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The effect of *Allium sativum* aqueous extract on *Echinococcus granulosus* protoscolices

Esraa AH Al-samir, Shaimaa A Alsamir*, Eman MA AL-Rubaaie, Sundus W Alabdullah, Mohammed A Mahdi
Department of Biology, University of Basrah, Basrah, Iraq

Abstract
Allium sativum is one of the most famous medicinal plants. It has been commonly used throughout history for its medicinal importance. The aim of this paper is to evaluate the consequences of Allium sativum aqueous extract as an *in vitro* lethal agent used for Echinococcus granulosus protoscolices. Protoscolices were brought from infected sheep livers. The results have been obtained using three different concentrations of Allium sativum aqueous extract (50, 100, and 150 mg/mL) for 5, 10, and 15 minutes. The viability was 100% in the control group. At a concentration of 50 mg/ml, it was 62.85, 51.11, and 32.01 after 5, 10, and 15 minutes, respectively. Also, the viability rate was 45.95, 34.49, and 21.32% at a concentration of 100 mg/ml after the same time of extract exposure. Finally, the viability rate was found to be 01.86, 01.45, and 0.00% percent at 150 mg/mL after 15 minutes of contact. These results indicated that Allium sativum has lethal effects on protoscolices *in vitro*.

Keywords: Hydatid cyst disease, hydatidosis, medicinal, allium sativum

Introduction

Hydatidosis, is a parasitic infection of Echinococcus granulosus metacestode. This causes a disease of cystic infection in human or sheep lungs or liver through the intake of polluted water or food containing the egg stage that is released in stool from the adult tapeworm that lives in the dog intestine (Landa-Garacia *et al*., 1997) [16].

Treatment of *Echinococcus granulosus* infection is usually done by surgical ways. However, in operations, the accidental spreading of the hydatid fluid viable protoscolices to the nearby tissues may cause reinfection (Arandes and Bertomeu, 2012) [6].

Allium sativum extract has been used for its medicinal properties all throughout history. Its primary ingredients are phenolic derivatives and volatile oil. Because of its several bioactive components, which include organic sulfides, saponins, phenolic compounds, and polysaccharides, it is grown as a prominent medicinal plant in many parts of the world (Stavělíková, 2008) [23]. Further, China has been used garlic as a traditional treatment (Jacob Song and Milner, 2001) [22]. Garlic's remarkable biological properties, such as its antibacterial, anti-inflammatory, anti-obesity, anti-inflammatory, anticancer, and anti-diabetic, have been the subject of numerous studies in recent decades (Tsai *et al*., 2012) [25]. It is used as a treatment for a number of common illnesses, including the flue, the common cold, snake bites, and high blood pressure (Agarwal, 1996) [24]. It, furthermore, has a medicinal uses for treating lung conditions, whooping cough, stomach issues, colds, earaches, and helps against cardiovascular disease (Mallika *et al*., 2014) [17]. Based on epidemiological data from human clinical studies, allium sativum and its active compounds play a major role in lowering the ability to get cardiovascular diseases and diabetes, and it also protects the immune system from microbial, fungal, cancer agents (Ambati, 2013) [5]. Various sulfur compounds have been reported to be present in raw garlic and its converted derivatives have been used in a variety of preparations (Dethier *et al*., 2013) [12].

Materials and Methods

Protoscolices Collection

Sheep liver hydatid cysts were sterilised with 70% alcohol, Protoscolices have been collected from it by washing the cyst fluid with a sterile phosphate buffer saline pH 7.2 three times. protoscolices finally have been collected by centrifuging them at 3000 rpm for 15 minutes.wet mount drop was examined under a microscope to check for the occurrence of alive protoscolices in the cystic fluid (Smyth and Barrett., 1980) [21]. Staining them with 0.1% aqueous eosin stain which was considered a vital stain was done. After 5 minutes, the protoscolices were examined under a microscope, uncolored protoscolices were considered alive and stained protoscolices were considered dead. The protoscolices that were not treated (with plant extracts) were considered as the control group. The viability rate was calculated (as a ratio of number of viable protoscolices to total protoscolices).

