RESEARCH ARTICLE

# Effect of the exposure period and concentration of aqueous and alcoholic zingiber (Zingiber officinale) extract on the mean of mortality for hydatid cyst's protocolises

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## Abstract

**Objective:** To determine the anti-protocolises effect of aqueous and alcoholic zingiber extract.

**Method:** The present experimental study was conducted from 01, July 2021 to 15. December, 2021 at the College of Education for Pure Sciences, Iraq, and comprised 25mg/mL, 50mg/mL, 100mg/mL and 200mg/mL concentrations of both aqueous and alcoholic extracts of zingiber officinale that were prepared and used in vitro on hydatid cyst protocolises with 10min, 20min, 30min, 40min, 50min and 60min exposure times. The data was analysed using statistical package for the social sciences (SPSS) version 10 by using ANOVA and t- test to determine the differences between groups.

**Results:** The concentration of 200mg/ml was more efficient against the protocolises for aqueous extract, while the alcoholic extract was effective at both 100mg/ml and 200mg/ml concentrations. There was a positive relationship of the degree of killing with both exposure time and concentration.

**Conclusion:** Zingiber officinale could be a potential bioactive natural product and its active constituents could be isolated and used as anti-protoscolex without any side effects.

**Key Words:** Zingiber officinale, Echinococcosis, Variance, Ginger

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## Introduction

Protoscolex is a juvenile larval tapeworm (echinococcus-sgranulosus) that arises from the germinal layer of an echinococcal cyst that grows into an adult worm in the intestine of the final host (dogs)[1]. This worm and its larval stage pose health and economic problems in different parts of the world, especially in the Middle East [2]. Hydatidosis has a high incidence among economic and domestic animals as well as humans in many countries, and data indicates that about 20% of sheep in many parts of the Middle East are infected with it, and may cause changes in structural tissue[3,4]. According to seroprevalence studies, the infection in some countries is as high as 4.8%[5].

Cystectomy is one of the most important treatment options available, in addition to chemotherapy with albendazole or mebendazole before and after human surgical treatment, which involves the use of cyclic agents to kill infectious protoscoleces that may spread into the peritoneal cavity during surgery and potentially re-infect the patient[1]. The instillation of a scolicidal agent, such as hypertonic salt, into a hydatid cyst is the most common procedure to prevent this complication[6].

Studies have focussed on finding an alternative to chemical treatments, and identified some plants for their medicinal properties[7-9]. Zingiber is one such plant known for its richness of effective substances, as medicinal properties[10].

The current study was planned to determine the anti-protoscoleces effect of aqueous and alcoholic zingiber extract.

## Materials and Methods

The present experimental study was conducted from the first of July 2021 to 15th December, 2021, at the College of Education for Pure Sciences, Iraq. After approval from the institutional ethics review committee, zingiber rhizomes were obtained from the local market in Diyala province, and were peeled and dried at room temperature for 4 weeks. They were then grounded with an electric mill and the powder was stored in a glass bottle until use for the preparation of aqueous and alcoholic extracts.

Aqueous extract was prepared by adding 100g of powder to 1000ml boiling water, and was left for 72 hours at room temperature. The extract was passed through filter paper (Whatman No. 3, Sigma, Germany) to remove plant residues. Finally, the extract was concentrated in a vacuum at 50°C using an evaporator (Heidolph,

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Germany), and the powder was stored at -20°C.

Alcoholic extract was prepared by adding 100g of plant powder to 1000ml of 80% methanol, and then it was treated with the same method that was used for the aqueous extract.

The solution of hydatid cyst was collected from the liver of sheep that were slaughtered locally (Figure 1). In all procedures related to the animals, institutional standards were followed.

The aspirated liquid was allowed to stabilise in a sterile flask, and the supernatant was discarded. The sediment containing protoscoleces was used for subsequent experiments. The viability of protoscoleces was tested by using 0.1% eosin staining under light microscopy[11].

The effectiveness of the extracts was assessed in the light of literature[12] using 25mg/mL, 50mg/mL, 100mg/mL and 200mg/mL concentrations of both aqueous and alcoholic extracts of zingiber officinale that were prepared and used in vitro on hydatid cyst protoscoleces with 10min, 20min, 30min, 40min, 50min and 60min exposure times. Zinginger extract solution was prepared with 25mg/mL, 50mg/mL, 100mg/mL and 200mg/mL concentration by dissolving 0.25g, 0.5g, 1g and 2g of extracted powder in 10mL of normal saline, respectively.

A drop of sediment containing at least 200 protoscoleces was added to a test tube containing 1ml of extract of the concentrations in each experiment tube, and gently mixed before being incubated at 37°C. The mortality of protoscoleces was tested under light microscope at 10min, 20min, 30min, 40min, 50min and 60min by adding 1ml of 0.1% eosin stain. The percentages of dead protoscoleces were counted. Furthermore, 4 concentrations of albendazole were used as positive controls. The test of mortality was repeated 3 times.

