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Evaluation Activity of Boiled Aqueous Extract of Broccoli Flower Plant (*Brassica Oleraceae L. Var. Italic*) And Albendazole Drug Against the Protoscolices *in Vitro*

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Abstract

The present study aimed to testing the efficacy of the boiled aqueous extract of broccoli flowers and albendazole drug against the viability of protoscolices *In vitro*. The results showed the percentage of viability of the protoscolices were 94% at zero time then become (0%) after nine days after the start of the experiment when using culture media of (Kreb ringers and the cyst fluid) (4 : 1) respectively as control group for comparison. . the aqueous extract of boiled for the flower of (*Brassica oleraceae*) with concentration of 300 mg / ml showed most effective in eliminating viability of protoscolices after 168 hours and the albendazole drug after 120 hours at percentage 0 % for both.

Keywords: *In vitro*, Evaluation, *Brassica oleraceae*, albendazole drug, protoscolices

Introduction

Hydatid cystic disease is an important disease that is common between humans and animals, (zoonotic disease)⁽¹⁾, known by many names, it is Echinococcosis , cystic echinococcosis Hydatidosis unilocular and hydatidosis⁽²⁾ it is a difficult endemic disease⁽³⁾.

This disease is widespread in the countries of the Middle east, especially the village societies in which humans live and have close contact with herbivores (intermediate hosts) and dogs, as dogs are final hosts⁽⁴⁾. This disease poses an economic dilemma in almost all parts of the world especially in middle east and Iraq as it affects the productivity of sheep, cows, goats and camels because their affected organs become unfit for human consumption and this causes weight loss and poor health status of the affected animals ⁽⁵⁾.

To reduce or prevent the occurrence of this disease in all its developmental stages, whether by final or

intermediate hosts, different types of medication have been used, These include albendazole, an oral anthelmintic wide spectrum, is used as the drug of choice for hydatid disease⁽⁶⁾. Albendazole carbamate-2-bropylthiobenzimidazole– (6) 5-Methyl is a highly absorbent compound. It is considered one of the preferred drugs in the treatment of hydatid cyst disease, as it works to destroy the layer, contraction and decay, as well as the disappearance of the structural cysts daughter cysts inside the cyst ⁽⁷⁾.

Surgical removal is one of the best methods to treat this disease, although it is sometimes difficult, impossible, or may cause secondary infection to the body Therefore, the interest in finding alternative means such as using plants to provide them and contain effective medicinal materials, as the researchers deliberately sought many plants to know their components and extract their effective compounds from these plants⁽⁸⁾. One of these plants is broccoli (*Brassica oleraceae L. var. italic*), as it is one of the winter vegetables belonging to the family Brassicaceae , which is a herbaceous plant of dark green color, morphologically similar to cauliflower plant, but it has hard stems and soft flowers, It contains a number of mineral elements such as potassium, phosphorus, etc., and is rich in vitamins such as thiamine, beta-carotene

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,etc. .It also has a role in protecting the cells of the body from cancer symptoms due to the high levels of biochemically active pharmaceutical compounds⁽⁹⁾. The current study aimed to a pharmacological evaluate the effectiveness boiled water extract of (*Brassica Oleracea* var. *Italica*) and use albendazole , then comparison with it , and their effect as a model of anti-therapy against protoscolices *In vitro*.

Materials and Methods

this study included collection of 30 samples of the hydatid cysts for humans and animals (sheep and cows), human samples collected from Babylon province and from private external laboratories (Dr. Ali Mohsen Hussain and Dr. Ahmed Raji Al_Janabi laboratory), and from hospitals of Al- Najaf , Baghdad and Al-Diwaniyah provinces, whereas animal samples were collected from the abattoirs of the Al- Qadisiyah and Al-Najaf provinces.

The protoscolices were collected stored in conservativemedia (KRP=HCF 4 : 1) solution for use in the present study experiment., the viability of protoscolices was determined using eosin stain (1%) technique⁽¹⁰⁾. Then use albendazole, which is commercially called zental.it is in the form of an emulsion 10 ml at a concentration 400 mg, Concentrations (300,200,100) mg / ml were prepared by formula($N1V1=N2V2$)⁽¹¹⁾.

the broccoli plant (*Brassica oleraceae*) obtained from the local markets in Babylon province Al-Hilla city . The boiled aqueous extract was prepared by dry the plant, then grind it well and dissolve 10 g of it in 200

ml distilled boiled water and left it for 24 hours, then filtered by gauze, then toke the leachate and the solution was placed in electric oven (40-45) C° for the purpose of obtaining the dried active compounds of the extract., Kept in glass containers in the refrigerator until use⁽¹²⁾.

15 gm of dry dregs were taken separately for each extract and dissolved in 50 ml of distilled water, so the stock solution concentration became 30% or 300 mg / ml. From this the concentrations were prepared (300,200,100) mg / ml according to the formula :- ($N1V1=N2V2$)⁽¹¹⁾.

