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Comparative Study of Active Material for Algae Cladophora Crispate and Annona Squamosa L. on the Activity of Protoscolices of Echinococcus Granulosus in Vitro

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ABSTRACT

The present study was conducted from the January 2019 to March 2020 to evaluate the effect of active materials (alkaloids) of algae Cladophora crispate and Annona squamosa on the vitality of the protoscolices of the larval stage of Echinococcus granulosus In vitro as therapeutic study for these active materials alone or in mixing with albendazole (0.3 mg / ml). Protoscolices were collected from the livers of infected sheep in Al-Najaf Al-Ashraf abattoir. The suspention was added to the different concentrations of the therapeutic agents alone or with albendazole which were used for different time period. The vitality of these Protoscolices was measured using 0.1% aqueous eosin. This study showed in vitro that there are statistically significant effects of the agent, the drugs agent (alkaloids the Cladophora crispate and Annona squamosa with albendazole is the best in killing the protoscolices 100% at the second day at the 0.4 mg/ml, alkaloids A. squamosa killing the protoscolices 100% at the fifth day at the 0.4 mg /ml, while alkaloids C. crispate lead to killing the protoscolices 100% at the sixth day at concentration 0.4 mg /ml, but mix the alkaloids the C. crispate with albendazole and alkaloids the A. squamosa with albendazole killing the protoscolices 100% at the third day at the 0.4 mg / ml respectively.

Keywords: Echinococcus Granulosus, Cladophora Crispate, Annona Squamosa.

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INTRODUCTION

The Hydatid cystic disease or Echinococcosis is one health problems and dangeours epidemiology in the most of the world [1], 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, Lebenon, Palastin, and North of Africa and Al-Sudan and some of cities of South of America[2]. 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[3]. Many materials that use in the treatment the disease partly succeeded that make the researchers get many ideas for active of chemical treatement[4] study the active and strong effect when the mix the alkaloids plant of *Sophora moorcoftaina* with albendazole in the killed secondary cyst in the infected white mice and also effect on the vatility of the protoscolices that take from one cyst in the vitro.

Classification of Parasite

Classification of parasite *Echinococcus* is the most important that depend on the morphological and structural of parasite [5]. and the *E. granulosus* in the classification system is.:

Phylum: Platyhelminthes
Class : Cestoidea
Subclass : Eucestoda
Order : Cyclophylidae
Suborder : Taeniada
Family : Taeniidae
Subfamily : Echinococcinae
Genus : *Echinococcus granulosus*

Algae and Use it in the Parasite Treatment

Algae are groups of the organism that is form by photosynthesis process and change the photo powers from sun to chemical power in the form of chemical compounds and contain chlorophyll stain that important for this process[6]. Algae are capacity to produce large number from biological active compounds such as groups of alkaloids, peptides, tri trbines and tannins[7]. Algae extracted in the last years are very important to known drugs effected anti bacterial, antiviral, antifungal and anti parasite and also produce anti oxidant.

Studies about Algae Cladophora Crispata

In the study[8] use the compound 2-N, Ndimethylhyrazino cyclohexane carbonitrile and it is alkaloid compound extracted from *Cladophora crispate* and *Hapalosiphon aureus* that is anti parasite to first time in Iraq against Hydait cystic disease and showed these study activity this compound against protoscolices of *E. granulosus*. Either study[9] the compound 2-N, Ndimethylhyrazino cyclohexane carbonitrile and Pyridine 2,3,4,5 tetrahyro extracted from *Cladophora crispate* compounds are against effect of protoscolices Hydait cystic disease compared with albendazole.

Active Compound in Plant Annona Squamosa L

The alkaloid compounds distributed in *Annona squamosa L* are Such as alkaloid compound Atisine in the root also other compounds are Alkaloids oxophoebine, reticuline, isocorydine, Flavonoid quercetin-3-o-glucoside methylcorydaldine.

MATERALS AND METHODS

Preparation of Solutions

Normal Saline Solution: Use
.Nacl 9 gm 1-
2-Distilled water 100

Kerbs–Ringer Solution.: Preparation this solution by use different materials [10].