The data was analysed using statistical package for the social sciences (SPSS) version10 by using ANOVA and t-test to determine the differences between groups. $P<0.05$ was considered statistically significant.

## Results

The concentration of 200mg/ml was more efficient against the protoscoleces for the aqueous extract, while the methanol extract was effective at both 100mg/ml and 200mg/ml concentrations, making methanol extract

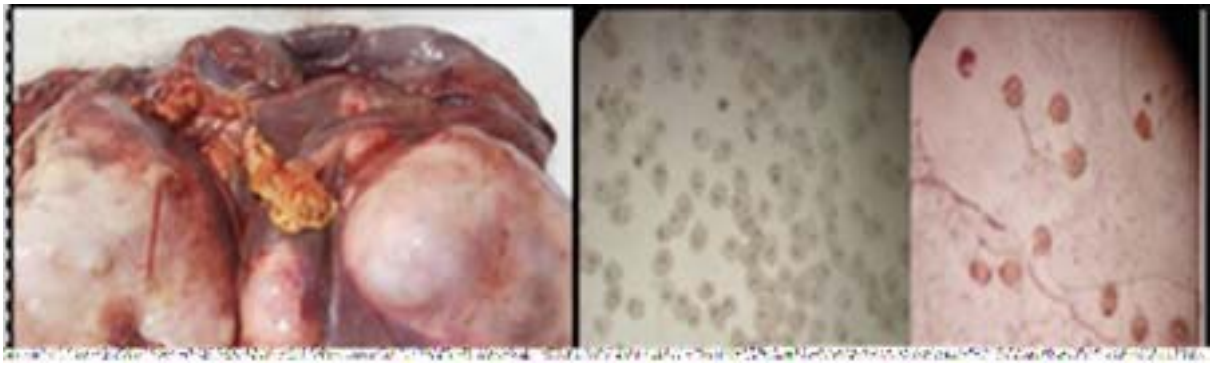

**Figure-1**: Liver infected with hydatid and the viability of alive and dead protoscolices.

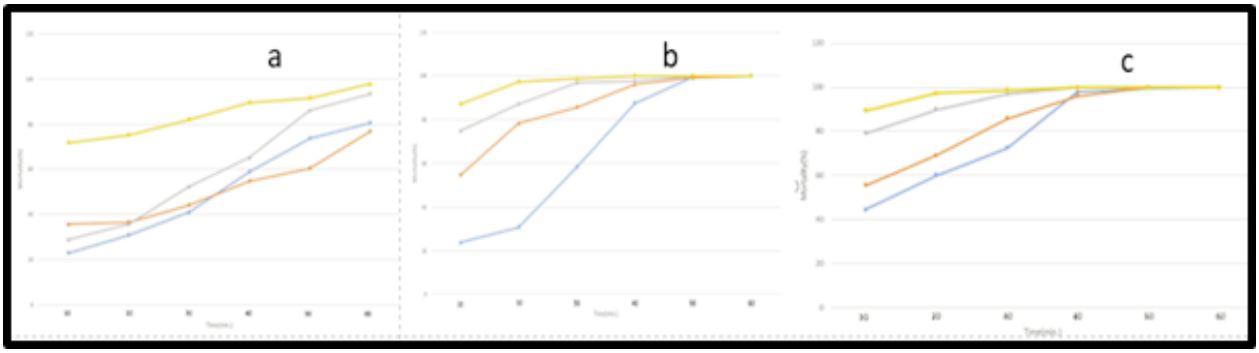


**Figure-2**: (a) The proportion of protoscolces killing increased with increasing concentrations of aqueous extracts and with increasing exposure periods. (b) The proportion of protoscolces killing increased with increasing concentrations of methanol extracts and with increasing exposure periods. (c) The proportion of protoscolces killing increased with increasing concentrations of albendazole and with increasing exposure periods.

**Table-1:** (A) Mean values of protoscoleces mortality exposed to different concentrations of aqueous extract for different time durations. (B) Mean values of protoscoleces mortality exposed to different concentrations of methanol extract for different time durations. (C) Mean values of protoscoleces mortality exposed to different concentrations of albendazole for different time durations

