitive host is the dog, in which adult tapeworms attach to the intestinal epithelium and undergo sexual reproduction, leading to the development of eggs. These eggs are shed into the environment with the faeces. The eggs contain an oncosphere, which upon ingestion by a suitable intermediate host and subsequent passage through stomach and intestine become activated, penetrate the mucosa, enter blood and lymphatic vessels and are disseminated in the body (3) .During the larval stage the parasite forming a cyst that fill with fluid (hydatid fluid) surrounded by three membrane layers and it grows in these organs (4and 5).Thus, prevention of the disease as the first step by providing the basic hygiene and treatment as the second step are the important parts of the approach to hydatidosis.There is still no consensus as to the optimal form of treatment. Medical treatment has been proven to be effective against larval E. granulosus (6).

Macrophage: It is non specific immune cell found predominantly in blood stream and as macrophages primarily in fixed tissue (usually reticuloendothelial system RES).

Its function accomplished by degradation of the antigen in the phagolysosome or by proteasomes. Its phagocytic activity is done through the action of lysosomal enzyme and generation of toxic forms of oxygen to kill the living organism (7).

## II. Materials And Methods

### II-1 Source of protoscolices

### II-2 Protoscolices collection and in vitro study

Echinococcus granulosus protoscolices were aseptically removed from hydatid cysts obtained from sheep at the abattoir in Baghdad/ Al-Rusafa Province The protoscolices were washed several times with saline containing 100 kU/L benzylpenicillin and 100 mg/L streptomycin as described by(8) . The survival rate of the protoscolices exceeded 95% after these procedures. Percentages of protoscolices viability or survival were determined by examining them by compound microscope for permeability of aqueous eosin stain (0.5%) as a

vital stain of protoscolices-, Green protoscolices: Viable, Red protoscolices: Dead (9) .These Protoscolices which isolated from hydatid cysts were exposed to three pH concentrations (4, 7, and 9) for 6, 24, 72 hours. Then four mice groups were injected intraperitoneally (I.P.) with protoscolices treated these concentration after 72 hrs plus hydatid cyst fluid as a positive control group. Then the macrophages are isolated after 6 weeks according to (9) method to see viability by dye exclusion of trypan blue. After three months the mice group are killed and dissected for the protoscolices infectivity assessment.

## III. Results

It can be seen from Table 1 that the protoscolices viability was affected by different concentrations of the pH, but this effect was not significant ($P > 0.05$) at pH 7 concentration,

while lower pH (4) and higher pH(9) concentrations cause highly significant decrease ($P<0.01$) in the protoscolices viability after 6 hours exposure which were (55.5±2.44, 47.25±2.32 ) respectively in comparison

with hydatid cyst fluid positive control group which was (91.00±1.21).This effect was highly significant $P<0.01$ after 24 and 72 hours exposures to lower and higher pH concentrations which were (41.50 ±3.87, 30.00

±2.60) and ( 34.25 ±2.21, 29.5 ±1.83) respectively comparison with hydatid cyst fluid treatment (90.5 ±2.21, 86.00±2.67) Table(1).The results showed that the attenuated protoscolices by lower(4) and higher (9)pH concentrations increase highly significant $P<0.01$ the macrophages viability which were (88.00±2.32, 80.±2.62)

respectively in comparison with positive control and pH 7 concentration which were (42.50±2.21, 48.00±1.93) respectively table (2).The attenuated protoscolices especially lower and higher pH concentration showed high significant ($P <0.01$) decrease in the numbers and sizes of cysts recovered from mice injected with different pH treated protoscolices after three months in comparison with cysts recovered from positive control group which all protoscolices significantly to develop and grow. Table (3).

The main result of this study is that lower and higher pH concentrations were able to inhibit the protoscolices viability which cause the activation of macrophage which in turn prevent the cyst to grow and develop.

