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# THE EFFECTIVE RANGE FOR SOME PLANT EXTRACTS ON ACTIVITY PROTOSCOLECES FOR *E. GRANULOSUS* IN VITRO

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**Abstract**

Hydatosis is an endemic disease in Iraq and neighboring countries. Its danger is that it cannot be diagnosed in the early stages of infection because the initial symptoms of the disease do not appear until after the hydatid cysts increase in size and put pressure on the tissues adjacent to it, and surgery is still the best available method to get rid of the disease, and since There are some cases in which it is not possible to perform surgical operations because the patient is not qualified for anesthesia or surgery. Therefore, research has directed towards the use of natural substances and compounds extracted from plants that have a healing efficacy. The results indicated that the extracts of *Mela azedrach* and *Capsicum annum* in eradicating Protoscoleces. It was also shown that the extract of *Capsicum annum* fruit was superior to that of *Mela azedrach* fruit extract, where the concentration of 15 mg/ml caused the kill of Protoscoleces 100% in the two times (60, 40) minutes, and showed that significant differences at the probability level ($p<0.01$) between all used concentrations and the general average for all exposure times.

**Key words:** hydatid cysts, *Mela azedrach*, *Capsicum annum*.

## Introduction

Hydatosis is a common disease that affects humans, caused by infection with the parasite *Echinococcus granulosus* [2,1]. This disease represents a health and epidemic problem in Asia, the Mediterranean, Africa, and South America [3]. It is an endemic disease in Iraq and neighboring countries, or hyperendemic in most Arabic countries due to the presence of large numbers of free dogs infected with adult worms, which are in direct contact with the intermediate hosts such as domestic animals, including humans [4]. The danger of this disease lies in the fact that it does not show pathological symptoms in the initial stages until after the cyst increases in size so that it puts pressure on the tissues adjacent to it, in addition to the loss of preventive means against this disease [5]. Although surgery is still the best available method for getting rid of Hydatid cysts [6], it may not be possible in many cases, when the patient is not qualified for anesthesia or surgery due to old age or other serious diseases, as the cyst may fall into organs that cannot reach it easily without possible side effects (dangerous Surgical intervention), as in cerebral hydatid cysts or attached to the heart or spine.

In view of the partial success of the drugs used in the treatment of hydatosis [7], research has strived towards the use of natural materials and compounds, especially medicinal plants, with healing efficacy for many diseases. Medicinal plants are among the important natural medicinal and therapeutic sources that played an important role in alleviating the suffering of people from

diseases, and about 70% of people these days practice the folk method, which is to resort to herbal medicine [8,9].

Studies in recent years have focused on many medicinal plants for efficacy, safety, and economic factors, as well as the fact that the plants contain compounds that are difficult to find a substitute for, whether by chemical or pharmaceutical methods (9). The current study came to investigate the effect of some extracts on the fruits of plants *(Mela azedrach , Capsicum annum*) on the viability and growth of Protoscoleces of *Echinococcus granulosus* in vitro.

**Material and method**

Hepatic hydatid cysts of human origin were obtained from hydatosis patient's disease after their surgical removal in Al-Diwaniyah Teaching Hospital and the various private hospitals in Al-Diwaniyah Governorate, after making sure that the cysts were not treated with protoscoleces before the eradication process. These cysts were transferred into refrigerated plastic containers and opened upon arrival to the laboratory. Smyth method [10] was used to obtain Protoscoleces and then the vitality of Protoscoleces was estimated by Eosin exclusion according to Smyth and Barret method [11], The movement of Protoscoleces was also taken into account, which is one of the important signs of vitality examination. The percentage of their live vitality in the calculated sample was calculated to the total number of Protoscoleces x 100. The process was repeated three times and the Survival rate was calculated, and the percentage of Protoscoleces vitality was calculated after each exposure time In this study the use of Protoscoleces with a vitality of <90%.

The fruits of *Mela azedrach* were collected from different areas in the city of Al-Diwaniyah, dried in the shadows, and then crushed using the electric grinder to obtain the fruits powder. The fruits of the *Capsicum annum* plant were also obtained from the herbs market in the center of Al-Diwaniyah Governorate. The samples were cleaned well, then dried and ground to obtain the fruits powder.

For the purpose of determining the effect of plant extracts on the vitality of in vitro Protoscoleces within a certain period of time and a certain concentration, the experiments were designed so that each concentration included three replicates, in addition to the control group containing the same number of Protoscoleces and not treated with the extract. Each tube was placed 1 ml of the dissolved plant extract in PBS. The concentrations and times of the aqueous extract containing approximately 1000 Protoscoleces were calculated, then the tubes were placed in a water bath at a temperature of 37 °C, and then the test tubes were lifted from the water bath and according to the specified times, and then the Protoscoleces treated with the plant extract were washed three times with PBS solution to get rid of the effect of The plant extracts were then examined, and they live and dead Protoscoleces were enumerated, and the results were recorded in the light of changes and morphological distortions, and the Protoscoleces were stained with eosin dye.

