



EFFECT OF THE GARDEN CRESS, *LEPIDIUM SATIVUM* L. LEAF EXTRACT ON PROTOSCOLICES OF *ECHINOCOCCUS GRANULOSUS* OF SHEEP ORIGIN IN *IN VITRO* CONDITIONS

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Abstract

In vitro, the bioactivity of the leaf extract of the garden cress, *Lepidium sativum* L. (Brassicaceae) on the viability of the protoscolices *Echinococcus granulosus* of sheep origin was investigated. The protoscolices were significantly affected at the applied concentrations of 25, 50, 75 and 100 mg.ml⁻¹, but the concentration 100 mg ml⁻¹ caused 100% mortality within 15 minutes. The viability inhibition was proportional to extract concentration and exposure time.

Key words: *Echinococcus granulosus*, hydatid cyst, plant extract.

Introduction

Now, the hydatid cyst disease is a fatal epidemic health problem at the local and global levels (Pawlowski, 1997, Perez-Serrano *et al.*, 1997). Hydatid disease is considered one of the endemic diseases in the Middle East, as Iraq, Palestine, Lebanon, Syria, Turkey and some countries of the Arabian peninsula, North Africa and Sudan, in addition to some countries in North America (Deplazes *et al.*, 2017, Mansouri *et al.*, 2016, Thopson, 2017) in addition to some countries North America. Also, Middle Europe, Russia, China, New Island, Today infection with this disease is expanding in new areas through the people journeys (Moazeni, 2011). The infection in rural areas increases wherein sheep and cattle domesticated and may. The people job contributes the infection percentage, for instance, in Lebanon, the dogs' faeces used in the leather tanning industry, so faeces of the definitive host (dogs) may contain the parasite's eggs, therefore, the collectors may be contaminated and infected more than the others (Roberts and Janovy, 2009). Budke *et al.*, (2017) show continuing successes in cystic and alveolar hydatid treatment, in spite of the disease challenges. In the present time, Iran is most middle east countries with permanent and continuous *Echinococcus* spp. Incidences (Carmena and Cardona, 2014) Hydatid cyst diseases and caused by the active larval stage of

different species of the genus *Echinococcus* and important two of this genus, *E. granulosus* cause cystic echinococcosis, which includes many strains according to their molecular structure, the other species is *E. multilocularis* which causes multilocular echinococcosis or alveolar echinococcosis (Deplazes *et al.*, 2017, Fatemi *et al.*, 2018).

The distribution of the infection percentage between the hosts' organs is depending on the host species and method of transmission, the principle, the liver infection is (50-70)% while (20-30)% lungs infection and less infection percentage in other organs as kidneys, spleen, brain, heart and muscles (Celebrook and Lightowers, 1995, Aghajanzadeh *et al.*, 2008). On the other side, Ahmed *et al.*, (2010) were found unexpected positions of hydatid infections as neck and breast sites, the sub-axillary infection is rare. The liver and lungs are mainly infected with the hydatid cyst, In human, the danger of this disease increases in brain, heart and bone column infection (Paredes *et al.*, 2007, Rahimi *et al.*, 2011). Othieno *et al.*, (2017) studied the correlation between human fatal factors of hydatid cyst disease and agricultural and grazing regions in Uganda. This disease can be diagnosed either, accidentally by X-ray, cyst explosive and give up anaphylactic reactions, nor by carrion anatomy after death. The carnivorous, especially domestic dogs and other species of Canidae family as

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the definitive host, there were, adult worms present in their intestines, the intermediate host is represented by a group of herbivorous as sheep, cattle and donkey, the human may accidentally become inter-mediate host. Infection of intermediate hosts through faeces of the definitive host (Zhang *et al.*, 2012, Budke *et al.*, 2013).

