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**Fadel YS Al-Arabi**  
Dr. Rafiq Zakaria College for  
Women, Aurangabad, Dr.  
Babasaheb Ambedkar  
Marathwada University,  
Aurangabad, India

**Gozif Mohammed N Omar**  
Department of Biochemistry, Dr.  
Babasaheb Ambedkar  
Marathwada University,  
Aurangabad, India

**Mansour Abdulnabi H Mahdi**  
Dr. Rafiq Zakaria College for  
Women, Aurangabad, Dr.  
Babasaheb Ambedkar  
Marathwada University,  
Aurangabad, India

**Vidya Pradhan**  
Dr. Rafiq Zakaria College for  
Women, Aurangabad, Dr.  
Babasaheb Ambedkar  
Marathwada University,  
Aurangabad, India

#### Correspondence

**Fadel YS Al-Arabi**  
Dr. Rafiq Zakaria College for  
Women, Aurangabad, Dr.  
Babasaheb Ambedkar  
Marathwada University,  
Aurangabad, India

## The effect of *tamarindus indica* extracts on larval stage of *Echinococcus granulosus* parasite *in vivo* and *in vitro* study

**Fadel YS Al-Arabi, Gozif Mohammed N Omar, Mansour Abdulnabi H Mahdi and Vidya Pradhan**

#### Abstract

It is known that medicinal plants are the most important alternative to chemical drug resources, especially for antimicrobial and parasite targets and the treatment of various diseases. The present study aimed at determining the effect of aqueous and alcohol extracts of the leaves of the *Tamarindus indica* plant on the vitality of the protoscolices of sheep *in vitro*. The results study show that alcohol and aqueous extract solutions used had an inhibitory effect on protoscolices compared with control group over all concentration range. The percentage of homicide was measured by using the eosin stain test, which depends on changing the green color of the alive protoscolices to red after killing it. The study revealed that the concentration of 100 mg / ml was the optimal focus for killing the protoscolices after 4 hours of exposure. It was observed that when laboratory animals were injected with the protoscolices treated with the extracts, the protoscolices failed in the formation of secondary hydatid cysts.

**Keywords:** Hydatidosis, *Echinococcus granulosus*, protoscolices, *Tamarindus indica*

#### Introduction

Cystic echinococcosis or hydatidosis is a silent cyclozoonotic infection of humans and domestic animals produced by the larvae of the cestode *Echinococcus granulosus* [1]. The larval stage of granulosus parasite is the cause of the disease, which belongs to the Platyhelminthes phylum of the Cestoda species infect the small intestine of the carnivorous [2]. Hydatid cyst spreads throughout most of the world, especially in areas where sheep are found in high rate and is endemic in Asia, North Africa, South and Central Americas, North America, Canada, and the Mediterranean region. In many countries, hydatid disease is more prevalent in rural areas where there is close contact between people, dogs, and various domestic animals which act as intermediate hosts [3].

Therefore the studies in recent years have been directed towards investigating many diseases using the folk medicine known as a herbal medicine for its effective, safe and economic factors [4].

The importance of this study is to show the effect of the alcohol and aqueous extract of the leaves of the plant *T. indica* in the vitality of the protoscolices of *Echinococcus granulosus* of sheep origins *in vitro* and their growth *in vivo*. There is a growing interest of research on medicinal plants due to their potential to cure many diseases, because of low prices and lower incidence of side effects when compared to synthetic drugs [5].

*T. indica* which belongs to the family Leguminosae (Fabaceae), commonly known as Tamarind tree, is one of the fruit tree species that is used as traditional medicine. Tamarind tree is found especially in the Indian subcontinent, Africa, Nigeria, Bangladesh, Pakistan, and most of the tropical countries. It is preferred to be used for diarrhoea, abdominal pain, and dysentery, some bacterial infections and parasitic infestations, constipation, wound healing, and inflammation. It is a rich source of most of the essential amino acids and phytochemicals, and hence the plant is reported to possess antidiabetic, antimicrobial, antivenomic, antioxidant, antimalarial, cardio protective, hepatoprotective, antiasthmatic, laxative and anti-hyperlipidemic activity. *T. indica* has ameliorative effects on many diseases. It can also be favoured as a nutrient addition for malnourished patients as it is cheap and easy to access [6].

