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Article

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. This study aimed 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. In three replications, the control group received one milliliter of distilled water and all of the extract and drug concentrations stated above. The viability rates of the protoscolices were determined for (0, 24, 48, 72, 96, 120, 144, 168, 192, 216) hours. According to the findings, the proportion of viable protoscolices was 84 % at zero time and 0 % on the ninth day after the experiment began. 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 to chemotherapy in treating cyst hydatid infection.

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

INTRODUCTION

Echinococcosis or hydatidosis caused by the larval stage of the tapeworm Echinococcus granulosus is one of the most important zoonotic diseases for humans and domestic animals [1]. 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 [2].

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 [3].

Cystic Echinococcosis (CE) can infect various human organs, and the treatment is extremely difficult, with surgery required to remove the cyst from the affected organs [4]. Various medications have been used, including mebendazole, flubendazole, praziquantel and ivermectin [5, 6].

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, and acceptability, and assessed to be more harmless than synthetic medicines [7], 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 with therapeutic value [8], numerous previous studies conducted in Iraq, such as [9, 10, 11, 12,] and 13 were developed. Therefore, this study complements previous studies to compare the efficacy and application of the anti-protoscolices treatment model of Brassica oleracea boiled water extract as a natural product to albendazole as a chemical drug In vitro.

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. In contrast, 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 [14].

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 [15] as an aqueous extract by cutting red leaves into very small pieces and placing them 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. The filtered mixture was allowed to completely dry at room temperature to obtain the crude concentrate.

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. [16].

Then take albendazole (also known as Albenda) to treat the problem. Concentrations (300,200,100) mg\ml were made using the formula (NIV1=N2V2) as an emulsion of 10 ml at a concentration of 400 mg [16]. 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 (0,24,48,72,96,120,144,168,192,216) hours. The dead protoscolices were shown in red color as a result of eosin dye (0.1%) penetration. The treated tubes were maintained in laboratory settings at 25 Cº, and the live protoscolices were revealed in bright green color. [10].

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 (LSD) at level 0.05.

RESULTS

According to the results obtained from Figures 3 and 4, it was found that when different concentrations of B. oleracea var. capitata extract were used, 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 200 mg/ml concentration and 300 mg/ml, they proved more effective in killing than 100 mg/ml. Thus, 300 mg/ml concentration was the most effective for protoscolices viability, at 48 hr. effective in killing than the 200 mg/ml concentration to 42.37%.

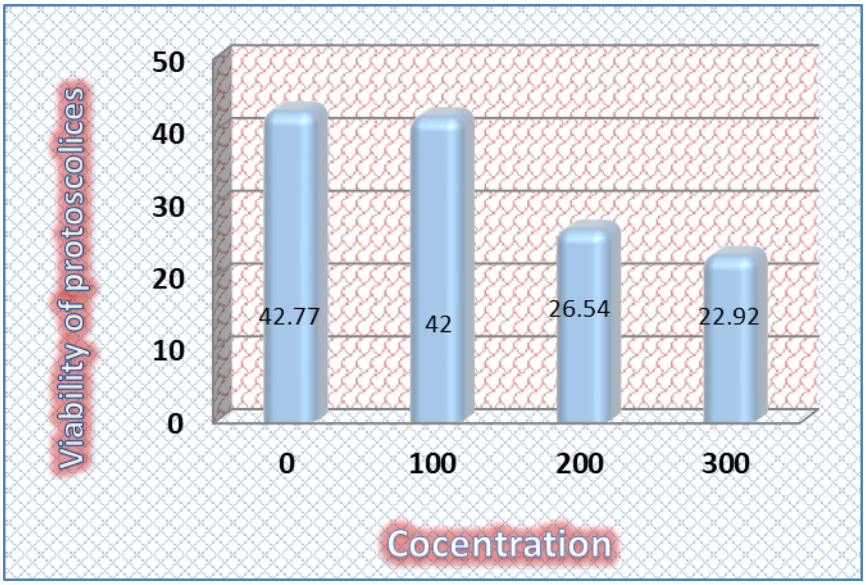

Figure 3. Effect of *Brassica oleracea* extract concentration factor on the number of *Echinococcus granulosus* protoscolices. (LSD value = 4.401 for least significant differences at level 0.05).

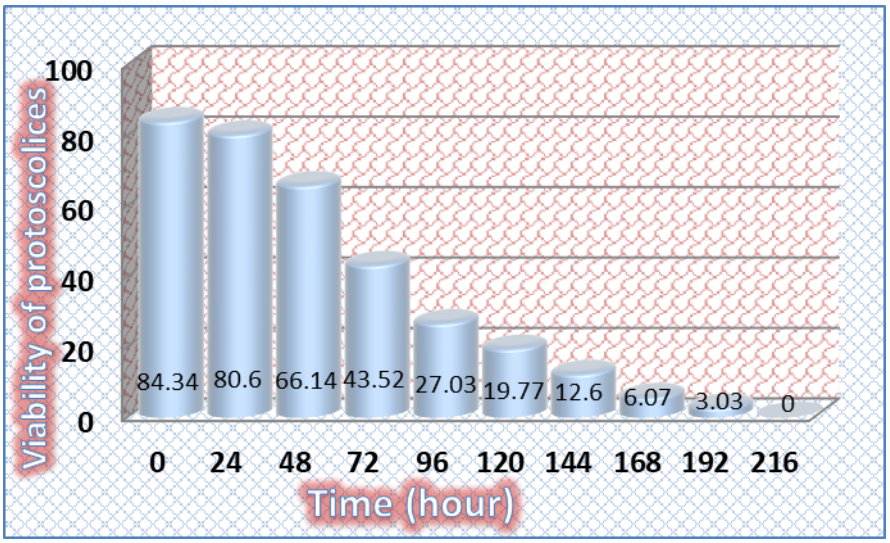

Figure 4. The length of time affected the number of protoscolices treated with *Brassica oleracea* extract (LSD value least significant differences at level 0.05 =7.011).

