Comparison of the Activity of Boiling Water Extract of Purple Cabbage Leaves and Albendazole Drug Against Protoscolices Of *Echinococcus Granulosus in Vitro* Study

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Abstract

Hydatid cystic disease is a financial burden in Iraq because it reduces the productivity of sheep, goats, cows, and camels by leaving their afflicted organs unsuitable for human consumption, causing weight loss and poor health. The goal of this study was to evaluate the efficacy and applicability of Brassica oleracea boiling water extract as an in-vitro anti-protoscolices therapy model to albendazole. Fresh leaves of purple cabbages (Brassica oleracea var. capitata) were extracted comprehensively by maceration in boiling water, and the required 100, 200, and 300 mg/ml concentrations were tested. Hydatid (human or animal) samples were collected from the hospital and external laboratories in Babylon province and stored in Kreb ringers culture media and cyst fluid (4:1) until use. For comparison, several concentrations of albendazole were generated. The control group received one milliliter of distilled water and all of the extract and drug concentrations stated above in three replications. The viability rates of the protoscolices were determined for (0, 24, 48, 72, 96, 120, 144, 168, 192, 216) hours. The proportion of viable protoscolices was 84 % at zero time and 0 % on the ninth day after the experiment began, according to the findings. The boiling water extract for purple cabbages with a concentration of 300 mg/ml was shown to be the most successful in removing protoscolices viability at a percentage of 0.67% after 96 hours, compared to the albendazole medicine at a percentage of 0% after 120 hours. The findings of a boiling water extract from Brassica oleracea var. capitata revealed that protoscolices' viability had been effective. It can also be used as an alternative for chemotherapy in the treatment of cyst hydatid infection.

Keywords: Purple cabbages, Protoscolices, Echinococcosis, Albendazole, plant extract

1. Introduction

Echinococcosis or hydatidosis caused by the larval stage of the tapeworm *Echinococcus granulosus* is one of the most important zoonotic diseases for human and domestic animals(Hama *et al.*, 2015). This disease leads to many medical, veterinary and economic problems Iraq is regarded as one of the countries plagued by the endemic sickness of hydatidosis (Abdulhameed *et al.*, 2019).

The pressure exerted by the cyst on the surrounding organs, which affects their growth and functions, as well as the cyst's explosion and spilling of its contents to the outside, which causes dead shock and the emergence of secondary cysts, are the causes of the clinical symptoms of infection in general (Zeibig, 2013).

Cystic Echinococcosis (CE) can infect a variety of human organs, and the treatment is extremely difficult, with surgery being required to remove the cyst from the affected organs (Sadjjadi *et al.*, 2013). Various medications have been used, including mebendazole, flubendazole, praziquantel and ivermectin (Hendrix and Robinson, 2006; Arziak *et al.*,2008).

As alternatives, medicinal plant extracts have been used to treat many diseases, including hydatid cysts, medicines herbals generally are used due to low cost, availability, acceptability, and assessed to be more harmless than synthetic medicines (Ozioma and Chinwe, 2019), As well as those plants, produce secondary compounds for treatment like phenolic, alkaloid, and terpinate compound (Wickzkowski *et al.*, 2012).

Due to the appearance of various phytochemical components which have therapeutic value (Gangola *et al.*, 2017), there are numerous previous studies conducted in Iraq such as Al-Maliki (2008), Al-

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Hamairy (2010), Al-Tai (2014), Saeed (2021) Al-Hasnawi (2022). Therefore, this study is complementary to previous studies to compare the efficacy and application of the anti-protoscolices treatment model of *Brassica oleracea* boiled water extract as natural products to albendazole as a chemical drug *In vitro*.

2. Materials and Methods

Human samples were collected from Al-Qadisiyah province and private external laboratories, and hospitals in Al-Najaf, Babylon, Baghdad, and Al-Qadisiyah provinces, while animal samples came from abattoirs in and Al-Najaf provinces (Figure 1). The protoscoleces were collected and stored in a conservative medium Kreb ringers solution for the current experiment. The viability of the protoscoleces was assessed using the eosin stain (1%) approach (Smyth, 1985).