Plant Material

A plant taxonomist from the University of Basrah, Department of Biological Science verified the garlic, which had been obtained commercially. Allium sativum aqueous extract has been prepared by crushing the garlic then weighing 15g, joining it with 30 ml of distilled water, and finally struggled it through filter paper type Watman. The mixture was kept at 20°C till used as a stock solution (Amagase *et al*., 2001) [4].

In vitro study

Allium sativum aqueous extract stock solutions were employed in concentrations of (50, 100, and 150) mg/ml. Sets of twelve tubes were done for the *in vitro* enquiry.

Each tube held 1.0 ml of live protoscolices (about 3000 viable protoscolices). Each tube also held 1.0 ml of plant extract for each concentration. The tubes were gently combined and allowed to incubate at 27 Cº. The control group had a protosclices solution without any extract plant. For every experiment, the percentages of viable

protoscolices or survival were calculated after five,10 and 15 minutes.the number of viabile protoscolices was counted using haemocytometer in a three replications' mean value served as the basis for the data.

GC-MS.

Using GC-MS to identify the bioactive substances that were found in the garlic aqueous extracts. Five grams of Iraqi garlic were crushed in a plastic filling and left for the indicated times. 10 ml of petroleum ether solvent were then added to the extract, and they were allowed to stand at room temperature for thirty minutes. Next, one ml of the Iraqi garlic extract was injected into a GC-MS (split at ratio of one and a half) capillary column (cross band 5% diphenyl-95% dimethylpolysiloxane) with a 30 ml (L)×0.32 (i.d.) and a 0.25µm film thickness; the injection temperature was 250 °C. Finally, one ml of the extract was injected into a capillary column in the Rtx-5MS capillary column (cross band 5% diphenyl-95% dimethylpolysiloxane); the recorded intervals ranged from 30 to 170 m/z. The separated peaks were found to match the spectra database in the package library NIST08.LIB.

Statistical Analysis

The data of the study were assessed using the Least Significant Differences (L.S.D.) test at the level of ($P<0.05$) to indicate the significance of the results.

Results

Macroscopically, liver containing viable hydatid cysts displayed irregularly shaped, fluid-filled, oval to spherical, gray-white patches that ranged in size from 1 to 10 cm (Fig 1). These areas held yellowish-white granules (hydatid sand).

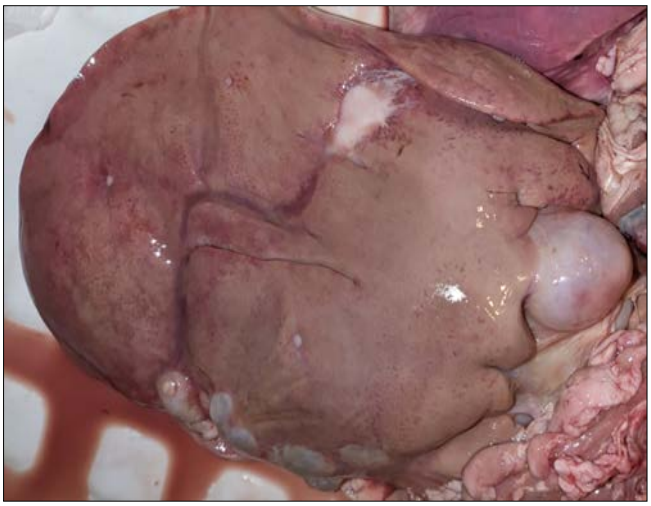

Fig 1: Fertile hydatid cyst-filled liver: a multitude of overlapping, pale, and projecting multifocal lesions are visible on the organ's exterior

The protoscolices treated with an extract of 50 mg/ml started to show a decline in viability 100% at zero concentration to 62.8,51.11, and 32.01% at time 5,10, and 15 minutes respectively and those have been treated with 100 mg \ ml showed a survival rate of 45.95,34.49, and 21.32% at 5, 10,15 minutes respectively. Finally It is clear from table (1) that the concentration with 150 mg/ml was the concentration that had the most impact on reducing the viability of the protoscolices showing a survival rate of 01.86,01.45 and 0.00% at 5,10, and 15 minutes respectively. The statistical analysis (L.S.D.) proved that there were a significant differences in $P<0.05$.