In order to show the effect of the effectiveness of the plant extract for Mela on the vitality of Protoscoleces, at certain concentrations and during specific periods of time until reaching the effective concentration and within the shortest period of time, the study was carried out at four concentrations, which are 300, 200, 100, 50 mg / ml at different times (12). And concentrations 1,

1,25, 1,50, 1,75, 2, 1, 2, 25 mg /ml to show the effect of the extract of the fruits of *Capsicum annum* (13) .

**statistical analysis**

The results were statistically analyzed using Complete Radomized Design (CRD) and Test Duncans̀ Multiple Rang at a probability level of (p 0.05) to determine the differences between different concentrations and times in Vitality of Protoscoleces (14).

**Results**

Tables (1,2), which represent the analysis of variance test, ANOVA, indicate that there are significant differences between the treatments at the probability level ($p \leq 0.01$) for the effect of each of the aqueous extracts of the fruits of *Mela azedrach* and *Capsicum annum* in the vitality of Protoscolece in vitro, respectively.

**Table (1): ANOVA analysis of the effect of aqueous extract of the fruits of *Mela azedrach* on the vitality of Protoscoleces in vitro at the probability level ($p \leq 0.01$).**

| Source of variance | df | Sum of squares (ss) | Mean squares (Ms) | F calculated |
|---|---|---|---|---|
| **Coefficients** | 22 | 63125.60 | 2231.52 | 341.42 |
| **Concentration** | 4 | 62229.80 | 12033.08 | 1501.62 |
| **Time** | 3 | 1813.71 | 602.22 | 72.10 |
| **Time× Concentraion** | 13 | 627.02 | 42.26 | 5.10 |
| **Experimental error** | 44 | 388.01 | 8.22 | |
| **Total** | 70 | 62521.25 | | |

**Table (2): ANOVA analysis of the effect of the aqueous extract of *Capsicum annum* fruits on the vitality of Protoscoleces at the probability level ($p \leq 0.01$).**

| Source of variance | df | Sum of squares(ss) | Squares(Ms) Mean | F calculated |
|---|---|---|---|---|
| **Coefficients** | **22** | **52807.50** | **2228.08** | **65.22** |
| **Concentration** | **4** | **53002.05** | **10021.0** | **228.30** |
| **Time** | **3** | **1642.32** | **610.42** | **15.02** |
| **Time×Concentration** | **13** | **202.20** | **12.62** | **0.25** |
| **Experimental error** | **44** | **1231.88** | **32.02** | |
| **Total** | **70** | **52568.62** | | |

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Duncan's test was conducted with the results shown in Tables (3,4) to clarify the effect of the extracts under study on the vitality of (Protoscoleces) and different concentrations showed different effects at different times, and this effect directly proportional with the concentration and exposure time. 30 mg/ml of *Mela azedrach* extract killed all Protoscoleces 100% at times 45,60 and 30 minutes and these times did not show a significant difference from the 15 minute time in which the vitality of Protoscoleces decreased to 2.00% at probability level ($p<0.01$), As in Table (3), it was observed that when Protoscoleces were exposed to the aqueous extract, the dye entered the Protoscoleces and colored it red.

**Table (3): Effect of aqueous extract of *Mela azedrach* leaves on viability of Protoscoleces in vitro according to Duncan test.**

| Time / Concentrations | Control 0minute | Average number of live protoscoleces after | | | | Generl average |
|---|---|---|---|---|---|---|
| | | 15minute | 30minute | 45minute | 60minute | |
| 5mg/ml | 95.00 | 87.00 o | 82.00 n | 80.11 n | 77.33 mn | 81,61 F |
| 10mg/ml | | 74.44 m | 67.22 l | 62.00 k | 61.11 jk | 66,16 E |
| 15mg/ml | | 57.00 j | 50.33 i | 44.22 h | 40.11 g | 47,91 D |
| 20mg/ml | | 37.22 g | 30.11 f | 20.99 e | 10.99 bc | 24,82 C |
| 25mg/ml | | 16.22 de | 14.00 cd | 10.77 bc | 8.00 b | 12,24 B |
| 30mg/ml | | 2.00 a | 0.00 a | 0.00 a | 0.00 a | 0,5 A |
| Overall average time | | 41,14 D | 40,61 C | 36,46 B | 32,92 A | |

Also, the concentration of 15 mg/ml of *Capsicum annum* fruit extract caused the death of Protoscoleces by 100% in the time 60 and 45 minutes, which did not differ significantly from the 30 and 15 minutes time, which caused the reduction of Protoscoleces vitality to 8% and 7%, respectively, at the probability level ( $p<0.01$) was followed by the rest of the concentrations shown in Table (4), and it was observed when Protoscoleces were exposed to the aqueous extract of the fruits of Capsicum annum that morphological distortions, shrinkage of Protoscoleces and the ingress of dye inside them.