*T. indica* is a plant that can be used traditionally in wound healing, snake bite, colds, helminth infections, and fever. It may also play a role as antimicrobial, antidiabetic, antiinflammatory and effects on the control of satiety, playing a potential role in the treatment or prevention of

obesity and other chronic diseases. These effects are probably due to the presence of polyphenols as n-Hexacosane, eicosanoic acid, b-sitosterol, octacosanyl ferulate, 21oxobehenic acid, and pinitol and phenolic antioxidants for proanthocyanidins. *T. indica* includes a variety of bioactive compounds in the leaves, seeds, bark, pulp, and flowers with beneficial effects to human health and the possibility of application in the pharmaceutical industry [7].

## Materials and methods

### Plant source

Leaves of the plant under study were obtained from the campus of Dr. Rafiq Zakaria College for women and is authenticated. The leaves are dried under shadow and grinded to powder.

### Preparation of plant extracts

#### Aqueous extract

The powder of leaves of *T. indica* (40 g) was taken in beaker and 400ml of distilled water. The mixture was stirred by magnetic stirrer for 24 hours. Then the mixture was filtered through four pieces of medical gauze and poured into the centrifuge tubes at speed of 3000 rpm for 10 minute. After that mixture was put in Petri dishes and placed in oven at 40 C° to dry. Finally the extract was scraped and collected in clean glass vials and kept in refrigerator for use [8].

#### Alcoholic extract was prepare

The plant leaves powder (40g) was taken in soxhlet apparatus. Then 400 ml ethyl alcoholic (70%) was added. It was left undisturbed. The solvent was evaporated by rotary evaporation. The mixture was finally placed in clean Petri dishes as the aqueous extract.

### The source of the hydatid cyst

The hydatid cyst were obtained from the sheep of the butchery of Al-Basateen, of Aden, Yemen. The cysts were then transferred to the laboratories of the Faculty of Science, University of Aden, Yemen (Fig. 1).

### Collecting the protoscolices

According of method [9] was used to obtain the protoscolices where the hydatid cyst was sterilized twice with ethyl alcohol (70%), and then the cyst fluid was removed by a sterile syringe. The cyst was washed internally with pH 7.2 and the antibiotic was penicillin IU20000 and Streptomycin 1 g/liter, the liquid was discarded in the test tubes, then it was centrifuge to at 3000 cycles/minute, and the protoscolices were examined under the microscope.

### Evaluating the vitality of the protoscolices

Protoscolices were estimated using aqueous eosin stain (1%) 20 microliters of the primer suspension was taken and same amount of the eosin stain was added to a clean glass slide. It was examined under the microscope. Which kept the color oblique of the green and prevented the entry of the stain they are a live protoscolices whereas the red was counted dead for its color pigmentation (Fig.2).

The percentage of live protoscolices in the sample was calculated by dividing the number of live protoscolices in the sample to the total number of calculated headings x 100. The procedure was repeated three times in a row and the survival rate was taken. The percentage of the vitality of the protoscolices was calculated after each exposure period [10].

## Solutions used

Phosphate buffer solution (PBS), Hank solution and aqueous eosin stain (1%) was used for present investigation.

## Laboratory animals

In this study, white albino rats *Rattus norvegicus* were used. The rats grew up in the laboratory of the Faculty of Science, University of Aden-Yemen. They grew and reproduced in the conditions of the animal house and were provided with water and food, which was a concentrated mash of added protein and dry milk. The floors of the plastic cages were covered with sawdust and were usually changed weekly to keep the rats clean.