Penicillin	4000 I.U.
Streptomycin	200 mg.
NaH_2Po_4	0.097 gm.
Na_2HPo_4	0.490 gm.
$Mg\ So_4$	0.072 gm.
$NaHCo_3$	0.281 gm.
KCl	0.157 gm.
NaCl	0.480 gm.
$CaCl_2$	0.0137 gm.

Phosphate Buffer Saline Solution (PBS).: Preparation by use these materials.:

KH_2PO_4	0.2 gm.
$Na_2HPO_4.12H_2O$	2.89 gm.
KCl	0.2 gm.
NaCl	8 gm.
Distilled water	

Eosin Aqueous Stain (% 0.1).: Preparation By Use Method[11]

Distilled water 10 ml
Eosin Powder 0.01 gm

Samples Collection of Hydaitd Cysts and Protoscolices Preparation

Collection infected livers and brought to the laboratory by blastic cysts and doing preparation during two hours. Use method[12] 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.

Numbering Protoscolices and Determination Viability

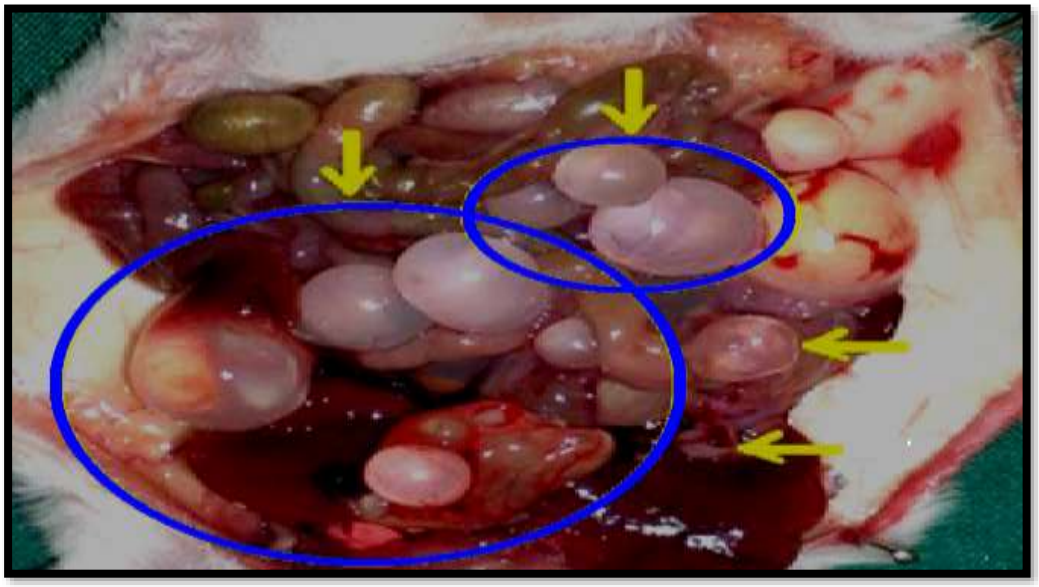
Numbering protoscolices by use method volume transport by micropipite 10 microliter and the numbering all the protoscolices by microscope under power 20 x and testing viability of protoscolices by use Eosin Aqueous Stain (% 0.1) and coloring the dead protoscolices by red color and life protoscolices by green color[13].

Collection and Diagnostic Algae Cladophora Crispate and Annona Squamosa

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

RESULTS

Diagnostic the parasite isolation from the infected livers in larval stage (Hydatid cyst) and it is *Echinococcus granulosus* to these cycle cysts and no branched in the picture (1).



Picture 1: Show cycle cysts

Estimation the Protoscolices Viability

Show the table (1) the protoscolices viability in 10 microliter for five replication and the average for the protoscolices viability in the hour zero (30.6 ±) but this average was in hour 96 (18 ±).

