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Research Article

THE EFFECT OF *ALOE VERA* EXTRACTS ON THE VIABILITY OF *ECHINOCOCCUS GRANULOSUS* PROTOSCOLICES

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ABSTRACT

The study included the preparation of alcohol and aqueous extracts of *Aloe Vera* leaves at concentrations 50-75-100 mg / ml to study the effect of the viability protoscolices of the parasitic *Echinococcus granolosus* in *vitro* and their growth in *vitro* in order to reach an effective focus in killing the protoscolices during the shortest period of time. The results showed a significant decrease in the treatment of the alcoholic and aqueous extracts *Aloe Vera* leaves. Total killings for protoscolices at 100 mg / ml at 24 hours followed by concentration 75 and 50 mg / ml at 48 hours exposure period.

Keywords: *Echinococcus granulosus*, Protoscolices, Extracts, *Aloe vera*.

INTRODUCTION

Hydatid cyst disease is parasitic disease common in humans and ruminants and is a health and economic problem in most parts of the world.[1] Hydatidosis is particularly prevalent in rural areas where cattle, especially sheep and dogs, are abundant. The parasite needs to complete its life cycle to a host of sheep, goats, camels, pigs, horses, donkeys, monkeys and other animals of the platoon to survive alive. [2] It is a parasitic disease which transmitted from carnivores to humans and animals, Herbivorous [3], cause the larval stage of *Echinococcus granulosus* parasite the disease, which belongs to the Platyhelminthes phylum of the Cestoda species infect the small intestine of the carnivorous. [4]

The Hydatid cyst has also posed a serious health problem for human life, and the disadvantage is the disease does not appear clearly until after the development of infection and increase the size of the cyst. [5] There are no definitive cure for this disease so far and surgery is considered the best way to treat and it is difficult to work in some cases because of the location of the cyst, as that the substances that are used to kill protoscolices during surgery having side effects, therefore studies in recent years have directed to many diseases using the folk Medicine known as a herbal medicine for effective, safe and economical. [6] Medicinal plants are better than medicines for two reasons. The first is that plants are considered as a repository of medicines that can be tested on humans without any side effects and the second is its availability in large quantities in most countries of the world.[7] The importance of this study to show the effect of the extract of alcoholic and aqueous of the leaves of the plant *Aloe vera* in the vitality of the protoscolices of *Echinococcus granulosus* of sheep origins *in vitro* and their growth *in* vivo.

The plant *Aloe vera* has a history back to the old time which, belongs to the family Liliaceae .[8] *Aloe vera*, a semi-tropical plant is one of the 400 species of *Aloe* .[9] *Aloe vera* has been used for medicinal aims in several applications for millennia: India, Egypt, Greece, Mexico, Japan, and China, It is utilized in functional foods especially for the preparation of health drinks with no laxative effects. It is also used in other food products including milk, ice cream, confectionery, etc. *Aloe vera* gel is also used as flavoring component and preservative in some foods .[10]*Aloe vera* is being generally used in herbal medicine as an antiviral, antibacterial, antifungal, and anti-inflammatory agent. Its ant diabetic effect in rats is also reported is also used as a treatment for many skin diseases; Looking at its large medicinal outcomes .[11]

MATERIALS AND METHODS

Plant source

The leaves of the plant under study were obtained from the campus of Dr Rafiq Zakaria college for women of is authenticated.

Preparation of plant extracts

Aqueous extract

Adapted method has been followed .[12] The aqueous extract of the leaves of *Aloe vera* plant was prepared by weighting 40 g of leaves powder and 400ml of distilled water .The mixture was stirred by magnetic stirrer for 24 hours. Then the mixture was filtered through four pieces of medical gauze and poured into the centrifuge tubes at speed of 3000 rpm for 10 minute. After that mixture was put in Petri dishes and placed in oven at 40 C° to dry .Finally the extract was scraped and collected in clean glass vials and kept in refrigerator for use.

Alcoholic extract

Alcoholic extract was prepare by using Soxhlet apparatus 40gm of the plant leaves powder was taken and placed in the apparatus. Then 400 ml ethyl alcohol concentration of 70%,was added. It was left undisturbed. The solvent was evaporative by rotary evaporative. The mixture was finally placed in clean Petri dishes as the aqueous extract.

The source of the hydatid cyst

The hydatid cysts were obtained from the sheep of the butchery of Al-Basateen, the city of Aden/Yemen. The cysts were then transferred to the laboratories of the Faculty of Science, University of Aden. Yemen (Fig. 1).
A permission of ethics was granted to the researchers by the faculty of science, University of Aden.

