

## Study of the effect of *Cladophora crispate* and *Annona squamosa* L., alkaloids without or with albendazole on weights of mice infected with hydatid cysts

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### Abstract

The current study is aimed to test the incidence of infection at injection mice with protoscolices and evaluate the effectiveness of the effective compound derived from the algae *Cladophora crispate* and plant *Annona squamosa* L and compare it with albendazole at the infected with hydatid cysts.

The results of the current study show that there is a decrease in the average weight of infected mice and treatment with active substances alkaloids without or with albendazole compared to the positive control group as the effect of the mixed drug (algae, plant and albendazole). Concentrate (0.6 mg / ml) had given a therapeutic efficacy amount (82.35%) and gave a percentage of cystic fibers (80.0%) compared with the rest of the concentration the active ingredient drug.

This study found that the combination of both types of alkaloids algae *Cladophora crispate* and plant *Annona squamosa* L with albendazole had the greatest effect in reducing weights of infected mice with hydatid cysts.

**Keywords:** *Cladophora crispate*, *Annona squamosa*. L., Hydatid cysts, Albendazole

### 1-INTRODUCTION

The Hydatid cystic disease or Echinococcosis is one health problems and dangerous epidemiology in the most of the world<sup>1</sup>, and it is old disease and Zoonotic disease, this disease is epidemic in the some of the locations of the world such as Iraq, Syria, Lebanon, Palastin, and North of Africa and Al-Sudan and some of cities of South of America<sup>2</sup>.

The Hydatid cystic disease is the distribution disease in Iraq and it is infected large numbers specially in the location this as result of distribution of the sheep and dogs and consider to complete parasite life cycle which need intermediate host such as (sheep and goats) and the final host such as dogs<sup>3</sup>. Many chemical materials that use in the treatment the disease partly succeeded that make the researchers get many ideas for active of chemical treatment<sup>4</sup>. Others researchers was use many ideas to active the process drugs treating by using effect materials isolated from different sources<sup>5</sup>. The larval phase of hydatid cyst that growth from onchosphere from *Echinococcus*<sup>6</sup>.

### 2-MATERIALS AND METHODS

#### Protoscolices Preparation:

##### 2.1-Samples collection of Hydatid cysts

Collection infected livers and brought to the labrotary by blastic cysts and doing preparation during two hours. Use method<sup>7</sup> by collection the protoscolices clean the external surface of cyst by ethanol 70% and clean the nidle by heat and opened the

cyst to getting of germinal layer and put in baker 250 ml and cleaning by washing bottle that contain (PBS) and collect the hydatid fluid for use.

## 2.2-Injection of Mice with Viable Protoscolices

After knowing the appropriate number of live protoscolices in a specific volume of protoscolices suspension and saline phosphate buffer, 2000 protoscolices were injected by a medical syringe with a volume of 1 ml and a 21-gauge needle in to the cavity after draining the injection area with ethyl alcohol when injecting each of the experiment mice<sup>8</sup>.

## 2.3- Collection and diagnostic algae *Cladophora crispate* and *Annona squamosa*

Collection the algae during February 2019 from location Al-Mishkab and transported to the labrotary and diagnostic the algae under microscope, cleaning the sampling after drying and crushing and storing in the bottles. *Annona squamosa* brought to the labrotary and drying the seeds. Collection from the market and crushing after storing in the bottles.

## 2.4-Crud Alkaloides Compound Extracts Preparation

The extraction of alkaloids compounds for algae *C. crispate* and plant *A.squamosa* followed a method<sup>9</sup>. It took 10g of dry matter powder and extracted it in 200 ml of ethyl alcohol 96% for 24 hours in Soxhlets and then concentrated the resulting substance in Rotary evaporator, after that the resulting substance was dissolved in 5 ml of ethyl alcohol and then added to the alcohol extract 30 ml of acetic acid concentration 2%, then the Rotary evaporator was used to get rid of the ethyl alcohol and the acidic solution was left behind. The alkaloids were detected using the Draconderf detector<sup>10</sup> as he gave the test an orange or is required when adding a drop of the detector to a drop of acidic solution, this solution has been added 10% ammonium hydroxide and then developed base in the separation pad and then 10 ml of Chloroform and shake are several times, let the mixture until two layers form take the bottom layer that contains alkaloids.