| Time(min.) Conc.(mg/ml) | 10 | 20 | 30 | 40 | 50 | 6060 |
|---|---|---|---|---|---|---|
| | | | Mortality (Mean±SD)(%) | | | |
| **25** | 44.3±9.29 | 59.66±8.50 | 78±18.08 | 112.33±13.20 | 140.33±31.78 | 153.33±25.16 |
| | 23.00 | 31.05 | 41.05 | 58.94 | 73.68 | 80.52 |
| **50** | 77.33±10.59 | 79.33±4.93 | 95.33±16.28 | 118.66±4.50 | 130.66±16.77 | 165.33±5.50 |
| | 29.01 | 36.74 | 44.18 | 54.88 | 60.46 | 76.74 |
| **100** | 56±18.73 | 69.66±17.55 | 101.33±18.23 | 126±5.29 | 166.33±19.03 | 182±22.11 |
| | 35.81 | 35.75 | 52.33 | 65.28 | 86.01 | 93.30 |
| **200** | 158±0.00 | 167.33±8.14 | 182±12.16 | 199.66±1.15 | 170±60.82 | 217.66±4.50 |
| | 71.81 | 75.22 | 81.98 | 89.63 | 91.44 | 97.74 |
| **p-value** | | | **P<0.05** | | | |
| | | | **A** | | | |
| **25** | 48.66±5.507 | 62±4.582 | 118±5.196 | 117.33±7.094 | 191.33±7.09 | 202±7.55 |
| | 23.76 | 30.69 | 58.41 | 87.62 | 99.55 | 100 |
| **50** | 111.33±21.221 | 159.66±30.27 | 174.33±33.501 | 195±36.715 | 201.66±39.14 | 203±38.43 |
| | 54.67 | 78.32 | 85.71 | 96.05 | 99.01 | 100 |
| **100** | 164±7.93 | 190±8.32 | 212.33±12.503 | 214±13 | 218±10.01 | 208.66±10.01 |
| | 74.88 | 87.21 | 96.80 | 97.71 | 100 | 100 |
| **200** | 196±7.21 | 219±2.64 | 222.33±4.50 | 222.33±4.50 | 225.33±4.50 | 225.33±4.50 |
| | 87.11 | 97.33 | 98.66 | 100 | 100 | 100 |
| **P-valuevaluep** | | | **P<0.05 0** | | | |
| | | | **B** | | | |
| **25** | 55.00±5.00 | 63.67±5.51 | 121.67±2.09 | 184.00±3.61 | 198.67±0.58 | 207.00±6.08 |
| | 40.01 | 60.00 | 73.05 | 99.90 | 100 | 100 |
| **50** | **1**17.00±15.39 | 177.00±6.08 | 195.67±4.93 | 213.67±6.43 | 217.67±13.65 | 223.33±5.77 |
| | 55.80 68.90 | 97.80 | 100 100 | 100 | | |
| **100** | 168.00±8.89 | 194.667±9.29 | 213.00±13.53 | 213.00±13.53 | 218.67±10.02 | 218.67±10.02 |
| | 79.90 | 89.99 | 99.05 | 100 | 100 | 100 |
| **200** | 200.00±2.00 | 222.333±2.52 | 223.33±2.89 | 223.33±2.89 | 226.67±2.89 | 226.67±2.89 |
| | 89.99 | 96.01 | 99.60 | 100 | 100 | 100 |
| **P-value** | | | **P<0.05** | | | |
| | | | **C** | | | |

SD: Standard deviation, P<0.05

more effective than aqueous extract (Table 1).

There was a positive relationship of the degree of killing with both exposure time and concentration (Figure 2).

## Discussion

The current findings were in line with earlier results reported by Sarvestani et al.[13], Al-Juboori et al.[14] and Al-Arabi et al.[15].

In the current study, the potency of the alcoholic extract was greater than that of the aqueous extract, and it was closer to the albendazole controls. the level of efficiency depended on exposure time and concentration. Furthermore, the extract graph differed significantly at 200mg/ml from the rest of the concentrations. These results are in agreement with Mahmoudvand et al.[16].

In previous studies, alcoholic zingiber extracts showed killer activities against protoscoleces. Ethanol extracts of zingiber killed all protoscoleces after 40 min[12]. AL-Mosa et al.[11] reported that ethanol zingiber extract activity increased with the increase in concentration and time of

exposure. Houshmand et al.[17] showed that at the concentrations of 200mg/ml and at 30 minutes, 100% protoscoleces were dead.

The scolicidal activity of zingiber officinale extracts can be attributed to its active substances, such as volatile oils and other components, such as zingibrene, zingiberol, bisapoline, and sesquiterpene, and amino acids, such as arginine cysteine and aspartic acid, which play an important role in its medical proprieties[18]. According to Amri and Touil-Boukoffa[19], the extract of zingiber may casue significant alterations in protoscoleces, and loss of spontaneous movement of protoscoleces[19].

It is noteworthy, that daily treatment with ginger powder at different dosages for more than a month did not relate to any rat's side effects, such as behaviour, growth, abnormality, or death, according to Rong et al.[20]

## Conclusion

Zingiber officinale could be a potential bioactive natural product and its active constituents could be isolated and used as anti-protoscolex without any side effects.

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