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

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
LSD value for interference at probability level 0.05 =11.42										

Table 1. The effect of varying doses of *Brassica oleracea* extracts on the quantity of *Echinococcosis granulosus* cysts *In vitro* throughout time.

Figures 5 and 6 showed a significant difference between the concentrations 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.

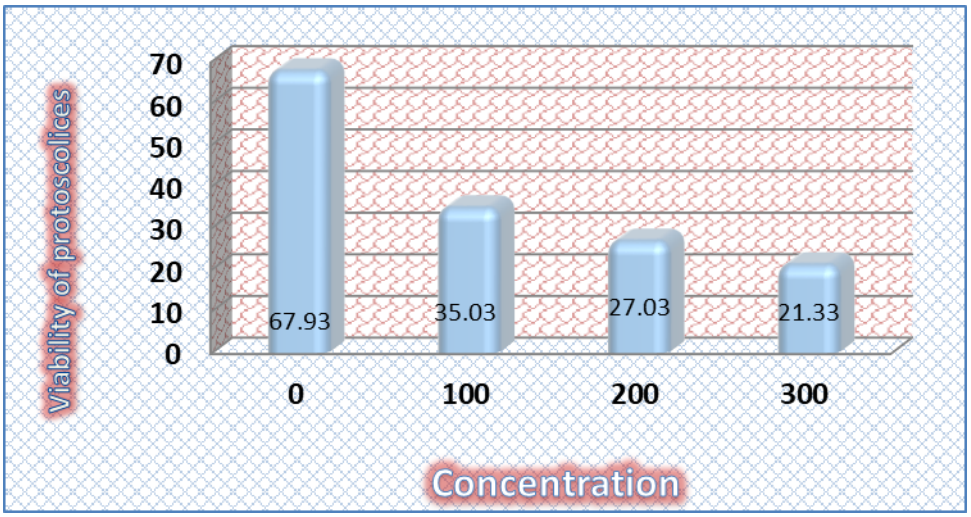

Figure 5. Effect of Albendazole concentration factor on the number of *Echinococcus granulosus* protoscolices. (The LSD value for least significant differences at level 0.05 = 2.042).

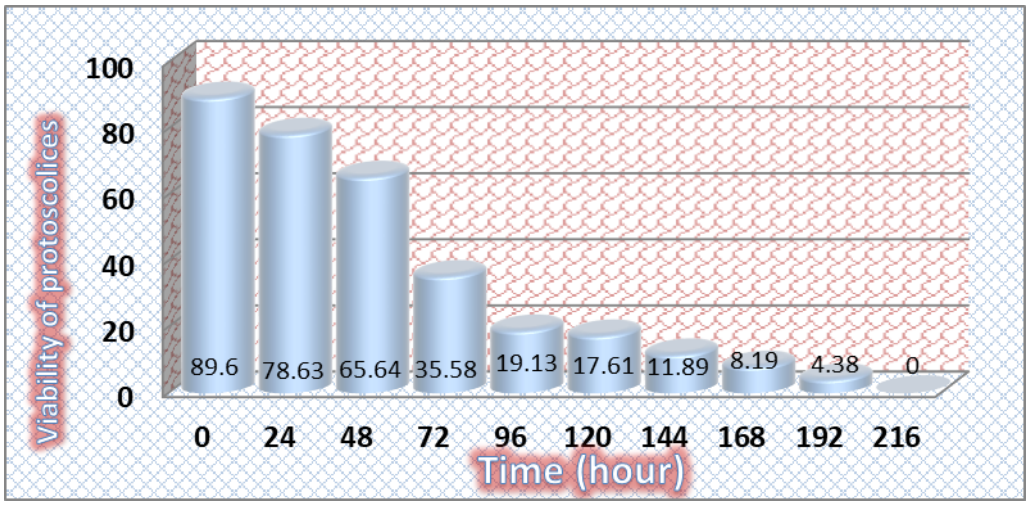

Figure 6. Effect of time duration on the number of protoscolices treated with albendazole. (The LSD value for least significant differences at level 0.05 = 7.012).

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.

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
LSD value for interference at probability level 0.05 =6.54										

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

The current study 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. from the effect extract best of the albendazole, so the plant extract can be used as an alternative in treating hydatid cysts.

DISCUSSION

The reason for the decrease in the viability of protoscolices is the presence of active compounds, Phenolic dissolved in water. This result is not compatible with Al-[17]. The active Phenolic compounds have a role in weakening the protoscolices membranes of the parasite [18, 19].

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 [10]. Medicinal plants with reliable therapeutic properties were important to natural herbal and medicinal systems of modern discovery. Plants may serve as a direct medicinal source of agents, and these bioactive products act as raw materials, producing more complex semisynthetic chemical compounds. Isolated medicinal plant compounds can contribute to new medicines being discovered [20].

CONCLUSION

According to the current study, the results of boiled water extract of Brassica oleracea var. capitata were successful in the viability of protoscolices. 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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