## Implantation in laboratory animals

In order to determine the effect of the plant extract used in this study on the vitality of the protoscolices of sheep origin, on the growth and development *in vivo* [11], rats were injected with the protoscolices treated with plant extract at the highest concentration in specific period of time. This was based on the results of screening the effect of this extract on the protoscolices *in vitro*. The detail procedure involved is as follows. I Protoscolices of sheep origin were treated with the acoholic extract of the *T. indica* plant at (100 mg /ml) for 4 hours and then injected in four rats in the peritoneum (2000 protoscolices/ rat). Similarly Protoscolices of sheep were treated with aqueous extract of *T. indica* plant (100 mg / ml) for 4 hours and then injected in four rats in the peritoneum (2000 protoscolices/ rat), and Protoscolices not treated with *T. indica* extracts injected in four rats in the peritoneum (2000 protoscolices/ rat). This was used as a control group.

## Anatomy of rats

The rats that were injected with the protoscolices of sheep origin were treated with the extracts under study after three months of the secondary hydatid cysts were investigated in the peritoneum, liver, lungs, kidneys and other areas of the body using a magnifying lens. Pictures were taken for the rats of two groups.

## Statistical analysis

The appropriate statistical method used to analyze the results obtained from the experiments in this study is Genestat 5 program [12].

## Results

### Effect of aqueous and alcoholic extracts of *T. Indica* plant on protoscolices *in vitro*.

The results show a clear effect of alcoholic and aqueous extracts of *T. indica* leaves in the killing of protoscolices compared to control group. As represented in table (1). There were significant differences between concentrations and exposure periods at the probability level of  $p < 0.05$ . The concentration of 100 mg / ml for both aqueous and alcohol extract showed a greater reduction in the vitality of the protoscolices to zero at 4 hours compared with Control group with 94.33% and 96.33% respectively, at the concentration of by 75 mg / ml. The lowest rate of killing protoscolices was at the concentration 50 mg / ml at time of 1 hour for both aqueous and alcohol extract. The mortality rate was 48% and 54.67% respectively, compared to 95.33% and 93.67% in the control group respectively for aqueous and alcoholic extract. It is also observed that the exposure period of four hours surpassed the other exposure periods of time at the different

concentration rates used in this study. It is also noticed that when there is an increase in the concentration and the

exposure time, there will be more mortality rate of protoscolices (Fig. 3 and 4).

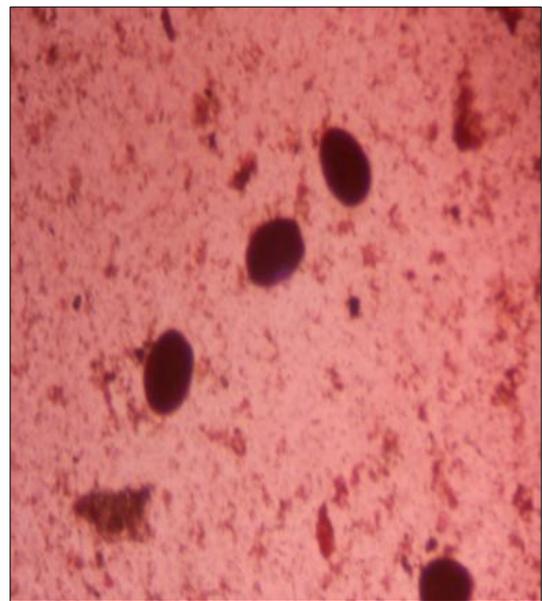
**Table 1:** Effect of *T. indica* extract on protoscolices *in vitro*.