**Table (1) The effect of pH on protoscolices viability in vitro**

| pH concentration | protoscolices viability: Mean±SD | | |
|---|---|---|---|
| | After6 hrs | After 24 hrs | After 72 hrs |
| H.C.F(+ control) | 91.00±1.21 | 90.50±2.12 | 86.00±2.67 |
| 4 | #55.50±2.44 | #41.50±3.87 | #30.00±2.60 |
| 7 | *90.50±3.50 | *89.25±0.32 | ^87.25 ±0.23 |
| 9 | #47.25±2.32 | #34.25±2.21 | #29.50±1.83 |

^ S $P<0.05$ * NS $P>0.05$ # HS $P<0.01$

**Table( 2)The effect of protoscolices treated for 72hrs with pH concentrations on macrophages viability after 6 weeks in vivo**

| pH concentrations | MØ viability:Mean±S.D. |
|---|---|
| H.C.F.(+ Control) | 42.50±2.21 |
| 4 | ^88.00±2.32 |
| 7 | * 48.00±1.93 |
| 9 | $80.00±2.62 |

. $ HS= $P<0.01$ ^S = $P< 0.05$ * NS = $P>0.05$

**Table(3) Effect of pH treated protoscolices on cyst numbers and diameters after three months in vivo**

| pH concentration | Cysts numbers | Cysts diameters(mm) |
|---|---|---|
| | Mean ± S.D. | Mean ± S.D. |
| H.C.F(+ control) | 16.13 ± 2.00 | 4.213 ± 1.792 |
| 4 | ^4.88 ± 1.16 | ^1.088 ± 0.380 |
| 7 | *10.25 ± 3.44 | *4..813 ± 0.577 |
| 9 | ^4.13 ± 3.33 | ^1.275 ± 3.64 |

^ HS= P<0.01 * NS = P>0.05

## IV. Discussion

In this investigation, we studied the effect of different pH concentrations on protoscolices viability of Echinococcus granulosus for different period's exposures in vivo and in vitro. There were no studies have been found to investigate the effect of different concentrations of pH treated protoscolices on macrophage viability and protoscolices infectivity in vivo.

In the present study we found that the pH4 effect on the viability of protoscolices in vitro in comparison with HCF control group and this effect was increased with the high pH 9 concentrations which showed higher effect than those of pH4. The results showed that pH7 was a typical pH that not effects and keep the viability of protoscolices for a long period time.

In another view the hydatid cyst fluid was a more better and still the protoscolices alive longer than pH 4and 9 because this fluid has a nutritional factors and a numbers of minerals, compound such as triglycerides, protein, fatty acid, carbohydrates and others (10 and 11).

Macrophage is one of the non-specific responses termed antigen –presenting cells (APCs) which link that innate and adaptive immune systems. These cells recognize microbes through their pathogens- associated molecular pattern (PAMP) receptors which have broad specificity and a non –clonal distribution (12) ..They play very important role in the elimination and control of parasitic protozoa and worms by secreting substances called

monokines[(13)]

The attenuated protoscolices which inoculated I.P. unable to penetrate the host tissues and stay intraperitoneally in where exposed to macrophage activities which kill the protoscolices by adhesion and destroy

them by lysozymes or by cytophilic antibodies with cooperation of macrophages and T cytotoxic lymphocytes which destroy the protoscolices(14 and 15) .

Attenuated protoscolices with lower (4) and higher (9) pH concentrations stimulate the immune system causing increment of macrophages viabilities especially after 72 hours which prevent protoscolices to develop by increasing in number and grow by increasing in diameters, while in contrary with pH7 which was a typical pH that keep the viability for a long period time. This long term protoscolices viability causing suppression of macrophages viability in vivo and let the protoscolices to penetrate the tissue and give them well establishment (15) .

## V. Conclusion

The results showed that the higher pH concentrations (9) and low pH (4) decrease significantly the viability of protoscolices in comparison with the hydatid cyst fluid positive control group. This decrease in viability has direct influence on the macrophage viability and protoscolices infectivity in vivo.the protoscolices viability and increase the macrophage viability which decrease the number of formed cysts and its diameters in vivo

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