Evaluate effectiveness of aqueous extract of the *Eucalyptus glubules* plant leaves on the growth and vitality of Hydatid Cysts Protoscolices of *Echinococcus granulosus* In Vitro

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Abstract. The present study aimed to evaluate the activity of different concentrations from aquatic extracts Eucalyptus globules leaves on the vitality of the protoscolices of *Echinococcus granulosus*. which collected from the infected sheep livers in Al-Najaf Al-Ashraf abattoir. protoscolices suspension was added to each concentration (2.5, 5, 10) mg/ml for different time periods, and the percentage of the viability of protoscolices was measured by using the eosin aqueous stain (0.1%). The results of the In Vitro. study revealed that the percentage of the viability of protoscolices was decreased at the concentration (2.5 / mg/ml. from 95 % to 27.24% in 96 hours, while the concentration (10) mg/l. decreased from 94% to 2.5% in 96 hours.

1. Introduction

Hydatid cystic disease (H.C.D) is one of the most serious epidemiological health problems in most parts of the world [1] It is known as the echinococcosis, cystic echinococcosis, hydatidosis, Hydiddosis Unilocular [2] is a common disease between humans and animals. The countries of the Middle East, North Africa, Sudan, the And the Caswian Sea Basin and some South American countries are highly endemic to the disease, Hyperendemic [3].

The disease in humans and other intermediates (sheep, cows, buffaloes, camels, horses and other animals) causes by larvae of *Echinococcus* parasite, which include many species, the most important of which are the genus *Echinococcus granulosus* and the *E. coli* type *E.multilocularis*. Member of the central host body [4].

In most rural areas where farm animals and meat foods are raised, the parasite's survival cycle requires the intermediate host and the final host (dogs, wolves, hyenas, leopards and other wild animals) [5].

The spread of hydatidosis disease can be attributed to two reasons: the first is the inability to detect the injury in the early stages because it shows symptoms only after the increase in the size of the sac, which leads to pressure on the tissues adjacent to it, the second reason is the loss of therapeutic means, The severity of the spread of metastasis [6].

The disease in Iraq is still an endemic and socially and economically affecting diseases, as well as its effects on the health of humans, which led many researchers to investigate the treatment methods,
although surgical intervention is one of the most important methods of treatment despite the serious problems experienced by the patient during surgery [7], because the patient is not surgically qualified due to age, anesthesia, or cyst in places where it is difficult for the surgeon to deal with it as in the cysts of the brain, heart or spine. Hence the importance of the Use of natural substances or extracts Various chemical may help in treating patients.

There are many materials used to treat the disease chemically against the Hydatidosis, it succeeded in part. The researchers focused on the activation of non-specific immunity in the infected host using isolated substances from various sources such as plant extracts [8] and fungi [9] And bacteria [10] Others studied the effect of platelet carbohydrates [11]. And proteins of primary and microbial and platelets [12] while others used sex hormones (estrogen and progesterone) because of their role in stimulating the immune system, such as immune rates against the hydatid cystic [13].

The aim of the present study is to evaluate the effectiveness of different concentrations of eucalyptus leaf extract on the vitality of the protoscolices of \textit{E.granulosus}, , isolated from the hydatid cyst fluid in infected sheep's livers.

2. Materials and Methods

\textbf{2.1. Collection of hydatid Cysts and Preparation of Protoscolices}

The hydatid Cysts were obtained from the livers of sheep which is naturally infected in Al-Najaf abbatoire (Fig. 1). They were placed in blastic containers and transported to the biology laboratory at the College of Education for girls. These organs were washed from the outside with water for the purpose of removing the blood and to suspend material during slaughter. The method [14] was used to collect Protoscolices, the surface of the hydatid Cysts was first sterilized using ethyl alcohol 70% and sterile 10 ml syringe with a 21-gauge needle. The collection process was performed under sterile conditions. The hydatid cyst fluid was withdrawn with the swim Protoscolices and placed in a flask 250 ml. Then open the cyst using scissors and forceps for the extraction of the generated layer and placed in a sterile container containing the sterile saline solution (Fig. 2) It was then washed with a washing bottle containing a saline phosphate solution (PBS).

