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Short note

Growth inhibition of the larval stage of *Echinococcus granulosus* of sheep origin by apricot, *Prunus armeniaca* seed extract *in vitro*

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ABSTRACT. Cystic echinococcosis is one of the serious disease that affecting humans and this domesticated animals. *In vitro*, the bioactivity of the apricot, *Prunus armeniaca* seed extract on the vitality of the larval stage (protoscolices) of *Echinococcus granulosus* of sheep origin had been investigated. The protoscolices were significantly affected by applying concentrations of 50, 100, 150 and 200 mg.ml^{-1}. The concentration 200 mg.ml^{-1} caused 100% mortality within 15 minutes. The vitality inhibition was proportioned to extract concentration and time exposure.

Keywords: *Echinococcus granulosus*, hydatid cyst, plant extract, *Prunus armeniaca*

Introduction

Cystic echinococcosis is one of the serious disease that affecting humans and this domesticated animals, and so it is classified among the zoonosis [1]. The disease result from the larval stages of the *E. granulosus* (unilocular cyst) or the *E. multilocularis* (multilocular cyst) [2,3]. It is often affects the vitality of infected organs such as the lungs and liver, thereby reducing their efficiency [4]. Which lead to economic and material losses in livestock, such as animal deaths and their lack of production of meat, milk and wool [2,5]. Recently, and as a result of continuous research, *E. granulosus* has shown important complex variations in the molecular characteristics in addition to their life cycle [2,6,7]. These *E. granulosus* are able to form two types of hydatid cysts, either they are cysts containing the larval stage (protoscolices) inside them and these are fertile cysts and other animals infected with cystic echinococcosis without protoscolices and these are considered sterile [6,8]. The fertility of the neocyst is related to its size [9]. And the as for neo cysts that are too small to be classified as fertile or sterile, the cellular and molecular mechanisms involved in cyst fertility remain unknown [10]. And in cattle, the fertility rate of hydatid cyst ranges from 0–96% in various parts of the world [7]. This disease is widespread in the world, including the countries of South America with high endemicity, eastern and southern Africa, the Caspian Sea, southern and western Europe, as well as its spread in many Arab countries, including Libya, Egypt, Sudan, Syria, Lebanon, Palestine and Jordan [11,12] with its widespread endemicity in Iraq, Iran and Turkey [13,14], respectively. This disease spread in areas that were previously absolutely free of it, such as Canada and North America [15]. The incidence of this disease increases in childhood as a result of playing and contacting children with dogs, especially the affected ones, compared with the elderly [16]. This disease showed a great degree of similarity in the severity of carcinomas spread in the metastases stage [17,18] thus, the hydatid cysts spread in all parts of the body except for hair and nails [19]. This led to a multiplicity of treatment methods according to the degree of infection, places and number of cysts, including surgical treatment, which is one of the best treatment methods [20]. It is advised to neutralize the hydatid cyst before removing it or opening it using effective killers [21,22]. Despite the inability to perform it in some cases and resort to chemotherapy, especially in early injuries, as it

Table 1. Effect of the aqueous seed extract of *Prunus armeniaca* on *Echinococcus granulosus* protoscolices viability of sheep origin

Time	C.	% viable protoscolices after				General average of concentration
Conc.	0.0	15 min	30 min	45 min	60 min	
50 mg.ml^{-1}	94%	91.00jk	87.00i	76.67gh	70.00g	81.17D
100 mg.ml^{-1}		88.33cj	71.00g	61.67f	47.33b	67.08C
150 mg.ml^{-1}		55.33e	29.66c	2.67ab	0.00a	21.83B
200 mg.ml^{-1}		0.00a	0.00a	0.00a	0.00a	0.00A
Average of time		58.58D	46.92C	35.25B	29.33A	

Explanations: C: control 0.00 min; Duncan's test $P<0.01$: values fallowed by different litter are significant; similar letters mean there are no significant differences; different letters mean there are significant differences

gave healing results for many cases [23]. This led to the importance of using extracts or substances of a different chemical nature that may help in treating patients [24]. Therefore, researchers have tended to find safe killer alternatives to the protoscolices, including plant extracts that have an effective and safe effect, especially those that are ingested by humans or animals [25].

This study was aimed to show the *in vitro* effect of aqueous extract of seeds of apricot plant on the vitality of the larval stage of *E. granulosus*.

Materials and Methods

Sample collection

Hydatid cyst liver samples were obtained of sheep origin, from butchery in Mosul city, Iraq and brought to the laboratory immediately after the slaughter of the infected sheep on the same day in plastic containers containing ice [26]. In order to obtain the larval stage, the exterior surface of the hydatid cysts was sterilized twice with 1% medical alcoholic iodine soaked cotton [27].

Estimation of the viability of the protoscolices

The vitality of the protoscolices were calculated according to the method by [28]. The bright green protoscolices were considered alive, compared to the dead protoscolices which had been became stained red. Among the important indications of the vitality, that was taken into consideration, the movement of the protoscolices. The vitality of the protoscolices at time zero were calculated according to the following:

Percentage protoscolices of vitality = Number of live protoscolices/total number of protoscolices in the calculated sample ×100 [29]. In the present study, the protoscolices with vitality > 94% were used.