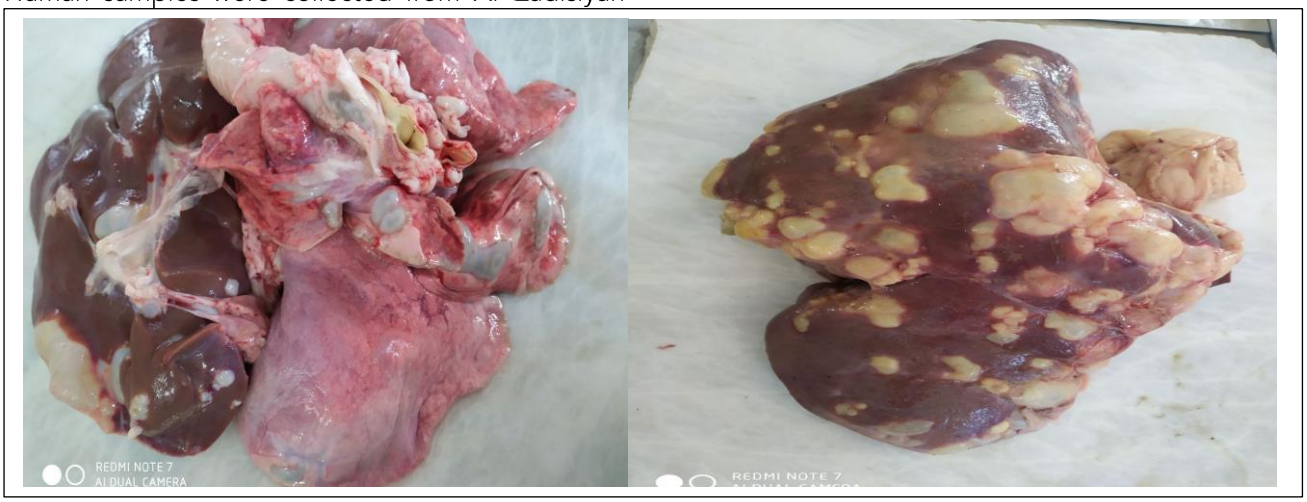

Figure (1): Hydatid cyst of infected sheep liver

Collection of Plant and Preparation of Extraction

Brassica oleracea var. *capitata* was collected from Hilla city in Babylon province (Figure 2) and extracted according to Harborne's maceration method (1998) as an aqueous extract by cutting red leaves into very small pieces and placing it in a conical flask with 500 ml of boiling water, stirring the mixture for two hours with an electric shaker, then allowing it for 24 hr. to macerate. The mixture was filtered via gauze after 24 Hr. and deposited in a tube before centrifugation. To filter the supernatant, Whatman No.1 filter paper was used. To obtain the crude concentrate, the filtered mixture was allowed to completely dry at room temperature.

Figure (2): Brassica oleracea var. capitata

The stock solution concentration became 30%, equivalent to 300 mg/ml after 7.5 gm of the crude plant was dissolved in 25 ml of distilled water. The concentrations (200,100) mg/ml were produced from this solution using the formula N1V1 = N2V2. (Al-Nakeeb, 2004).

Then take albendazole (also known as Albenda) to treat the problem. concentrations (300,200,100) mg\ml were made using the formula (NIV1=N2V2) in the form of an emulsion of 10 ml at a concentration of 400 mg (Al-Nakeeb, 2004). The control group was given one milliliter of distilled water and all of the extract and medication concentrations mentioned above, with three replications. The protoscolices' viability tiny was then estimated during a time period of (0 ,24,48,72,96,120,144,168,192,216) hours. The dead protoscolices were shown in red colour as a

result of eosin dye (0.1%) penetration. The treated tubes were maintained in laboratory settings at 25 C°, and the live protoscolices were revealed in bright green colour. (Al-Hamairy, 2010).

Statistical Analysis

To produce findings, the experimental research was conducted as factorial trials with a completely randomized design and significantly evaluated with the least significant difference (L.S.D.) at level 0.05.

3. Results and Discussion

According to the results obtained from Figures (3) and (4), it was found that when used different concentrations of *B. oleracea* var. *capitata* extract, There was no significant difference between control and concentration 100. While the 200 mg/ml concentration was effective for protoscolices viability, falling from 85% at the start of the experiment to 20.67 % after 72 hr.

Although there was no significant difference between the concentration of 200 mg/ml and 300 mg/ml, they proved more effective in killing than the concentration of 100 mg/ml, thus 300 mg/ml concentration was the most effective for protoscolices viability, at 48 hr. effective in killing than the concentration of 200 mg/ml to 42.37%.