Table 1: Percentage of viability of *Echinococcus granulosus* protoscolices after being exposed to different concentrations of aqueous extract of Allium sativum for different time periods *in vitro*

Concentration Mg/ml	Survival rate			Mean of survival rate in concentrations	Comparison groups
	5min	10 min	15 min		
0	100%	100%	100%	100	p>0.05
50	62.85%	51.11%	32.01%	50.11	p<0.05
100	45.95%	34.49%	21.32%	33.90	p<0.05
150	01.86%	01.45%	0.00%	1.43	p<0.05
Mean of survival rate in exposure times	61.47	52.20	52.20		

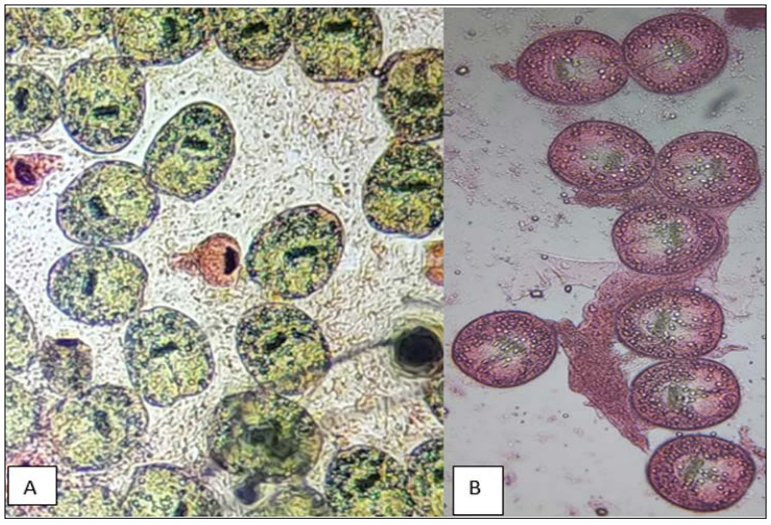

Fig 2: Microscopic images of Echinococcus granulosus Protoscolie, A-alive non-stained with 0.1% eosin stain (green colored), B- dead stained (red colored) after treatment with Allium sativum aqueous plant extract.

Diagnosing the bioactive Compounds of Allium sativum aqueous extract by GC-MS.

Results of the analysis of garlic by GC-MS have shown different bioactive compounds, as illustrated in fig 3.