Control group was treated with one militer of distilled water and the all concentration that mention above for all extracts with three replications for each (broccoli , albendazole), then the viability rate of the protoscolices were calculated over a period of (0,3,24,48,72,96,120, 144,168,192,216) hours of treatment with evidence of eosin dye(0.1%) penetration, The dead protoscolices were revealed in red color, and the live protoscolices were revealed in bright green color and the treated tubes were stored in laboratory conditions at 25C⁽¹³⁾.

Results and Discussion

The effect of boiled broccoli extract against the viability of protoscolices :

Table, figure (1) The percentage of viability of protoscolices in the conservative media (KRS + HCF 4: 1) after exposure to different concentrations of boiled broccoli aqueous extract in different periods time *In vitro*.

Tbale (1) The effect of the interference of concentrations of boiled broccoli extract on the viability of the protoscolices of the *E. granulosus* *In vitro* according to different time period

Time (hour) Concentration (mg/ml)	0	3	24	48	72	96	120	144	168	192	216
	The viability of protoscolices (%)										
Control (0)	94.33	83.6	82.6	68.6	68	62	61	59	55.33	46.66	0
100	94.33	69.4	57.3	54.4	47	44.3	40	40	37.6	36	0
200	94.33	58.16	35.6	32	29.33	27.83	26	24.56	21.33	0	0
300	94.33	58	33	22.9	23	22.2	14.5	6.9	0	0	0
L.S.D value for interference at probability level 0.05 = 9.754											

Table (1) show that the concentration of 300 mg / ml was most effective on the viability of protoscolices , decreased from 94.33% to zero at 168 hours from the start of the experiment, followed it the concentration of 200 mg / ml the viability decreased to zero but at 192 hours followed it a concentration of 100 mg / ml viability decreased to 36% at 192 hours.

may be due to the fact that boiling water extract is linked to the action of enzymes and thus the active substance in the broccli extract has turned into another substance more effective and thus has resulted in a reduction in the viability percentage of protoscolices of the *E. granulosus.*, from these compounds (phenols) which are among the active compounds Dissolved in water⁽¹⁴⁾. The results of the current study were agreed with the study Al-Shahery and Salih (2015), which demonstrated that broccoli help in treatment for Induced Polycystic ovary syndrome in Albino rats⁽¹⁵⁾. Also agreed with Mohammed *et al.*,(2019) study, that used of broccoli as a source of natural antioxidants or nutrients that protect the liver from the toxicity of lead acetate⁽¹⁶⁾.

The effect of albendazole drug against the viability of protoscolices :

Table, figure (2) The percentage of viability of protoscolices in the conservative media (KRS + HCF 4: 1) after exposure to different concentrations of albendazole drug in different periods time *In vitro*.

Table (2) The effect of the interference of concentrations of albendazole on the viability of the protoscolices of the *E. granulosus* *In vitro* according to different time period

Time (hour) Concentration (mg/ml)	0	3	24	48	72	96	120	144	168	192	216
	The viability of protoscolices (%)										
Control (0)	94.33	83.6	82.6	68.6	68	62	61	59	55.33	46.66	0
100	94.33	62.2	58.5	49.8	39.9	39	17.3	11.06	8	1.1	0
200	94.33	56.3	54.8	42	28.13	27.8	3.66	3.3	0	0	0
300	94.33	49.3	39.2	38.9	18.5	9.8	0	0	0	0	0
L.S.D value for interference at probability level 0.05 = 7.012											

Table (2) show that the concentration of 300 mg / ml was most effective on the viability of protoscolices , decreased from 94.33% to zero at 120 hours from the start of the experiment, followed it the concentration of 200 mg / ml the viability decreased to zero but at 168 hours followed it a concentration of 100 mg / ml viability decreased to 1.1% at 192 hours.

May be due to the compounds of benzimidazole causes decrease glycogen by affecting the glucose absorption mechanism at the membrane of hydatid cyst,

degenerative changes that occur at the endoplasmic reticulum and mitochondria of germinal cells and the increase in the number and the activity of the lysosomes cause cellular autolysis , Albendazole is widely used as a primary treatment in hydatid disease of the liver and successful results have been reported^(17,18).

Through the two experiments in the current study, we notice when increase of the concentration and the longer the exposure period (time period), the viability of protoscolices are decreased, The results are consistent

with the Al-Omari' study (2005)⁽¹⁹⁾, which found that increased concentration has a significant impact on the loss of protoscolices, and it also agrees with Al-Hamiary (2010)⁽¹³⁾, Al-Musawi (2012)⁽²⁰⁾ and Al-Shimary(2020)⁽²¹⁾.

Conclusions

From the present study, it was found that the effect of broccoli (*Brassica oleraceae*) boiled aqueous extract 300 mg/ml at 168 hours, and its ratio 0, the same effect of albendazole at the same concentration (300 mg/ml), but at 120 hours. The boiled broccoli extract can be used as an alternative to chemotherapy to treat hydatid cysts disease and recommended to conduct other studies on broccoli effect especially in vivo study among mice or rats.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Science for Women and all experiments were carried out in accordance with approved guidelines.

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