Table 1: Show the protoscolices viability in 10 microliter for five replication

Hour	Number protoscolices in 10 ML of five Replication					M ± SD	SE
	1	2	3	4	5		
0	40	33	27	25	28	30.6±5.38	2.41
12	35	30	32	28	30	31±2.32	1.04
24	28	26	29	22	20	25±3.46	1.55
48	30	25	27	20	22	24.8±3.54	1.58
72	25	23	18	20	21	21.4±2.41	1.08
96	16	18	20	19	17	18±1.41	0.63

Determination the Rate of Protoscolices Viability

Determined the rate of protoscolices viability in 50 microliter of five replication, show the rate of all number protoscolices between (151-141) and the rate of the life protoscolices was between (145-135), and the rate of dead protoscolices was between(13-5), so the rate of protoscolices was (94.96) table (2).

Table 2: Show the rate protoscolices viability in 50 microliter for five replication

Replecation	1	2	3	4	5	Sum	M± SD
Rate of all number of protoscolices	150	144	141	149	151	735	147±3.6
Rate number of life protoscolices	145	138	135	141	139	698	139.6±3.3
Rate number of dead protoscolices	5	6	7	8	13	39	7.8±4.7
Rate %	96.66	95.83	95.74	94.63	92.05	94.96	

Study Effect of Albendazol in Protoscolices Viability in Vitro

Show the table (3) this clear scientific different from 0.1 mg / ml low the protoscolices viability from (94.7) to zero in hour 168 in seven day from testing while low protoscolices viability when use (0.3,0.2) mg/ ml from (93.5, 92.2) to zero in hour 120 (five day) and show (L.S.D) 0.3 mg/ ml is high effect.

Table 3: The rate protoscolices viability when explored to different from albendazol and different time in vitro

Hour / Mg/ml	The rate protoscolices viability							
	0	24	48	72	96	120	144	168
Control	95.2	87.2	80.6	75.9	70.5	63.8	55.5	50
0.1	94.7	65.4	50	30	.16.4	7	0	0
0.2	93.5	45.5	28.3	18.5	8.9	0	0	0
0.3	92.2	25.2	15.4	11.8	6.4	0	0	0
L.S.D= 15.4								

Alkaloids Effect of Cladophora Crispate in Protoscolices Viability in Vitro

The rate of protoscolices viability is low when the explore to different from alkaloids *Cladophora* if when 0.2 mg/ ml low from (94) in hour zero to (8.9) in seven day, while in 0.4 mg / ml the rate of protoscolices viability in hour zero was (90) compared of viability in six day was killed of all them.

Table 4: The rate protoscolices viability when explored to different from and alkaloids Cladophora crispat a different time in vitro

Time(hour) Mg/ ml	The rate% of protoscolices viability							
	0	24	48	72	96	120	144	168
Control	95	93.5	91.2	88.4	79.6	76.8	64.6	62.5
0.1	94	60.5	49.9	34.84	25.11	18.8	13.76	8.9
0.3	92	45.7	30.55	20.57	12.44	9.55	6.66	2.77
0.4	90	33.03	23.89	14.96	7.8	2.4	0	0
L.S.D =12.39								

Alkaloids Effect Annona squamosa in Protoscolices Viability in Vitro

When the use of different from alkaloids *Annona squamosa* show the deference in protoscolices viability starting in hour zero was the rate of viability (93) in concentration (0.2 mg/ ml) compared with the rate of viability in five day with concentration 0.4 mg/ ml killed all the protoscolices table (5).

Table 5: The rate protoscolices viability when explored to different from and different time in vitro alkaloids Annona squamosa

Time(hour) Mg/ ml	The rate% of protoscolices viability							
	0	24	48	72	96	120	144	168
Control	96	92.7	86.7	81.3	76.5	70.9	65.8	60.9
0.1	93	50.1	40.1	27.9	20.3	10.5	6.9	4.2
0.3	92	45.1	30.1	26.5	18.2	10.4	5.3	3.1
0.4	91	40.1	25.1	15.8	10.3	0	0	0
L.S.D = 6.86								

Alkaloids Effect of Cladophora Crispate with Albendazole in Protoscolices Viability in Vitro

The result showed in table (6) that was scientific differences and the rate was (95) in hour zero and concentration (0.1 mg / ml) but this rate low in five day to zero from treatment and the viability low in third day from treatment with concentration (0.4 mg / ml) to zero the analysis proof the concentration moor effect is (0.4 mg / ml).