Extract	Concentration	Control	Time /Hour				Mean
			1	2	3	4	
			M%	M%	M%	M%	
Alcoholic Extract	100mg/ml	96.33	82.33	88.33	98.00	100.00	93.00
	75mg/ml	94.67	68.00	76.67	89.33	96.00	84.93
	50mg/ml	93.67	54.67	63.33	77.00	88.33	75.40
Mean		94.89	68.33	76.11	88.11	94.78	84.44
Aqueous Extract	100mg/ml	94.33	77.33	86.67	97.00	100.00	91.07
	75mg/ml	93.00	64.33	72.67	84.00	92.67	81.33
	50mg/ml	95.33	48.00	57.33	70.00	84.00	70.93
Mean		94.22	63.22	72.22	83.67	92.22	81.11
Con*T	100mg/ml	95.33	79.83	87.50	97.50	100.00	92.03
	75mg/ml	93.83	66.17	74.67	86.67	64.33	83.13
	50mg/ml	94.50	51.33	60.33	73.50	86.17	73.17
Mean of Time		94.56	65.78	74.17	85.89	93.50	
LSD 5% Ex= 3.044, C=3.728, T =4.813, Ex*C =5.272, Ex*T= 6.806, C*T=8.336, Ex*C*T =11.789, CV= 8.7							

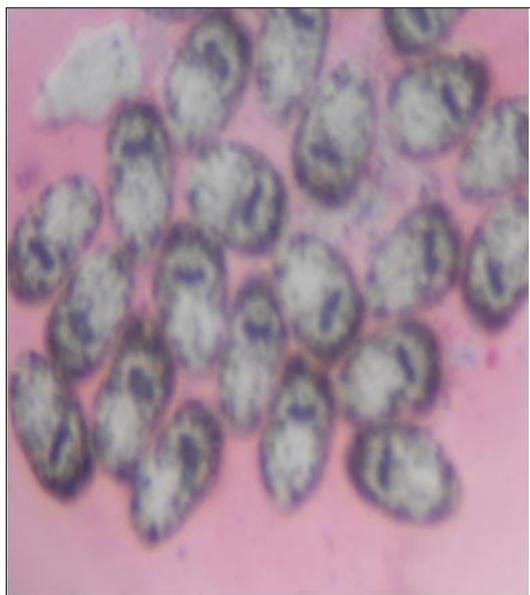
\*M: Mortality



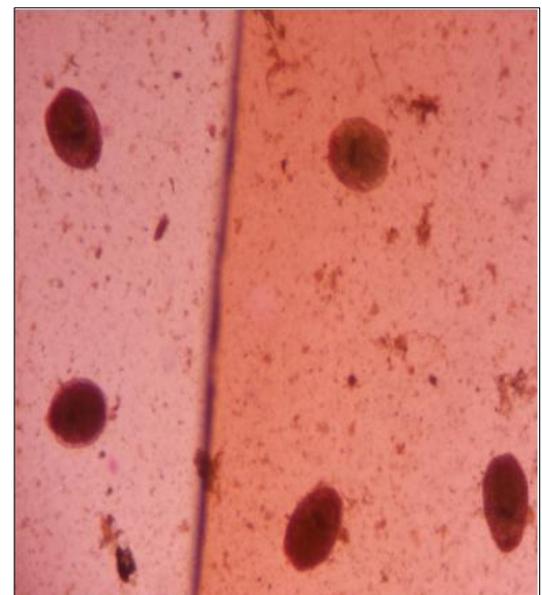
**Fig 1:** The hydatid cysts in liver of sheep.



**Fig 3:** Protoscolices treated at alcoholic extract *T. indica*



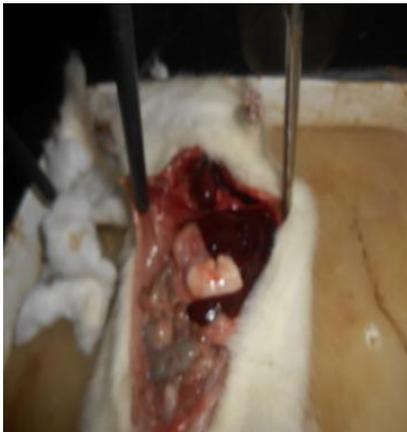
**Fig 2:** Vitality of the protoscolices.