## Determine the Lethal Dose (LD50) for *C. crispate* and *A. squamosa* l. 2-5-

An experiment was conducted to identify the half-lethal dose after taking 1g of dry matter for everyone who dissolved in a few drops of ethyl alcohol and then completed the volume to 100ml with distilled water, thus the concentration of the original solution became 1%, equivalent to 10 mg / ml.

Drunk the white mice Balb / c orally and monitored these mice within 24 hours and noticed signs of inactivity or death, starting with very few concentrations and upward until reaching the half-lethal dose<sup>11</sup>.

## 2-6-Deisgan the experiments study

The mice Balb/c with age 6-8 weeks after six months from infected to six groups 24 mice for every group. While mice control 24 mice for 8 mice to each group, the first group positive control group infected by protoscolices and not treatment, second group positive control group that infected by protoscolices and treatment with albendazole, third group negative control group that not infected by protoscolices but injected with:-

First test:- Alkaloids :- preparation three concentrations (0.4, 0.3, 0.2) mg / ml from each *C. crispate* and *A. squamosa* L, the mice division to groups 8 mice to each concentration 24 mice for each algae and plant. After six months from infected treatment the mice daily by oral during month.

Second test:- Alkaloids with albendazole :- take (0.4, 0.3, 0.2) mg / ml from alkaloidal extracted for each *C. crispate* and *A. squamosa* and mixed with

albendazole 0.2 mg/ml, the mice division to groups 8 mice to each concentration 24 mice for each algae and plant, treatment the mice daily by oral during month.

Third test :- Mixed alkaloids *C. crispate* and *A. squamosa* (0.2, 0.3 , 0.4) mg / ml together half by half 8 mice to each concentration (24) mice and oral treatment during month .

Fourth test:- mixed alkaloids each from *C. crispate* w and *A. squamosa* together (0.2, 0.3 , 0.4) mg / ml together with albendazole 0.2mg/ml(24) mice and oral treatment during month .

## 2-7-Statistical Analysis

Analyze the product statistically using contrast analysis Anova with Least significant Differences ( LSD) at significant level  $p < 0.05^{12}$  .

## 3-Results

### 3-1-Weighting of mice treatment with alkaloids

Table (1) shows the rate of increased and decreased in weights the infected mice with hydated cysts and treatment by different concentration of alkaloids of algae and plant . The results have been showed that losing of weight when treating mice increased with increase the concentration of extracting materials from algae and plant .Concentration (0.4mg/ml) was the best of alkaloids of algae and plant .The increasing rate of negative and positive control grouping was high compared with the treatment groups.

**Table (1) Rate weighting the infecting and non infecting mice treatment with alkaloids of *Cladophora crispate* and *A.squamosa L.* for 30 days**