Collecting the protoscolices

The method of [13] was used to obtain the protoscolices where the hydatid cyst was sterilized twice with ethyl alcohol (70%), then the cyst fluid was removed by a sterile syringe The cyst was washed internally with pH 7.2 and the antibiotic was penicillin IU20000 and Streptomycin 1 g/liter, the liquid was discarded in the test tubes, then it was centrifuge of at 3000 cycles/minute, and the protoscolices were examined under the microscope.

Evaluating the vitality of the protoscolices

The protoscolices were estimated by using Smyth and Barrett's method. [14] 20 microliters of the protoscolices was taken and added the same size of the aqueous eosin stain in a clean glass slide and examined under the microscope. The green portoscolices were counted as a life whereas red ones were counted as dead. The vitality of the protoscolices was taken into consideration because they are important signs to examine the vitality (Fig. 2). The percentage of live protoscolices in the sample was calculated by dividing the number of live protoscolices in the sample to the total number of calculated headings x 100. The process was repeated three times and the survival rate was taken. The percentage of the vitality of the protoscolices was calculated after each exposure period.

Solutions used

Phosphate buffer solution (PBS), hank solution and aqueous eosin stain were used for present investigation.

Laboratory animals

In this study, white albino rats *Rattus norvegicus* were used. The rats grew up in the laboratory of the Faculty of Science, University of Aden-Yemen. They grew and reproduced in the conditions of the animal house and were provided with water and food, which was a concentrated mash of added protein and dry milk. The floors of the plastic cages were covered with wood saws that were usually changed weekly to keep the rats clean.

Implantation in laboratory animals

In order to determine the effect of the plant extract used in this study on the vitality of the protoscolices of sheep origin, on the growth and development in vi*vo* [15], rats were injected with the protoscolices treated with plant extract at the highest concentration in specific period of time. This was based on the results of screening the effect of this extract on the protoscolices *in vitro*. The detail procedure involved is as follows. Protoscolices of sheep origin were treated with the acoholic extract of the *Aloe vera* plant at (100 mg / ml) for 24 hours and then injected in four rats in the peritoneum(2000 protoscolices / rat). Similarly Protoscolices of sheep were treated with aqueous extract of *Aloe vera* plant (100 mg / ml) for 24 hours and then injected in four rats in the peritoneum (2000 protoscolices / rat), and Protoscolices not treated with *Aloe Vera* extracts injected in four rats in the peritoneum (2000 protoscolices/ rat). This was used as a control group.

Anatomy of rats

The rats that were injected with the protoscolices of sheep origin were treated with the extracts under study after three months of the secondary hydatid cysts were investigated in the peritoneum, liver, lungs, kidneys and other areas of the body using a magnifying lens. Pictures were taken for the rats of two groups.

Statistical analysis

The appropriate statistical method used to analyze the results obtained from the experiments in this study are Genestat 5 program (1995).

RESULTS

Effect of aqueous and alcoholic extracts of *Aloe vera* plant on protoscolices *in vitro*

Table (1) shows the effect of alcoholic and aqueous extracts of *Aloe vera* leaves at concentrations of 100, 75 and 50 mg/ml on the protoscolices *in vitro* in comparison with the control group. The results showed significant differences at the probability level of $p \leq 0.05$ for all concentrations and in all times used when compared with control group.

The maximum concentration of 100 mg/ml showed the greatest effect on the vitality of the protoscolices. The total time for killing was 24 hours (100%) and the highest mortality rates were observed in composition with the control group which remained at vitality level of 94.33% with probability level of $p \leq 0.05$. followed by a concentration of 75 mg/ml. The lowest protoscolices mortality rate was observed with 50 mg/ml at the period of 6 hours, with 5% and 3% for alcoholic and aqueous extract, respectively, compared with control group.

There were also significant differences between the different periods of exposure. The 48 hours exposure period was the greatest. No significant differences were observed between the aqueous and alcoholic extracts (Fig. 3 and 4).