pk	Name	RT	Area	%	Formula
1	3-Furanmethanol	8.647	2243567	1.003507	C5H6O2
2	1,1-Divinyl-1-silacyclobutane	8.957	1027477	0.459572	C7H12Si
3	endo-Borneol	9.525	1164551	0.520883	C10H18O
4	2-Piperidinecarboxylic acid	9.783	1791093	0.801123	C6H11NO2
5	1-(3,3,3-Trifluoro-2-hydroxypropyl)piperidine	10.063	1092211	0.488526	C8H14F3NO
6	2-Furancarboxaldehyde, 5-methyl-	10.609	9684928	4.331892	C6H6O2
7	trans-3-Methyl-2-n-propylthiophane	11	1036040	0.463402	C8H16S
8	.epsilon.-N-Formyl-L-lysine	11.398	983053	0.439702	C7H14N2O3
9	Benzaldehyde, 3-benzyloxy-2-fluoro-4-methoxy-	12.151	1435521	0.642082	C15H13FO3
10	2-Butene, 1,4-bis(ethylthio)-, (E)-	12.49	740345	0.331143	C8H16S2
11	1,2-Benzenediol, 3-fluoro-	12.814	5616381	2.512105	C6H5FO2
12	1,2-Benzenediol, 3-fluoro-	12.94	3633258	1.62509	C6H5FO2
13	4-Mercaptophenol	13.441	525207	0.234916	C6H6OS
14	2-Azido-2,4,4,6,6-pentamethylheptane	13.64	548866	0.245498	C12H25N3
15	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	14.112	17351300	7.760921	C6H8O4
16	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	14.732	2435483	1.089347	C6H6O4
17	.gamma.-Dodecalactone	14.916	1321702	0.591173	C12H22O2
18	4-Hexenal, 6-hydroxy-4-methyl-, dimethyl acetal, acetate, (Z)-	15.197	1942930	0.869037	C11H20O4
19	5-Hydroxymethylfurfural	15.477	41084383	18.3763	C6H6O3
20	Melezitose	16.318	564242	0.252375	C18H32O16
21	2,2,6-Trimethyl-12-oxabicyclo[8.2.1]trideca-3,6,10(13)-triene-5,11-dione	16.48	2408023	1.077065	C15H18O3
22	cis,trans-5,9-Cyclododecadiene-cis-1,2-diol	17.018	1162848	0.520121	C12H20O2
23	Melezitose	17.151	529250	0.236724	C18H32O16
24	Pyrrolizin-1,7-dione-6-carboxylic acid, methyl(ester)	17.468	506355	0.226483	C9H11NO4
25	trans-Traumatic acid	18.213	1002221	0.448275	C12H20O4
26	(4S,8aR,9R,12S,12aR)-9,12-Dihydroxy-4-methyldodecahydro-2H-benzo[d]oxecin-2-one	19.12	542886	0.242823	C14H24O4
27	(4S,8aR,9R,12S,12aR)-9,12-Dihydroxy-4-methyldodecahydro-2H-benzo[d]oxecin-2-one	19.423	741850	0.331816	C14H24O4
28	(4S,8aR,9R,12S,12aR)-9,12-Dihydroxy-4-methyldodecahydro-2H-benzo[d]oxecin-2-one	19.865	627637	0.280731	C14H24O4
29	Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-	20.027	701897	0.313946	C9H9F3O2
30	(4S,8aR,9R,12S,12aR)-9,12-Dihydroxy-4-methyldodecahydro-2H-benzo[d]oxecin-2-one	20.182	605325	0.270751	C14H24O4
31	(4S,8aR,9R,12S,12aR)-9,12-Dihydroxy-4-methyldodecahydro-2H-benzo[d]oxecin-2-one	20.75	772139	0.345364	C14H24O4
32	Benzeneethanamine, 2-fluoro-4,5-dimethoxy-	21.067	2661712	1.190535	C10H14FNO2
33	(4S,8aR,9R,12S,12aR)-9,12-Dihydroxy-4-methyldodecahydro-2H-benzo[d]oxecin-2-one	21.429	674895	0.301868	C14H24O4
34	12-Hydroxy-14-methyl-oxa-cyclotetradec-6-en-2-one	21.65	682954	0.305473	C14H24O3
35	12-Hydroxy-14-methyl-oxa-cyclotetradec-6-en-2-one	21.768	841811	0.376527	C14H24O3
36	Oleic Acid	21.842	862976	0.385993	C18H34O2
37	(4S,8aR,9R,12S,12aR)-9,12-Dihydroxy-4-methyldodecahydro-2H-benzo[d]oxecin-2-one	22.1	1612036	0.721034	C14H24O4
38	Oleic Acid	23.73	736236	0.329305	C18H34O2
39	n-Hexadecanoic acid	23.929	9068211	4.056046	C16H32O2
40	Oleic Acid	25.677	49971293	22.35125	C18H34O2
41	Oleic Acid	25.824	4792548	2.14362	C18H34O2
42	Oleic Acid	26.532	543767	0.243217	C18H34O2
43	Oleic Acid	27.521	1203379	0.53825	C18H34O2
44	9-Octadecenoic acid (Z)-, oxiranylmethyl ester	28.421	674253	0.301581	C21H38O3
45	2,3-Dihydroxypropyl elaidate	29.918	1173122	0.524716	C21H40O4
46	2,3-Dihydroxypropyl elaidate	30.117	4173320	1.86665	C21H40O4
47	9-Octadecenoic acid (Z)-, oxiranylmethyl ester	33.576	878276	0.392837	C21H38O3
48	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	36.275	1657846	0.741524	C57H104O6
49	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	37.905	5246992	2.346884	C57H104O6
50	rac 1-Oleoyl-2-palmitoylglycerol	43.282	26917402	12.03967	C37H70O5
51	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	45.642	3376683	1.510329	C21H40O4
52			2.24E+08	100	