|                               | Concentrations<br>Mg/ ml                        | Rate of weight<br>Before treatment( g) | Rate of weight<br>after treatment (g) | Rate% weight<br>increase and<br>decrease |
|-------------------------------|-------------------------------------------------|----------------------------------------|---------------------------------------|------------------------------------------|
| <i>Cladophora<br/>crispat</i> | 0.2                                             | 30.6 $\pm$ 2.4                         | 32.0 $\pm$ 2.1                        | 4.57+                                    |
|                               | 0.3                                             | 32.2 $\pm$ 2.7                         | 2.8 32.3+                             | 0.30+                                    |
|                               | 0.4                                             | 32.3 $\pm$ 2.8                         | 32.1 $\pm$ 2.7                        | 0.61-                                    |
| L.S.D                         |                                                 |                                        |                                       | 0.6*                                     |
| <i>Annona<br/>squamosa L</i>  | 0.2                                             | 3.2 $\pm$ 31.1                         | 31.6 $\pm$ 3.4                        | +1.60                                    |
|                               | 0.3                                             | 32.7 $\pm$ 2.4                         | 33.1 $\pm$ 2.9                        | 1.22+                                    |
|                               | 0.4                                             | 33.5 $\pm$ 3.1                         | 34.2 $\pm$ 2.7                        | 2.08+                                    |
| L.S.D                         |                                                 |                                        |                                       | 0.3*                                     |
| Control<br>groups             | Positive group<br>treatment with<br>albendazole | 2.4 32.8 $\pm$                         | 33.8 $\pm$ 2.9                        | + 3.04                                   |
|                               | Positive group ( +)                             | 35 $\pm$ 1.2                           | 36.2 $\pm$ 2.5                        | 3.03+                                    |
|                               | Negative group ( -)                             | 33.2 $\pm$ 3.07                        | 34.1 $\pm$ 3.7                        | 2.6+                                     |

$P < 0.05$

### 3-2- The mice treatment by alkaloids with albendazole 0.2 mg/ ml

The table (2) shows the rate weighting the infected mice with hydatid cysts and treatment by different concentration of alkaloids of algae and plant with albendazole 0.2 mg / ml and percentage . The results showed the mice treatment with alkaloids of *A.crispata* with albendazole ( 0.5 , 0.6) mg / ml decreasing in weight to against from alkaloids the algae with albendazole the lose in weight at 0.6 mg/ml .

**Table(2) Rate weighting the infecting and non infecting mice treatment by alkaloids of *Cladophora crispata* and *A.squamosa* L. with albendazole for 30 days**

### 3-3The mice treatment with mix from alkaloids the algae and plant without or

|                                                | Concentrations<br>Mg/ ml                        | Rate of weight<br>Before treatment( g) | Rate of weight<br>after treatment (g) | Rate% weight<br>increase and<br>decrease |
|------------------------------------------------|-------------------------------------------------|----------------------------------------|---------------------------------------|------------------------------------------|
| <i>Cladophora<br/>crispata</i><br>+albendazole | 0.4                                             | 32.7±2.4                               | 33.1±2.9                              | 1.22+                                    |
|                                                | 0.5                                             | 32.3±2.8                               | 32.5±2.7                              | 0.30-*                                   |
|                                                | 0.6                                             | 32.3±2.8                               | 32.1±2.7                              | 0.61+                                    |
| L.S.D                                          |                                                 |                                        |                                       | 0.3*                                     |
| <i>Annona<br/>squamosa</i> L<br>+albendazole   | 0.4                                             | 31.2±3.1                               | 31.6±3.4                              | 1.60+                                    |
|                                                | 0.5                                             | 34.2±2.7                               | 33.5±3.1                              | 2.04+                                    |
|                                                | 0.6                                             | 32.0±2.1                               | 30.6±2.4                              | 4.37+                                    |
| L.S.D                                          |                                                 |                                        |                                       | 0.5*                                     |
| Control<br>groups                              | Positive group<br>treatment with<br>albendazole | 32.8±2.4                               | 33.8±2.9                              | + 3.04                                   |
|                                                | Positive group ( +)                             | 35±1.2                                 | 36.2±2.5                              | 3.03+                                    |
|                                                | Negative group (-)                              | 33.2±3.07                              | 34.1±3.7                              | 2.6+                                     |

**P< 0.05**

### with albendazole 0.2 mg / ml

The table (3)shows compared between the weight infected mice with hydatid cysts and treatment with different concentrations mix from alkaloids the algae and plant without or with albendazole 0.2 mg / ml for 30 days , and loss the weight of treatment mice has increased with increase in the concentration of the alkaloid mixture together as it showed significant differences in statistical analysis , and when using the algae and plant alkaloids mixes with albendazole , decreasing weights in all concentrations.