Plant materials

Fruit of apricot, *Prunus armeniaca* (Rosacea) is used in the treatment of infertility, eye inflammation, bleeding, and muscle spasms [30,31]. Apricot kernels contain chemical compounds with therapeutic benefits such as anti-oxidants such as vitamin A and C, lycopine, olulin, linoleic, hydrocyanide, sosterols, cambester, carotenoids and flavonoids [30,32]. Glycoside and amygdalin, which stimulates proper programmed death of the cell and inhibiting its reproduction, growth and cell cycle [33]. Phenolic acids (chlorogenic acid and cyanogenic) [34,35].

Results

The effect of aqueous extracts of apricot seed on the viability of *E. granulosus* protoscolices of sheep origin, *in vitro*, were performed using Duncan test (Tab. 1) to find their effects at different concentrations showed different effects according to the time used. He use the concentration of 200 mg.ml^{-1}, killed all protoscolices 100% in the period of 60, 45, 30 and 15 minutes, and these times did not show a significant differences at likelihood level ($P<0.01$) compared to control group with 94% viability. For the concentration of 150 mg.ml^{-1}, it caused the reduction of the vitality of the protoscolices to 100% on exposure for 60 minutes, which did not differ significantly from the time 45 minutes, which reduced the vitality of the protoscolices to 2.67% and differed with the time of

30 and 15 minutes, which differed significantly between them. The concentration of 100 mg.ml^{-1}, reduced the vitality of the protoscolices to 47.33% at a time of 60 minutes, which was significantly different at exposure for 45, 30 and 15 minutes, which led to a reduction in the vitality of the protoscolices to 88.33, 71.00 and 61.67%, respectively, and which differed morally between them. The concentration of 50 mg.ml^{-1}, the time of 60 and 45 minutes did not differ significantly, for those who reduced the vitality of the protoscolices to 76.67 and 70.00%, respectively, while they differed significantly with the times of 30 and 15 minutes and who caused the reduction of the vitality of the protoscolices to 91.0% and 87.0%, respectively. And who differed significantly between them as for the general average of the concentrations used, significant differences were found between all the concentrations at a probability level (P<0.01), as the concentrations 200, 150, 100 and 50 mg.ml^{-1}, reduced the vitality of the protoscolices to 0.00, 21.83, 67.08 and 81.16%, respectively, and for the average.

Discussion

The results of the current study showed that the aqueous extract of apricot seeds had a clear effect on the vitality of the protoscolices of *E. granulosus*, of sheep origin, and this effect was directly proportional to the increase in concentration and duration of exposure to this extract. The concentration of 200 mg.ml^{-1}, which caused 100% mortality of all protoscolices in 15, 30, 45 and 60 minutes, is identical to the result of [36]. Who used the aqueous extract of the seeds of the pumpkin plant, (*Cucurbita maxima*), at a concentration of 60 mg.ml^{-1},which caused the killing of all the larvae (protoscolices) of the *E. granulosus* in a 24-hour period, with the apricot plant superior to the *Cucurbita maxima* plant in the period, while the *Cucurbita maxima* outperformed the apricot plant in the concentration. A complete killing happened to larvae when exposed to a concentration of 150 mg.ml^{-1} for 60 minutes. The result is also agreed with the result of [37] who used the aqueous extract of um seeds at a concentration of 300 mg.ml^{-1}, which caused complete mortality of the larvae during the period of 45 and 60 minutes, with the apricot seeds being superior in duration and concentration. The result of this study is similar to the result of [38] who used the aqueous extract of the seeds of the *Nigella sativa* plant at a concentration of 15 mg.ml^{-1}, which caused the complete destruction of the larvae of the *E. granulosus* 100% in the time 45 and 60 minutes, and this shows the superiority of the seeds of the *Nigella sativa* plant over the seeds. The apricot plant was in focus, with the apricot plant better in duration. It was similar with the result of [39] who used the aqueous extract of the leaves of the parsley plant (*Petroselinum sativum*) at a concentration of 75 mg.ml^{-1}, which caused the complete destruction of the larvae of the protoscolices 100% in a time of 60 minutes. focus and for the same duration. And it converged with the result of [40] who used the essential oil of *Cannabis sativa* at concentrations 0.002 and 0.01 mg.ml^{-1}, which caused a decrease in the vitality of larvae of the protoscolices to 20.9 and 26.08%, respectively, in the period of 2 hours *Cannabis sativa* contains apricot seeds in concentration, with apricot seeds exceeding in duration. And it coincided with the result of [41]. Which used the aqueous extract of the leaves of the *Lepidium sativum L*. at a concentration of 100 mg.ml^{-1}, which caused the killing of the larvae by 100% in times of 15, 30, 45 and 60 minutes, and thus the garden crass, surpassed that of the apricot plant in concentration and at the same exposure times.

In conclusion, the aqueous extract of apricot seeds had a clear effect on the viability of the larval stage, which led to its reduction and decay, which was directly proportional to the increase in concentration and duration of exposure. The suggestion conducting a future study to extract the active compounds of the apricot seeds and to study the extent of their effect on the larval viability of the *E. granulosus* parasite *in vivo* and *in vitro*.

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