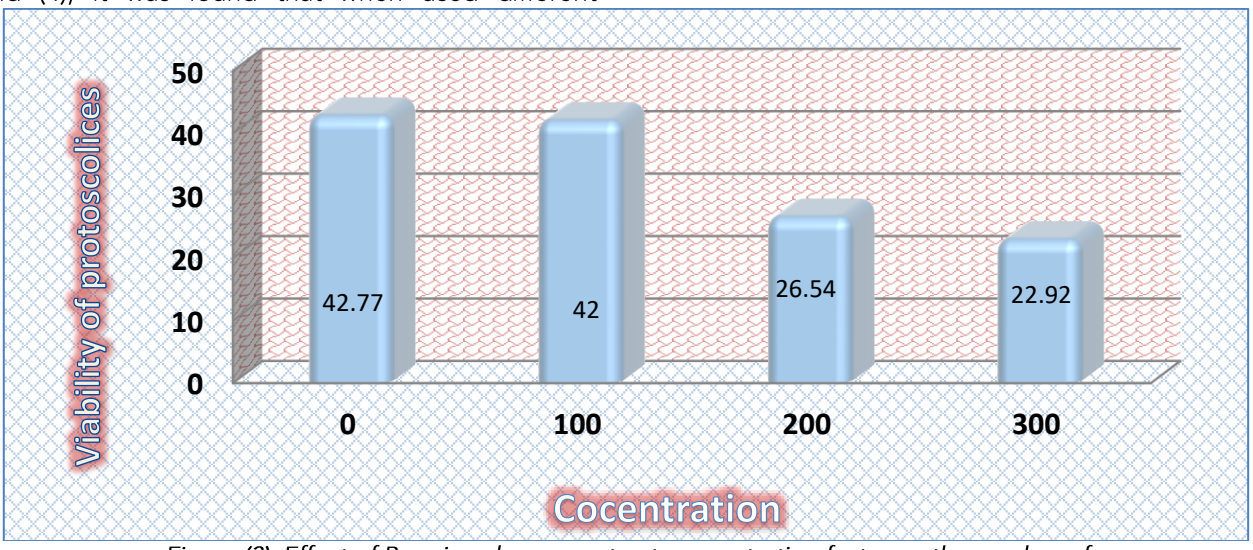

Figure (3): Effect of Brassica oleracea extract concentration factor on the number of Echinococcus granulosus protoscolices. (L.S.D value = 4.401 for least significant differences at level 0.05).

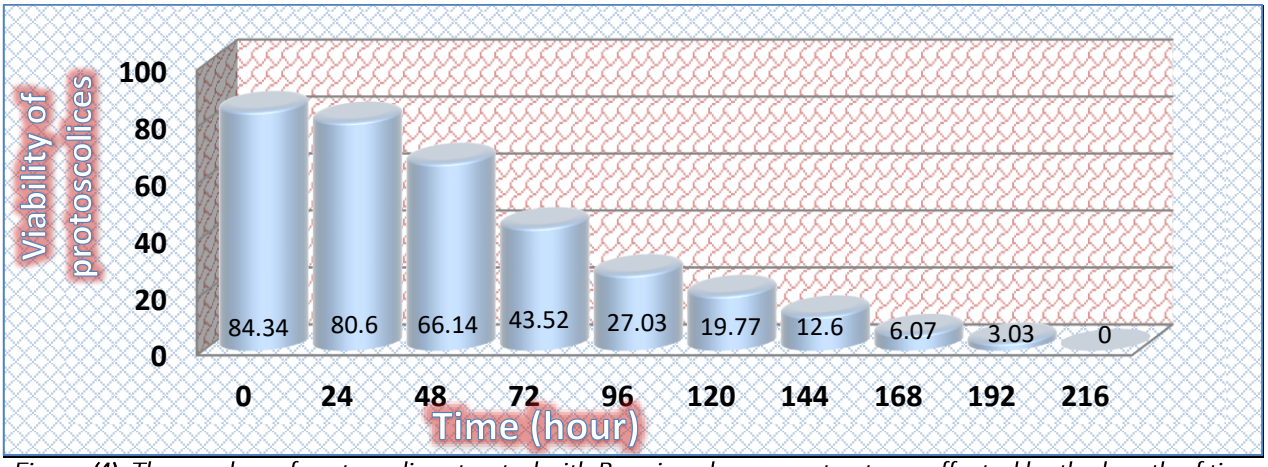

Figure (4): The number of protoscolices treated with Brassica oleracea extract was affected by the length of time (L.S.D value least significant differences at level 0.05 =7.011).