![Fig. 1: germinal layer of hydatid cyst](image)

![Fig. (2) Sheep liver infected with a number of hydatid cysts](image)

Then collected this extract, Which was obtained and added to the hydatid fluid, which was delivered to the fluid, then collected the Protoscolices in sterile test tubes for deposition with the device Centrifuge,
Hettich - Micro 120 three times and at 3000 cycles / minute and for 15 minutes per deposition, and antibiotics were added before Starting with the second wash (Crystalline Penicillin at 2000 units / L and Streptomycin at 1 g / L) to the PBS wash solution during the second wash. After the washing was completed, the leachate was poured and a small amount of sterile saline phosphorylation was added to the precipitate to calculate the number of Protoscolices. Then, biopsies were carried out and the number of Protoscolices was calculated.

2.2. Estimation Of Protoscolices Viability
The viability of the Protoscolices was estimated by mixing a certain volume of the Protoscolices suspension with a similar volume of the water eosin dye (0.1%). Using a micropipette and a well-drained solution, a drop was examined directly under the microscope Olympus Co., LDT. The percentage of the Protoscolices that appeared was bright green (Fig. 3a) was calculated relative to the dead Protoscolices (Fig.3- B) Five replicates [15].

2.3. Count of viable Protoscolices
The number of Protoscolices was calculated using a fixed-size transfer method with a 10-microliter pipette after the suspension of the Protoscolices suspension and the sterile local phosphate analyzer. The number of Protoscolices per milliliter was calculated as follows:
Average number of Protoscolices in fixed volume used (10 μl) = 28.6 Protoscolice as in Table (2)
Permission number of Protoscolices in one millimeter = 100 × 28.6 = 2860 Protoscolice.
The approximate number of 2000 Protoscolice. (0.7 ml) was bright green, and the red-painted Protoscolice. were neglected because they were dead.

2.4. Preparation of the plant extract of Eucalyptus plant leaves
prepared by adding 50 g of eucalyptus leaf powder to 500 ml distilled water and boiled to 100 m for one hour. The mixture is then stirred by magnetic hazar for one hour to explode the walls of the plant cells. The mixture is then left in the refrigerator at a temperature of 4°C for an hour for the purpose of soaking [16].
The extract is then filtered with a filter paper of 0.45 Twice in a row. The sample is then kept in sealed glass bottles. Then separate the filter with Centrifuge at 3000 rpm for 10 minutes.
Take the filter and place in a 50 °C heat oven to dry the extract and obtain it from the extracted sample. In order to estimate the biological efficacy of the extract under study, take 2 g dry extract of the
plant extract and solvent in 100 ml distilled water, then concentrate the stock solution (10%). The concentrations of 2.5, 5 and 10% were prepared according to the law $N_1 V_1 = N_2 V_2$.

3. Results:

3.1. Estimating the vitality of the Protoscolices

Table (1) shows the vitality of the Protoscolices in 10 microliters and five replicates calculated by the fixed-size method. The average mean of the vitality of the Protoscolices at the treatment was at Zero hour $(28.6 \pm 4.49)$, while at 96 hour $(18.8 \pm 1.72)$.

| Transaction duration (hours) | The number of protoscolices calculated in (15)microliters For five replicates | Average number of protoscolices S.D ± S.E. |
|-----------------------------|--------------------------------------------------------------------------------|------------------------------------------|
| 0                           | 36 30 25 23 29                                                               | 6.28 ± 49.4 01.2                        |
| 12 hour                     | 28 22 32 27 31                                                               | 28 ± 52.3 57.1                          |
| 24 hour                     | 25 21 29 26 29                                                               | 26 ± 96.2 32.1                          |
| 48 hour                     | 28 26 25 24 20                                                               | 6.24 ± 65.2 18.1                        |
| 72 hour                     | 22 21 19 20 21                                                               | 6.20 ± 62.1 45.0                        |
| 96 hour                     | 17 19 18 22 18                                                               | 8.18 ± 72.1 77.0                        |