There are of hydatidosis treatments. But surgery one of preferred treatment methods, in spite of operational difficulties and impossibilities in some cases (Oryan and Alidadi, 2014), the chemical treatment is useful in cases of impossible surgical interference, the Al-Bendazole is more drug treatment, but the drug with low effect on the infected organ due to cyst thickness and dose the dose increasing for long period may cause side effects as diarrhea, nausea, vomiting, increase in aminotransferase enzyme and decrease of leucocytes count (Moazeni, 2011, Deplazes *et al.*, 2017). Every day, investigating centres and the World Health Organization (WHO) discover the new hidden role of manufactured chemicals by human beings and their side effects, so some of them became as fatal drugs. Now, there is blacklist of poisonous drugs and gradually increased and sometimes common ones added to that list (Arab agricultural development organization, 1988). The hydatidosis is still and continues as fatal disease for together human beings and animals health, so that, the investigators are a focus on safe protoscoleces. It was found, the plant extracts have a safe bioactive effect, especially edible and eaten plants (Al-Abayday, 2012).

The garden crass, *Lepidium sativum* was tested for hydatid cyst disease treatment, the plant is annual herb, glabrous attaining a high up to 40 cm, seeds are smooth and brownish to reddish colour. The leaves are consumed by human beings salad (Kalipha, 2011). The plant is known to contain immidazole, Lepidine, gluconasturin, unsaturated fatty acids, B-carotenes, ascorbic acid, glucotropaelin and benzyl isothiocyanate, *L. sativum* have been widely used to treat several ailments in traditional system of medicine (Kalipha, 2011, Al-Haj, 2003, Al-Rubaiya, 1976).

The objective of the present study is to estimate *in vitro* how to range the secondary metabolites of aqueous extract of garden crass, *L. sativum* kills proto-scolices of *E. granulosus* of sheep origin with meaning full against the protoscoleces to be restricted their effects and distribution.

Materials and Methods

Source of hydatid cysts: The infected liver with protoscolices of *E. granulosus* were obtained from a butchery in Mosul city, Iraq (36° 20' 06" N 43° 07' 08" E).

After the infected sheep was sacrificed, the samples were soon brought to the laboratory by plastic container and proved with ice to being not affected by environmental temperature (Smyth, 1976). For obtain alive protoscolices, the outer surface of the hydatid cysts was twice sterilized by witted cotton with alcoholic iodine, after Smyth protocole (Smyth, 1985).

Estimation of protoscolices Viability: The viability of the protoscolices was estimated beyond Smyth and Barrett (1988), alive protoscolices have a brilliant green colour, while dead ones stained with red colour. Also, the movement of the protoscolices is one of the important indicators in the vital examination. The viability of the protoscolices was calculated as in time zero for control according to (Metcalf *et al.*, 1986).

$$\text{Viability} = \frac{\text{No of alive protoscoleces} \times 100}{\text{Total of counted protoscoleces in sample}}$$

In the present study, the protoscolices with viability 95% were used.

Plant material and extraction: Leaves of garden crass were brought from the local market, the leaves washed and dried in shelter grinded electric miller, mixed with distilled water and stirred for 24 hrs. then filtered through Whatman filter paper and evaporated on a rotary evaporator and reduced pressure to obtain the crude extract.

Statistical analysis: The experiments were designed with three replications in addition to controlling treatment. The aqueous extract of *L. sativum* was applied at 15, 25, 75, 100 mg.ml⁻¹ concentrations, for 15, 30, 45 and 60 min for every concentration. The results were analyzed by ANOVA variance analysis and Duncan's test with 0.01 probability.

Results and Discussion

The (Table 1) shows *in vitro* significant difference between the treatments at P<0.01 in the protoscolices viability, which treated with the aqueous extract of

Table 1: Effect of the aqueous leaf extract of garden crass on *Echinococcus granulosus* protoscolices viability of sheep origin.

Source	D. F.	Sum of squares	Mean Square	F. value
Model	19	69604.850	3663.413	28.39
Concentration	3	3174.050	1058.016	8.20
Time	4	63085.266	15771.316	122.23
Time × Concent.	12	3345.533	278.794	2.16
Error	40	5161.333	129.033	
Corrected Total	59	74766.1833		

ANOVA variance analysis (P<0.01).

Table 2: Effect of the aqueous leaf extract of *Lepidium sativum* on *Echinococcous granulosus* protoscolices viability of sheep origin.