Also, Table (1): Show the effect of the period on the protoscolices viability when the 200 mg/ml concentration was effective for protoscolices viability 20.67 % after 72 hr. getting to zero at 120 hr., but the concentrations of 300 mg/ml reached 42.37% at 48 hr. getting to zero at 120 hr.

Table(1): The effect of varying doses of Brassica oleracea extract on the quantity of Echinococcosis granulosus cysts In vitro throughout time.

Time (hour)	0	24	48	72	96	120	144	168	192	216
Concentration mg/ml	The number of protoscolices									
Control (0)	83.72	80.44	70.64	65.38	51.11	32.39	23.66	12.35	8	0
100	83.87	82.31	76.68	66	51.73	46.67	26.73	11.95	4.14	0
200	84.75	80.43	74.87	20.76	4.6	0	0	0	0	0
300	85.01	79.23	42.37	21.95	0.66	0	0	0	0	0
L.S.D value for interference at probability level 0.05 =11.42										

Figures 5 and 6 appeared a significant difference between the concentration that the 300 mg/ml albendazole drug concentrations were most effective for protoscolices viability, decreasing from 90 % to zero at 120 hr. from the start of the study. The viability declined to zero when accompanied by 200 mg/ml concentrations at 168 hr., although the 100 mg/ml viability after 192 hr.

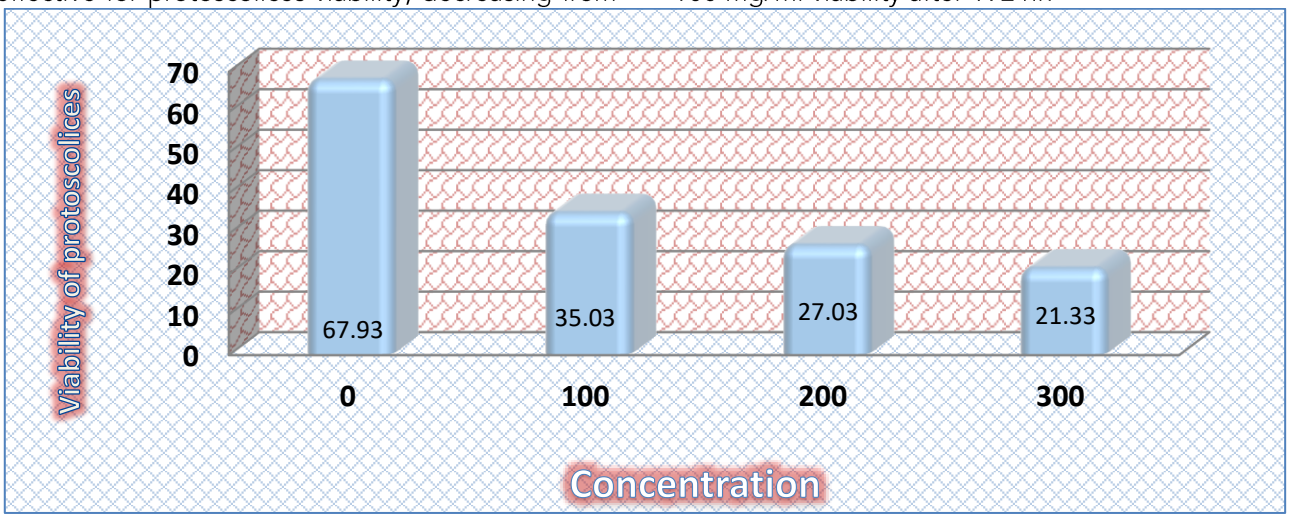

Figure (5): Effect of Albendazole concentration factor on the number of Echinococcus granulosus protoscolices. (The L.S.D value for least significant differences at level 0.05 = 2.042).