3.2. Determination of the percentage of vitality of the protoscolices

The percentage of protoscolices vitality was determined at (50) microliters and five replicates. It was found that the total number of protoscolices ranged between 141-151 and the average number of live protoscolices ranged between 135-145. The mean number of dead protoscolices ranged from (5-13). Therefore, the percentage of vitality of protoscolices was 94.83% as in Table (2).

| Duplicates | 1     | 2     | 3     | 4     | 5     | Total  | Arithmetic average S.D. |
|------------|-------|-------|-------|-------|-------|--------|--------------------------|
| Average number of protoscolices | 150   | 145   | 141   | 148   | 151   | 735    | 147 ± 3.6                |
| Average number of live protoscolices | 145   | 139   | 135   | 140   | 138   | 697    | 139.4 ± 3.3              |
| Average number of dead protoscolices | 5     | 6     | 6     | 8     | 13    | 38     | 7.6 ± 2.9                |
| percentage | 96.66 | 95.86 | 95.74 | 94.59 | 91.39 | 94.83  |                          |

3.3. Study of the effect of different concentrations of the aqueous extract of leaves of eucalyptus plant in the vitality of the protoscolices in vivo

It is noted from Table (3) that the percentage of protoscolices vitality decreased when the concentration of the extract was (2.5) mg / ml from 95 to 27.24 at 96 hours, while the concentration of 10 mg / ml decreased from 94 to 2.5 at 96 hour. As in Table (3).
Table (3) - Percentage of the vitality of the protoscolices after exposure to different concentrations of aqueous extract for leaves of eucalyptus plant and for different periods in vitro

| Conc. Mg/l | Percentage of vitality of protoscolices (%) |
|------------|-------------------------------------------|
| 0          | 96  92.7  91.4  86.11  80.55  77.8      |
| 2.5        | 95  70.4  59.75 48.92  35.1  27.24      |
| 5          | 94  61.1  44.14 32.84  19.19  8.66      |
| 10         | 94  54.3  32.62 23.18  12.92  2.5        |

4. Discussion

In the test of the vitality of the protoscolices, the current study was based on the phenomenon of the penetration of the water Eosin dye without relying on the evidence of the movement of the spin and the external displacement (Evagination) because of the difficulty of distinguishing between the moving and static protoscolices, which leads to errors in the count and the fact that the coup gives inaccurate readings of some Living protoscolices are not capable of multiplication for physiological reasons, so they are counted in the dead number, in contrast to the actual reality, whereas the phenomenon of the penetration of the Eosin dye is a competitive matrix related to the nature of the permeability of the biosphere.

Whereas the phenomenon of Eosin permeability is a competitive process related to the nature of the permeability of the biosphere.

When a physiological defect occurs, the dye is carried out through the walls of the casing, so that the dead protoscolices are painted red While living protoscolices retain their natural green color [17,18]. The Aqueous extract of the leaves of the eucalyptus plant had a significant effect on the reduction of the percentage of the vitality of the protoscolices of Echinococcus granulosus Parasite.

Increasing the concentration of the aqueous extract and increasing the length of time. The aqueous extract with a concentration of (10) mg / ml had a greater effect in reducing the percentage of the vitality of the protoscolices than the concentration (5-2.5) mg / ml.

This may be due to the possession of eucalyptus leaves on simple phenolic compounds, including Ellagic acid and Ferulic acid, and the presence of tannins and other compounds in small quantities (Devi et al., 1996).

These active compounds (phenols) break down the parasite's free membrane and its proteins And fat because of the susceptibility of these phenols to the deposition of proteins by the formation of hydrogen bonds between the groups of hydroxyl phenols and nitrogen compounds and proteins inhibit the work of enzymes necessary for the organism and thus lead to its destruction. (Reed, 1995).

Therefore, we conclude from the present study that the different concentrations of the aqueous extract of the leaves of the eucalyptus plant lead to a decrease in the percentage of the vitality of the protoscolices of the Echinococcus granulosus Parasite.

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