Table 6: The rate of rate protoscolices viability when explored to different concentration from alkaloids Cladophora crispata with albendazole different time in vitro

Time(hour) Mg/ ml	The rate% of protoscolices viability							
	0	24	48	72	96	120	144	168
Control	96	94.5	89.7	80.6	78.4	70.9	65.8	60.9
0.1	95	31.22	8.12	4.35	1.22	0	0	0
0.3	94	22.7	6.14	3.55	0	0	0	0
0.4	94	12.65	3.15	0	0	0	0	0
L.S.D=10.62								

Alkaloids Effect of Annona Squamosa L with Albendazole in Protoscolices Viability in Vitro

When use concentration from alkaloids *Annona squamosa* and mix it with albendazole 0.1 mg /ml was effect in low protoscolices viability, in hour zero and concentration 0.1mg / ml the rate was (95) but in fourth day was moor effect in killed all the protoscolices but in concentration 0.4 mg / ml was result to low the viability to zero in third day from treatment table (7).

Table 7: The rate of rate protoscolices viability when explored to different concentration from alkaloids Annona squamosa with albendazole to different time in vitro

Time(hour) Mg/ ml	The rate% of protoscolices viability							
	0	24	48	72	96	120	144	168
Control	95	91.5	83.72	77.6	70.4	68.9	65.4	60.9
0.1	95	30.22	15.1	3.25	0	0	0	0
0.3	93	20.7	5.14	2.95	0	0	0	0
0.4	90	10.65	1.15	0	0	0	0	0
L.S.D=5.88								

Effect the Mix Alkaloids Algae Cladophora Crispate and Plant Annona Squamosa L in Protoscolices Viability in Vitro

The result show if use all the types of alkaloids lead to low protoscolices viability in 0.1mg / ml and hour zero was (95) to (9.8) in seventh day from treatment, but this rate low from (92) to zero in sixth day from treatment and concentration 0.4 mg / ml.

Table 8: The rate of rate protoscolices viability when explored to different concentration from alkaloids algae Cladophora crispate with Annona squamosa to different time in vitro

Time (hour) Mg/ ml	The rate% of protoscolices viability							
	0	24	48	72	96	120	144	168
Control	95	88.5	83.2	77.6	72.4	69.9	63.4	61.9
0.1	95	35.2	25.1	33.2	28.4	20.7	16.3	9.8
0.3	93	25.7	15.14	22.95	19.9	17.4	10.6	5.1
0.4	90	20.6	12.15	7.5	4.3	1.2	0	0
L.S.D=11.74								

Effect the Mix Alkaloids Algae Cladophora Crispate and Plant Annona Squamosa L with Albendazol in Protoscolices Viability in Vitro

Show the result in table (9) that present scientific differences, in concentration 0.1mg /ml low the rate of protoscolices viability from(94) to zero in fourth day from treatment, also low the rate from 90 to zero in second day from treatment at the concentration 0.4 mg/ml.

Table 9: The rate of rate protoscolices viability when explored to different concentration from alkaloids algae Cladophora crispate with Annona squamosa with albendazol to different time in vitro

Time(hour) Mg/ ml	The rate% of protoscolices viability							
	0	24	48	72	96	120	144	168
Control	95	90.4	88.2	82.4	75.6	76.8	64.6	61.5
0.1	94	24.25	7.9	4.4	0	0	0	0
0.3	92	19.7	13.5	2.57	0	0	0	0
0.4	90	12.3	0	0	0	0	0	0
L.S.D=4.48								

DISCUSSION

The hydiat cyst disease is trough challenge for doctors and researchers in parasitological, it is fighting human life and infected animals so lead to large loose, so that, using different methods to from distribution after known the life cycle by programs of disease and away toughing the dogs, the complete many studies about disease in Iraq and world, in this study use protoscolices from sheep's so that strain is very active in infected the human[14]