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**Table (4): Effect of aqueous extract of *Capsicum annum* fruits on the viability of Protoscoleces in vitro according to Duncan test**

| Time<br>Concentrations | Control 0minute | Average number of live protoscoleces after | | | | Generl average |
|---|---|---|---|---|---|---|
| | | 15minute | 30minute | 45minute | 60minute | |
| 2.5mg/ml | 95.00 | 90.00 o | 82.00 no | 76.66 mn | 70.00 klm | 79,66 F |
| 5mg/ml | | 72.00 mn | 70.22 lmn | 69.00 klm | 63.00 jkl | 68,55 E |
| 7.5mg/ml | | 60.00 ijk | 58.00 ij | 53.39 hij | 52.66 hi | 56,025 D |
| 10mg/ml | | 45.32 gh | 40.00 fg | 38.22 fg | 30.62 ef | 38,54 C |
| 12.5mg/ml | | 26.00 de | 22.00 de | 18.00 cd | 10.26 bc | 7,065 B |
| 15mg/ml | | 8.00 abc | 7.00 ab | 0.00 a | 0.00 a | 3,75 A |
| Overall average time | | 50.22 D | 46.53 C | 42,54 B | 37.75 A | |

**Discussion**

There is no doubt that plants in general, and medicinal ones in particular, are an important source of many organic and inorganic compounds of pharmaceutical and pharmacological importance that can treat many diseases and bacterial and parasitic infections. In many cases, it was found that secondary substances in plants play an important role in Treatments Based on this characteristic, the medicinal plant drugs are sometimes comparable to the industrial drugs in their curative properties (15). The human being was forced to practice various methods to reduce this disease and its spread, including the use of plant extracts against Protoscoleces, which represents a transitional stage between humans, domestic animals and dogs, as a treatment for this the disease. As for the effect of plant extracts that were used in this study on the viability of Protoscoleces, the results agreed with (16) when they used aqueous extract of *Fagopyrum esculentum* seeds, seeds of *Trigonella foenum-graecum*, and recorded a complete death rate of Protoscoleces in 45 minutes, and we also agree with (17) When using the extract of *peganum harmala* and obtaining a phase of complete inhibition of Protoscoleces at a concentration of 500 mg / ml and within a period of 40

minutes. While the results of this study outperformed the (18) when used the aqueous extract of *Olea europaea* and *Christs thorn* at concentrations of 500,250,100,50 and 1000 mg/ml and obtained complete death of Protoscoleces in 72 hours for *Olea europaea* and 96 hours for *Christs thorn* in a concentration of 1000 mg/ml each, respectively, Regarding the effect of the fruits of the plant Mela azedrach on the vitality of Protoscoleces, its reduced the vitality of Protoscoleces to zero in a period of 30 minutes at a concentration of 300 mg / ml, and this is close to the results obtained from (17) when using tubers of Cyperus papyrus at a concentration of 500 mg / ml in a time of 3 hours Taking into account the superiority of the results of the current study in focus and time compared with (17).

The reason for the inhibitory effect of the mela azedrach aqueous plant extract may be due to the fact that its fruits contain active chemical compounds Salannin, Salammol, gedunin, deacetylsalanin, Salannolacetate, epoxyazaradion, which may have affected Protoscoleces by stopping metabolic processes or their effect on enzymes that occur cell cycles. Inside Protoscoleces (12). The results showed that the extracts of the fruits of *Capsicum annum* had a clear effect on the vitality of Protoscoleces of Echinococcus granulosus, and this effect was directly proportional to the increase in concentration and the increase in the duration of exposure to these extracts. This agrees with (13) who indicated that the concentration of 30 mg / ml of the aqueous extract of the leaves of *Teucrium polium* causes complete killing of Protoscoleces during times of 60, 45, 30 minutes. And with study (19) which recorded an extermination rate of 26.62% when using the aqueous extract of *peganum harmala* at a concentration of 500 mg / ml and a time of 30 minutes, as well as result of (20) with an extermination rate of 26.67% when using the aqueous extract of the fruits of the *Mela azedrach* plant at a concentration of 200 mg / ml and a time of 30 minutes, while the concentration of 1.25 mg / ml led to a decrease in the vitality of Protoscoleces to 77.00% and a time of 60 minutes. The results were higher than recorded by (21) when they used the aqueous extract of Fagopyrum esculentum seeds at a concentration of 25 mg / ml and a time of 45 minutes, where they recorded a percentage of vitality reduction for protoscoleces 35.66%, and (20) when they used the aqueous extract of the leaves of the plant Myrtus communis at a concentration of 300 mg / ml and a time of 15 minutes Which obtained a vital reduction rate for protoscoleces 35.67%. Also (19) when using the aqueous extract of *Thymus vulgaris* leaves at a concentration of 500 mg/ml for one hour, and the percentage of vitality reduction was 35.66%. The effectivity of *Capsicum annum* may be caused due to contains capsaicin, the alkaline substance, as well as it contains fatty acids, flavonoids, volatile oils, and dyes, as well as vitamin C, phosphorous, sulfur, vitamin B complexes, sodium, selenium, and proteins (13).

## Conclusion

The results indicated the superiority of the extracts of the fruits of the plants under study over the algal extracts in the negative effect on the vitality of Protoscoleces in vitro. The extract of *Capsicum annum* had the strongest and the fastest annihilating effect.Followed by *the Mela azedrach fruit extract*, While the algal extracts recorded a decrease in the vitality of Protoscoleces, but without killing them.

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