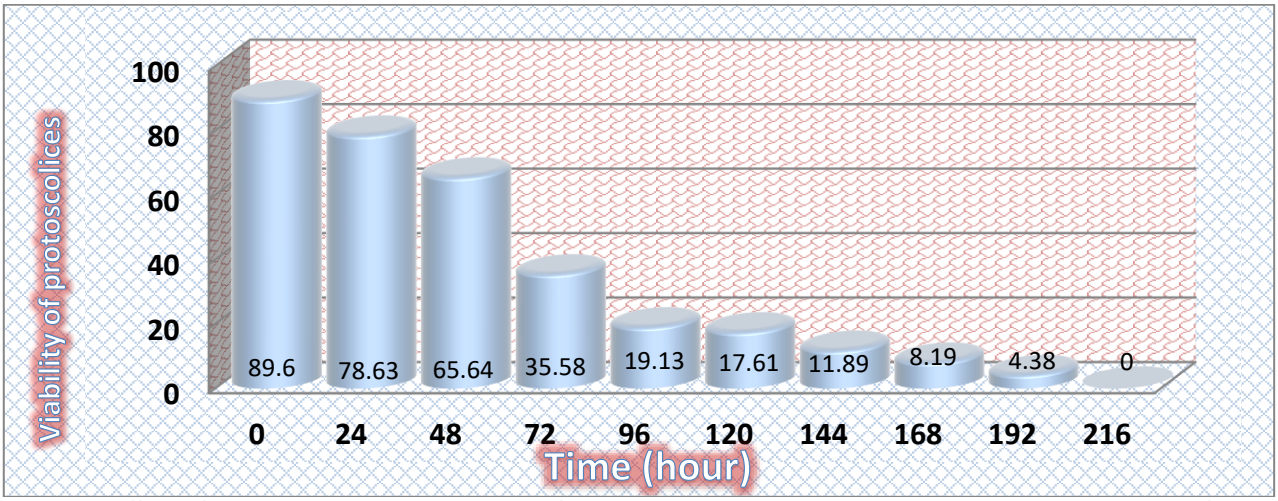

Figure (6): Effect of time duration on the number of protoscolices treated with Albendazole. (The L.S.D value for least significant differences at level 0.05 = 7.012).

Table (2): Shows the effect of the time period on the protoscolices viability for (100,200,300) mg/ml concentration (96, 72, 48) hr. to (33.93, 25.14, 58.62)% getting to zero at (192, 168, 120) hr. sequentially

Table (2): The effect of varying Albendazole concentrations on lowering the viability of protoscolices in *Echinococcosis granulosus In vitro* throughout different periods.

Time (h.)	0	24	48	72	96	120	144	168	192	216
Concentration mg/ml	No. of protoscolices									
Control (0)	89.06	78.31	62.75	52.25	42.85	40.44	37.41	32.7	17.51	0
100	88.89	80.42	74.65	62.34	33.93	12.44	10.03	0.08	0	0
200	89.99	81.03	66.53	25.14	22.93	17.55	0.11	0	0	0
300	90.45	74.74	58.62	2.6	0.16	0	0	0	0	0
L.S.D value for interference at probability level 0.05 =6.54										

The reason for the decrease of the viability of protoscolices for the presence of compounds active, Phenolic dissolved in water. This result is not compatible with Al-Mhaisen (2020). The active Phenolic compounds have a role in weakening the protoscolices membranes of the parasite (Gocemen,1993; Abdullha, 2013).

From the current study, it was found that the *Brassica oleracea* boiled water extract reduced the viability of the protoscolices to zero at 120 hr. for the concentration (200 and 300)mg/ml. Also, Albenazole reduces the viability of the protoscolices to zero at 120 hr. .reduce from that the effect extract best of the Albendazole .so the plant extract can be used as an alternative in the treatment of hydatid cysts.

Via previous research and the result of the current study, it is noted that the effect on the viability of

protoscolices decreases with increasing drug concentration since increased drug concentration contributes to an increase in the penetration of the active materials into the membranes of the parasite and then the fracturing of the membrane and the shattering of the parasite and then the weakness of the parasite (Al-Hamiary, 2010). Medicinal plants with reliable therapeutic properties were important to natural herbal and medicinal systems of modern discovery, Plants may serve as a director medicinal source of agents, and these bioactive products act as raw material, producing more complex semisynthetic chemical compounds. Isolated medicinal plant compounds can contribute to new medicines being discovered (Bhalshing and Maheshwar,1998).

4. Conclusion

The results of boiled water extract of Brassica oleracea var. capitata were successful in the viability of protoscolices, according to the current study. Instead of chemotherapy, a boiled water extract of *Brassica oleracea* var.*capitata* can be used to treat hydatid cyst infection.

Conflict of Interest

The authors declare that there is no conflict of interest

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