Effect the Activity of Albendazol in Protoscolices Viability in Vitro

Albendazol is from drugs that show effect in low protoscolices viability with increased the concentration, and this with studies[15] and Al-Nakeeb. [16]Albendazol is from

Benzimidazole that is medical importance against microorganism and parasite, albendazol contact with structure protein B-tubulin that is lead to closed Polymerization process in Microtubules that causes damages in transport and growth of parasite cells and cut absorption the glucose in the worms and larva and lead dead the parasite[17]. The result of these study the strong effect of albendazol on the killing of parasite in vitro, and increasing the concentration lead to reduction the number of protoscolices from first day until the seventh day, and was the concentration 0.3 mg/ ml from albendazol the greater effect in reduction protoscolices viability, so was albendazol the first in treatment the cases that not possible treatment surgically[18].

Effect the Alkaloids Algae Cladophora Crispate and Plant Annona Squamosa L without or with Albendazol in Protoscolices Viability in Vitro

Algae *C.crispata* is consider from rich plants with chemical compounds, as that use the ethanol in extraction process that works on solution many from active compounds such as alkaloids and was and result the statements is approach of study [19]on the *C.crispata* in it contain the alkaloids, carbohydrates, proteins, trpines and steroids, also approach with study [20] prof to present 44 chemical compound in algae *C.crispata* from seven alkaloid compounds such as 2-Piperidinone, N-4-bromo-n-butyl, Cyclohexane,1-1,5-dimethylhexy l-4- methylpentyl, 2-Myristynoyl-glycinamide, Hexahydropyridine,1-methyl-4-5-dihydroxyphneyl ,Benzohquinolone,2,4-dimethyl ,2-Ethylacridine ,5-Methyl-2-phenylindolizine these compounds have been activity of inhibition because interpenetration in serious the reaction metabolism of proteins necessary to continuity viability of microorganism and ability to destruction the cell wall and that is lead to dead of parasite. Show the result of current study that alkaloids clear effect in reduction rate of protoscolices viability whereas the reduction the viability with increased the concentreation and at the mix these alkaloids with albendazol 0.3 mg / ml [21], and that return to synergistic work of these alkaloids with albendazol addition to increase the absorpition. Either the plant *A.squamosa* contain many of chemical compounds that contain alkaloid compounds such as Atisine, oxophoebine, this study is showed the result of the current study sensitivity the protoscolices exposed to concentration 0.4 mg / ml and concentration 0.6 mg / ml with albendazol, and effect if mix the alkaloids of *A.squamosa* with albendazol large effect in reduction the protoscolices viability in the third day from treatment compared with the control that is return to synergistic work[22].

Effect the Mix Alkaloids Algae Cladophora Crispate and Plant Annona Squamosa L without or with Albendazol in Protoscolices Viability in Vitro

The current study was proved that mix alkaloids algae *Cladophora* and plant Annona together that more effect in reduction protoscolices viability and that to chemical contain and other compound contact with it such as taninnes flavonat and lacteines, that ability to denature proteins and stopped the work of enzymes responsible about series from the metabolism reaction and that lead the loose micro organs ability of life [23]. Either mix alkaloids of algae and plant with albendazol was best the concentration 0.6 mg / ml as lead to killed the protoscolices in the third day from test and that return to united these effect materials[24] and giving the uniform synergistic work during to effect on the metabolism process that contact with nitrogen and amino acid when necessary to building the mitochondria membrane, nuclei and golgi bodies necessary to micro organism, causes contact with proteins that lead to changes in chemical characters of cell wall so as change in the cell form and later dead the cell[24], and this result approach to study Khalaf *et al*., 2011 that test the compound 2-N,Ndimethylhydrazino cyclohexanecarbonitrile and it is alkaloid compound extracted from *C.crispata* and *Hapalosiphon* aureus agnasit infected by hydatid cysts.

CONFLICT OF INTEREST

None

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