.Table (3) the average weight of healthy, infected and treated mice with a mixture of algal and plant alkaloids without or with albendazole for a period of 30 days

|                                                               | Concentrations<br>Mg/ ml                        | Rate of weight<br>Before treatment( g) | Rate of weight<br>after treatment (g) | Rate% weight<br>increase and<br>decrease |
|---------------------------------------------------------------|-------------------------------------------------|----------------------------------------|---------------------------------------|------------------------------------------|
| <i>Cladophora<br/>crispata</i> + <i>A.<br/>squamosa</i> L     | 0.2                                             | 33.3±0.212                             | 33.8 ±0.158                           | 1.50                                     |
|                                                               | 0.3                                             | 34.0±0.866                             | 34.5±0.707                            | 1.47                                     |
|                                                               | 0.4                                             | 33.6±0.165                             | 34.0±0.866                            | 1.19                                     |
| L.S.D                                                         |                                                 |                                        |                                       | 1.09*                                    |
| <i>C.crispata</i> + <i>A.<br/>squamosa</i> L.<br>+albendazole | 0.4                                             | 32.5 ±0.395                            | 31.6±0.082                            | 2.76                                     |
|                                                               | 0.5                                             | 33.7±0.117                             | 33.5±0.353                            | 0.59                                     |
|                                                               | 0.6                                             | 32.6±0.293                             | 30.6±0.387                            | 6.13                                     |
| L.S.D                                                         |                                                 |                                        |                                       | 1.3*                                     |
| Control<br>groups                                             | Positive group<br>treatment with<br>albendazole | 2.4 32.8±                              | 33.8±2.9                              | + 3.04                                   |
|                                                               | Positive group ( +)                             | 35±1.2                                 | 36.2±2.5                              | 3.03+                                    |
|                                                               | Negative group (-)                              | 33.2±3.07                              | 34.1±3.7                              | 2.6+                                     |

P<0.05

### Discussion

This study has been used the alkaloids *C.crispate* and *A.squamosa* L each alone or mix together or mix each one of these alkaloids with albendazole or mix together with albendazole with orally treatment and different concentrations to period 30 days in treatment infected mice with protoscolices to *E.granulosus* parasite that showed the results decrease in weight rating , and the cause of this decrease may be as result to effect these materials when using in treatment and the results proofed as the albendazole is effect drug in treatment the hydatid cysts in mice that lead to reduced these cysts at 0.3 mg / ml by orally treatment to period 30 days or that cause low the number lymphocytes that important to growth hydatid cysts and against stimulating immune and production antibodies and important in dead the protoscolices<sup>13</sup> . the mixture alkaloids algae and plant with albendazole was the best to low the weight of mice treatment at concentration 0.6 mg / ml as result to effecting these effect materials on germinal surface in secondary hydatid cysts in treatment mice in the form of degeneration and sloughing in the cyst these results resemble to result study that is decrease in the rate weights the mice at treatment with phenols or alkaloids specially at mix with albendazole<sup>14</sup>, but in other study was the extraction the methanol and hexan from algae *C.crispate* that lead to low weighting the infected animal after treating comparision with albendazole<sup>15</sup>, but these do not resemble study<sup>16</sup> that found increasing in weights of mice treated with the alcoholic extractor and aqueous extract to *Punica granatum* and explained that increase because good nutrition and long test time for uninfected mice but the infected mice and treating was cause the increasing to liver and other organs overblown by infected with hydatid cysts.



Found when the mix alkaloids extracted from *Sophora moorcroftiana* with albendazole proof the mix effect together was strong on the secondary hydatid cysts<sup>17</sup>.