Fig 3: GC-MS. of Allium sativum aqueous extract showing bioactive Compounds

Discussion

During hydatid cyst infections, surgery is the most used choice for handling a number of treatments that are being used in various degrees of success. However, the metabolites of certain medicines such as mebendazole, benzimidazole, albendazole sulfoxide, and albendazole are possibly lethal in some foci (Hayajneh *et al*.,2014) [14]. However, the commercially used albendazole is poorly absorbed in the digestive tract, resulting in a low hepatic concentration, which lowers its efficiency because the liver is most affected organ during infection by this parasite (Rokni,2009). Uptake of albendazole may result in major side effects such as allergic purpura, influenza-like illness, encephalitis syndrome, drug rash. The treatment is, nonetheless, not successful. Thisresults came in agreement with previous reports presented by Karakay (2007) [15]. In this study, we investigated the effect of garlic aqueous extract on the hydatid cyst protoscolices. Water was used as a solvent in the extraction process for two reasons: 1) it is a neutral liquid that does not negatively or positively affect

garlic bioactive compounds; and 2) it does not interfere with them, compared to organic solvents that may cause neither negative nor positive interference with these compounds (Al-Hilli, 2000 [2]. Alkaloids, phenols, and terpenes are among the substances found in plant extracts that are effective as bioactive materials because they block the respiratory process and interfere with the metabolism of carbohydrates (Delorenzi *et al*., 2001) [10]. Observing the results of the study, it became clear that the alkaloids have a strong and important effect on Tumbling the viability proportion of the protoscolices. This depends on the difference in concentration. These results are similar to that of Delorenzi *et al*.,(2001) [10].furthermore, alkaloids could induce the damage of cell organelles and lead to the destruction of the cell nuclei and finally to death (Gidado *et al*., 2007) [13].On the other hand phenols are active on the enzyme (acetyle-choline esterase) that controls the cell membrane permeability and flexibility, they made the membrane lose its permeable feature's, yielding in loss of regulation and the entrance of various toxic component and finally the death of the parasite (Barzin *et al*., (2019) [7].Phenols have the ability to denature proteins and altering enzymes actions thar are responsible for a series of basic metabolic activities, thus causing the microorganism to lose its ability to continue living (Marizel *et al*., 2012) [18], They have effect in reducing protoscolices vitality proportion. This is consistent with the result of (Zainab alaa *et al*.,2024) which had a important consequence in dropping the viability of the acanthamoeba. This effect was depending on the period and concentration, as it led to a substantial reduction in the viability rate of the protoscolices. As for flavonoids, they work to reduce sugars, which leads to an imbalance in carbohydrate metabolism and thus a decrease of energy units (ATP) prepared for vital activities in the parasite (Sarkar *et al*., 1996) [20]. The effectiveness of flavonoids in the extract could be due to the presesnce of Quercetin, Kaempfprpl, and dihydroxyquercetin (Messaoudene *et al*., 2011) [19]. The process of inhibition done by alkaloid was clarified by the fact that they interfere in the series of protein metabolism reactions necessary for the continued vitality of the microorganism, and their ability to destroy the cell wall and the proteins and fats it contains, thus killing the parasite (Cowan, 1999) [9]. The study concluded that high concentration of bioactive compounds play an important role in minimizing protoscolices viability rate compared to low concentrations.The results are also reliable with what Al-Azzawi, *et al*., (1999) [1] found, that when concentration inceased,the reducing of parasite vitality will do so,The reason for this may be due to the plant extract bioactive compound content in a high level such as: terpenes, alkaloids, or it may be related to the high concentration providing more effectin killing the parasite comparing to a low concentration.Increasing the time period of exposure had an important role on the vitality of the protoscolices. This seems to be reliable with the results of Al-Omoran, (1995) [3] who found out that the effect on the vitality of the protoscolices increases with an instant increase in the exposure period. This explanation might be related to the increase in the penetration of the active substances by penetrating the parasite's membranes and then destroying them, or lead to the weakening of the parasite (Carrique-Mas *et al*., 2001) [8]

Conclusions

The *vitro* lethal effect of *Allium sativum* extract against *echinococcus granulosus* protoscolex was very valuable and could lead to the discovery of new medication that is safe for human, without any side effects. In addition, these protoscolices have also developed resistance to albendazole (Urrea-Paris *et al*., 2000). For this reason, this studysug gested that *Allium sativum* is a valuable medicinal therapy; its natural bioactive compounds works as an active agent in killing protoscolices.

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