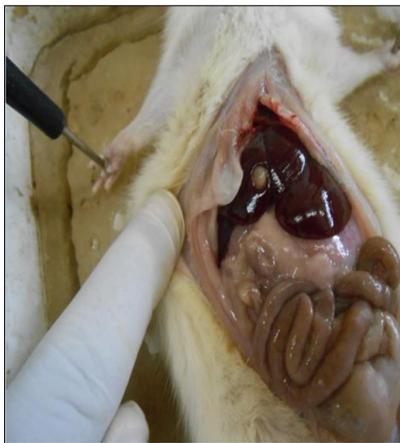
**Fig 4:** Protoscolices treated at aqueous extract *T. indica*



**Fig 5:** Rat treated by alcoholic extract *Tamarindus indica*



**Fig 6:** Rat treated by aqueous extract *Tamarindus indica*



**Fig 7:** Rat control

#### Effect of extracts of on protoscolices *in vivo* study

After performing the above mentioned experiments related with effect of alcoholic and aqueous extracts of *T. indica* plant on the vitality of the protoscolices *in vitro* and the observation of the mortality rate of the protoscolices *in vitro*. The protoscolices treated with the extracts under this study were injected into the peritoneum of the laboratory rats to check the effect of these substances on the mortality of the protoscolices *in vivo*. Three months later the rats were sacrificed and dissected to investigate the presence and growth of secondary hydatid cyst. (Fig.5 and 6). The hydatid cysts were clearly visible in the laboratory rats injected with the non-treated extracts using the substances used in this study, the control group shown the (Fig. 7).

#### Discussion

Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plant materials. Scientific studies indicate that the promising phytochemicals can be developed from the medicinal plants for many health problems. Moreover, the herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. These drugs are invariably single plant [13, 14].

The results of study were found superior in terms of exposure time with the study [15] when using the hot aqueous seed extracts of *Cucurbita maxima* plant a concentration of 60 mg / ml where they obtained the killing of the protoscolices after 24 hours of exposure. The results of the present study were also found to be superior to a study conducted [16], when boiling water was used to encroach the roots of pomegranate plant at a concentration of 20 mg / ml that obtained full death of the heads after 24 hours of exposure. It also surpassed the study carried out [17] when they used endophytic *Eupencillium* and *Chaetomium* isolates from *Azadirchta indica* as they obtained complete death of the heads at 6 hours exposure time. The results were found approximately similar to the result [18] when the raw alkaloids of the *Peganum harmala* were used at a concentration of 31%, which led to the destruction of the protoscolices at the time of 72 hours exposure.

The death of the protoscolices may be due to the fact that the *T. indica* plant contains the tannins, which have the ability to bind to the proteins inside the organism, preventing their degradation, which in turn leads to inhibiting the metabolism of nitrogen and amino acids that are essential to the survival of the organism, it may also be attributed to the fact that the tannins destroy the cellular membrane of the organism through the effect on the fat and proteins in it and then lose parasite ability to grow, and may penetrate the cell membrane and block the active substances of some enzymes inside the cell, which may be necessary for the growth of the parasite and proliferation [18, 19].

The mortality rate of the protoscolices treated by *T. indica* plant extracts can be attributed to its inclusion of active substances such as Alkaloids whose effect is a consequence of its reaction with the metabolic protein reaction required for the vitality of the protoscolices. Then this leads to the destruction of the cell wall and its proteins and fats till the protoscolices [20].

The death of the parasite can be due to Phenol substance which has an effect on the acetyl cholinesterase enzyme that controls the flexibility and permeability of the cell membrane. Phenols make the membrane lose its permeability which result in passing of various taxonic substances without regulating and this leads to the death of parasite [21].

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