#### References

- 1-Zhang, R ; Chen, X. and Wen, H. ( 2017). An improved experimental model of cystic hydatid disease in liver resembling natural infection route with stable growing dynamics and immune reaction . bioRxiv, 155168.
- 2-Pradhan, S., Dahal, R., Phuyal, S., & Ghimira, B., Singh, Y. (2017). Primary Hydatid Cyst of Pancreas. Retrieved from Case Report. Acta Scientific Medical Sciences., 101: 25-27.
- 3-Hayajneh, F. M. F., Althomali. A. M. H., & Nasr, A. T. (2014). Prevalence and characterization of hydatidosis in animals slaughtered at Al Taif abattoir, kingdom of Saudi Arabia. Open Journal of Animal Sciences, 4(10), 38.
- 4-. Polat, E. ; Aslan, M. ; Cakan, H. ; Saribas, S. ; Ipek, T. and kocazeybek, B. (2009). The effect of albendazole and povidone iodine for hydatid cysts protoscoleces , In vitro and vivo. Afri. J. Microbiol. 3(11) : 743-746.
- 5- Nunnari, G. ; Pinzone, M. R. ; Gruttadauria, S. ; Celesia, B. M. ; Madeddu, G. ; Malaguarnera, G. ; Pavone, P. ; Cappellani, A . and Cacopardo, B . (2012) . Hepatic echinococcosis : Clinical and therapeutic aspects . World. J. Gastroenterol.,18(13):1448-1458.
- 6-Falih, E. B. (2002). Parasitological, pathological and immunological studies on hydatidosis in mice and goats and the use of heat in naturally occurring the treatment of lesions of hydatidosis in animals and man. Ph.D. Thesis, Coll. Vet. Med. Univ. Baghdad. (Arabic).
- 7-Kwan, M. M. (1985). Life behavior of larvae (*Echinococcus granulosus* From intermediate host in mice immunocompromised , M. Sc. Thesis, Coll. Veterinary medicine. , Univ. Baghdad . 95pp.
- 8-Wangoo, A; Grangulyand, N. K. ; Mahajam, R.C. (1989). Phagocytic function of monocytes in murine model of *Echinococcus granulosus* of human origin. Indian. J.Med. Res. 89: 40 – 2.
- 9- Al-Samaraai , K, O. ( 1983).Distribution the alkaloids and importance the taxonomy in some type from Solanaceae in Iraq. M . SC. College of Sciences .Baghdad : 157 pp.
- 10-Harborne, J. B. (1984). Phytochemical methods. Chapman and Hall. New York. 2<sup>nd</sup> edn., : 288pp.
- 11-Litchfield, J .T. and Wilcoxon, F. (1949). A simplified method of evaluating dose effect experiments. J. Pharmacol. EXP. And Therap., 96: 94-113.
- 12-Al-Rawi, K & K. ( 2000) . Design and analysis of agricultural experiments. Book House for printing and publishing , Coll. Mou.
- 13- Yarsan, E.; Tanyuksel, M.; Babur, C. and Kutlu, I. (1999). Evaluation of albendazole on humoral and cellular immune response. Turkish Bulletin of Hygienic and Experimental Biology No.2.
- 14-Al-Humairy , A, K. ,( 2010). Evaluation of the activity *Datura stramonium* seeds extracts on growth and development of hydatid cysts for *Echinococcus granulosus* in white mice *Balb / c* ( Therapeutic , Histologic and

Immunologic study). Ph . D. thesis. College of Sciences . Babylon Uni, 167pp.

**15-Khalaf,A. K. (2012).** In vitro and in vivo studies of secondary metabolites extracted from some algae against protoscolices of *Echinococcus granulosus* in laboratory mice Balb/ c strain in comparision to albendazole activity. Ph.D . Thesis, College of Education. University of Basrah.

**16-Al-Mubarak, Z . A.(2006)** .Extraction effect of *Punica granatum* in treatment *Echinococcus granulosus* in mice . M. Sc. Coll, edu for girls . Uni .Ku. 98pp.

**17-Ma, X. M. ; Bao, G. SH. , Wan, J. M. ; Liao, D. J. ; Yin , SH.F.; Meng, X. Q. ; Zhou, G. K. ; Lu, X. M. and Li, H. Y. (2007).** Therapeutic effect of *Sophora moorcroftiana* alkaloids in combination with albendazole in mice experimentially infected with protoscolices of *Echinococcus granulosus* . Braz. J. Med. Biol. Res. , 40(10): 1463-1708.