Time Conc. mg.l ⁻¹	Control min. 0	The percentage of viable protoscoleces after				General average of concentration
		15 min.	30 min.	45 min.	60 min.	
25	95%	74.66 efg	71.33 def	42.33 bc	33.66 b	65.25 C
50		00.82 fg	33.67 def	66.56 cde	53.00 cde	55.50 C
75		34.00 b	35.33 eb	33.00 b	10.33 a	28.16 B
100		0.00 a	0.00 a	0.00 a	0.00 a	0.00 A
General average of Time		57.13 C	53.80 B	45.80 AB	38.40 A	

Duncan's test $P < 0.01$. Values followed by different litter are significant.

Lepidium sativum leaves. Duncans's test (Table 2) illustrates the effect of different concentrations of the aqueous extract with exposure times. The concentration 100 mg.ml⁻¹ killed the protoscolices with mortality 100% at all the exposure times (60, 45, 30 and 15 min). The concentration of 75 mg.ml⁻¹ was significantly decreased the protoscolices viability to 33.10% at the exposure time 60 min. and it significantly differs with other exposure times 45, 30 and 15 min. but not a significant difference between the last three concentrations. The viability of the protoscolices was significantly decreased at 50 mg.ml⁻¹ concentration to 53.00% after exposure time 30 min. but in case of exposure times 60, 45 and 30 min. significantly differences at 15 min. The viability of the protoscolices treated with the 25 mg.ml⁻¹ extract not exhibited significant differences between 60 and 45 exposure times, but all of them caused a significant decrease in viability to 33.66 and 42.38% respectively. Also, 25 mg.ml⁻¹ treatment, the times 60 and 45 significantly differ with 30 and 15 times and not significant differences between the later (30 and 15 min.).

For the total average of the concentrations, table 1 is exhibit significant difference at $P < 0.01$. The concentrations 100, 75, 50 and 25 mg.ml⁻¹ decrease the protoscolices viability to 0.00, 28.17, 55.50 and 65.25% respectively. The total average of exposure time appeared no significant difference between the times 60 and 45 min., but caused significant decreasing in protoscolices viability to 38.40 and 45.80% respectively. The treatment for 60 min. was significantly different with times 30 and 15 min., which decreased protoscoleces viability to 53.80 and 57.13% respectively. without a difference in effect viability between them. Not found a significant difference between the times 45 and 30 min. while significant decrease in viability of protoscoleces from 95% in control to 45.80 and 57.13% sequentially.

These results illustrate clear to effect the aqueous leaf extract of *L. sativum* on protoscolices of *E. granulosus* of sheep origin, also, this effect proportional increases with extract concentration and exposure time. The concentration of leaf extract 100 mg.ml⁻¹, which causes

100% mortality at the exposure time 15, 30, 45 and 60 min. agree with Al-Kashab (2014) results for the same concentration of *Rula graveolens* extract which caused mortality only at exposure times 60 and 45 min. The present extract less effective than Al-Shawani (2011) findings, who used *Punica granatum* leaf extract 60 mg.ml⁻¹ concentration for 45 and 60 min. then the protoscolices decreased by 11.66% for min. The present results a similar with Faris (20143) for 100 mg.ml⁻¹ for *Popular* sp. extract, which decreased protoscolices viability to 7.67% after 15 min.

The present results, nearly agree with Hosseini *et al.*, (2006), who used 50% glucose solution, which caused 97.3% mortality during 5 min. The present results differ with Al-Aboody (2001) study, where used the aqueous extract of *Plantago lanceolata* L. arrested the protoscolices viability after three days of the application. Also, Elissondo *et al.*, (2006) found 10 mg.ml⁻¹ Floabendazole more active and kill the protoscolices within 30 days. In contrast with Hameed (2010), the same concentration (100 mg.ml⁻¹) of the fungus *Aspergillus niger* caused only 0.125% mortality during 72 hours.

The inefficiency of biochemical ingredients gives the garden crass More activity in kill and inhibition of the protoscoleces of *E. granulosus* of sheep origin in relation to other plant extracts in previous studies.

Conclusion

The aqueous extract of the garden crass, *L. sativum* has conspicuous activity in kill and reduce the protoscolices viability. This is proportionally increased with the extract concentration and the exposure time table 2. Moreover, partitioning of the extract with suitable separation technique, will give us the specific active ingredients and show the mechanism of their effect on the viability of *E. granulosus in vitro*.

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