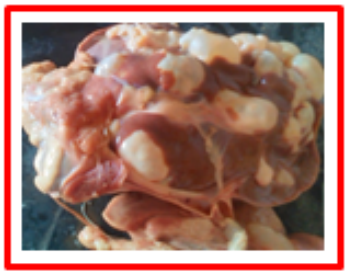

Fig.1. The hydatid cysts in liver of sheep

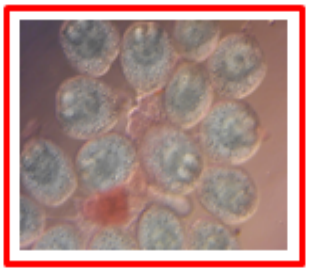

Fig. 2. Vitality of the protoscolices

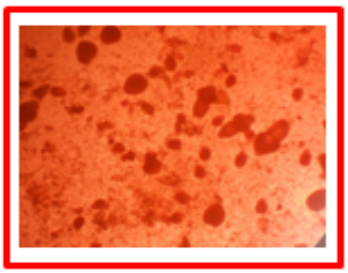

Fig.3. Protoscolices treated at alcoholic extract *Aloe vera*

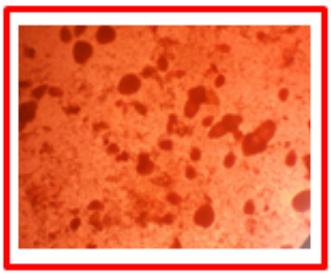

Fig.4. Protoscolices treated at aqueous extract *Aloe vera*

Table 1: Effect extract alcoholic and aqueous for plant *Aloe vera* on protoscoleces sheep origin *in vitro*

Extract	Concentrations	Control %	Time/Hours					Means
			6	12	18	24	48	
			M %	M %	M %	M %	M %	
Alcoholic	100mg/ml	94.33	19.33	42.33	57.33	100.00	100.00	68.89
	75mg/ml	94.33	11.67	26.00	39.67	94.00	100.00	60.94
	50mg/ml	92.67	5.00	13.33	23.00	74.33	100.00	51.39
Means		93.78	12.00	27.22	40.00	89.44	100.00	60.41
Aqueous	100mg/ml	93.67	17.33	39.00	54.67	100.00	100.00	67.44
	75mg/ml	95.00	11.33	24.33	37.33	88.00	100.00	59.33
	50mg/ml	92.00	3.00	13.00	25.00	67.67	100.00	50.11
Means		93.56	10.56	25.44	39.00	85.22	100.00	58.96
Interaction concentration × Time	100mg/ml	94.00	18.33	40.67	56.00	100.00	100.00	68.17
	75mg/ml	94.67	11.50	25.17	38.50	91.00	100.00	60.14
	50mg/ml	92.33	4.00	13.17	24.00	71.00	100.00	50.75
Means of Time		93.67	11.28	26.33	39.50	87.33	100.00	
LSD **5%**		Extract (EX) = 1.827, Concentration (C) = 2.238, Time (T) = 3.165, C × T = 5.483, EX×T= 4.476, EX×C×T = 7.752, EX×C= 3.165						
CV		8.0						

* M= Mortality

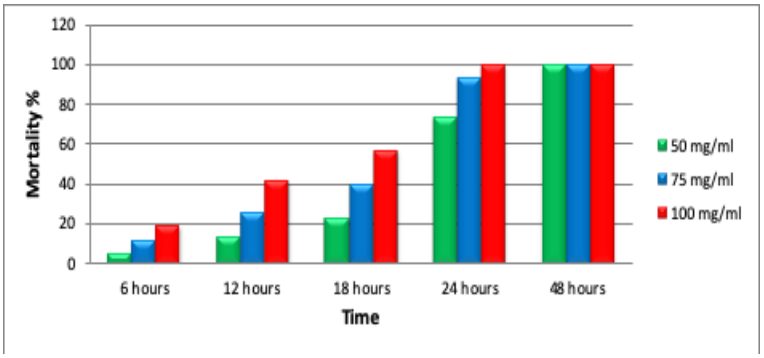

Fig. 5. Effect of extract alcoholic of plant *Aloe vera* on protoscoleces sheep origin *in vitro*

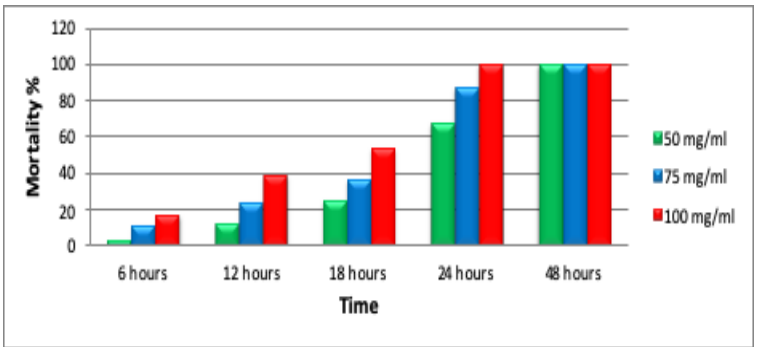

Fig. 6. Effect of extract aqueous of plant *Aloe vera* on protoscoleces sheep origin *in vitro*

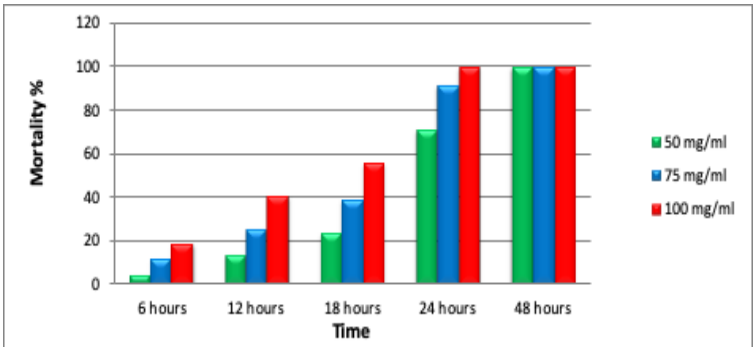

Fig.7. Average variation in inhibition capacity of extracts on protoscoleces in *vitro*

Effect of aqueous and alcoholic extracts of *Aloe vera* plant on protoscolices *in vivo*

After performing the above mentioned experiments related with effect of alcoholic and aqueous extracts of *Aloe vera* plant on the vitality of the protoscolices *in vitro* and the observation of the mortality rate of the protoscolices *in vitro*, The protoscolices treated with the extracts under this study were injected into the peritoneum of the laboratory rats to check the effect of these substances on the mortality of the protoscolices *in vivo*. Three months later the rats were dissected to investigate the presence and growth of secondary hydatid cysts in different the effect of the extract under this study on the mortality of the protoscolices as shown (Fig. 8 and 9). The hydatid cysts were clearly visible in the laboratory rats injected with the non-treated extracts using the substances used in this study, the control group as shown the (Fig. 10).

Fig. 8. Rat treated by alcoholic extract *Aloe vera*

Fig. 9. Rat treated by aqueous extract *Aloe vera*

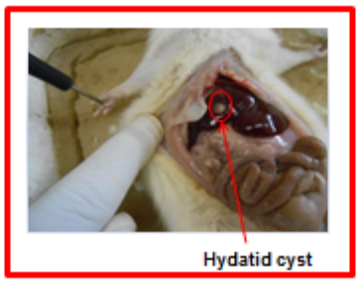

Fig. 10. Rat control

DISCUSSION

There is no doubt that plants in general and with medicinal properties in particular are an important source of many organic and inorganic compounds of pharmaceutical and medical importance that treat many diseases and bacterial and parasitic infections.

The results of this study were similar to the results obtained by Abdul-Jabbar-Mustafa F [16] in terms of time. When she used aqueous extract of *Cyperus longous* plant at concentration of 20% after the passing of 24 hours all the protoscolices were killed.

The results of study have also superior to the results obtained by Colebrook AL *et al* [17] who treated the protoscolices with the cyclosporine (CSA) at concentration of 100 µg /ml. These for the protoscolices were entirely killed after five days of exposure to the cyclosporine (CSA).

The study by Elissondo MC *et al* [18], when using Thymol with concentration of 10 µg/ml, as the vitality of the protoscolices decreased to 53.5% after 12 days of incubation.

These results also exceeded the results obtained by Al–azawi BM *et al* [19] who reported that aqueous extract of *Myrtus communis* and *Nigella sativa* at concentration of 45-50 mg/ml to treat the protoscolices and observed a mortality rate of 100% after three days of exposure period.

The mortality rate of the protoscolices treated by *Aloe vera* plant extracts can be attributed to its inclusion of active substances such as
Alkaloids whose effect is a consequence of its reaction with the metabolic protein reaction required for the vitality of the protoscolices. Then this leads to the destruction of the cell wall and its proteins and fats till the protoscolices die. [20] The mortality rate of the protosolices by *Aloe vera* death of the primates when treated with aqueous and alcoholic extracts of leaves of *Aloe vera* plant can also be attributed to the tannins which may penetrate the cell membrane and block the active sites of some enzymes inside the cell which are necessary for the growth of parasites .[21]

The death of the parasite can be due to Phenol substance which has an effect on the acetyl cholinesterase enzyme that controls the flexibility and permeability of the cell membrane. Phenols make the membrane lose its which result in passing of various toxic substances without regulating and this leads to the death of parasite. [22]

CONCLUSION

The alcoholic and aqueous extracts of *Aloe vera* leaves were able to kill the protoscolices *in vitro* and did not allow the formation of secondary hydatid cysts *in vivo.* Further studies on the *Aloe vera* and its effect on the parasite of *Echinococcus granulosus in vivo* are suggested to be done.

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