

Original paper

Effectiveness of aqueous extracts of pumpkins seeds in the initial hydatid vitality isolated from outside of the body of sheep

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ABSTRACT. This study includes testing the effectiveness of seed pumpkin extract with intensive concentration (0.25, 0.50, 0.75, 1.00 g/ml) referring to time periods (1, 6, 12, 24) an hour depending on the initial primates vitality *Echinococcus granulosus* in the glass in order to reach an effective focus on killing the initial primates in a shortest possible period of time. The results of the current study show that a decrease in the vitality of initial primates when using the extracted aqueous of the pumpkin seeds up to 98.84% when focusing on 1.00 g/ml at time 24 hours when treated with extract aqueous of pumpkins seed.

Keywords: pumpkins seeds, hydatid, extracted aqueous, echinococcosis, effectiveness

Introduction

Hydatid cyst are known since antiquity, and illness that is caused by these cysts are called hydatid disease or echinococcosis, cystic echinococcosis (CE) which represents a big dilemma for the man from two sides like medical and economic in most parts of the world [1]. Hydatid is a cyclozoonotic parasitic disease that is common to humans and animals. The disease is caused by larval stage of *Echinococcus granulosus* [2]. Furthermore, *Echinococcus* have wide spread in the global (cosmopolitan). Though, there are some types of *Echinococcus* have prevalent selected [3]. It is found in some countries, as well as in Middle Eastern countries such as Iraq, Syria, Palestine and Lebanon and a section of North Africa and Arabian Peninsula countries and Sudan and the Caspian basin as well as some South American countries [4]. It is the only type that causes a disease of hydatid cysts in Iraq, despite human cases of *Echinococcus multilocularis* cysts multi hydatid cabins and diagnosis of a new kind which is Iraqi *Echinococcus*

of stray dogs in Nineveh [5]. The primary infection happens with first echinococcosis as a result of human and animal swallow of the echinococcosis cysts and it incubates in the gut of hexagonal embryos thorns. It is impenetrable bowel and drifting with the blood to the liver, lungs and other areas where hydatid cysts crafted resides. Moreover the hydatid cyst or mechanically leads to secondary infection that hydatid cysts receive secondary echinococcosis [6]. The infection of hydatid cysts represents a type *E. granulosus* which is one of the most important infections, since the growth and development of the cysts cause disease or death sometimes. As well as economic losses in sheep and cattle [7]. It has been resorted to the use of extracts of different plant types to treat parasitic diseases. This is because of the side effects that caused by chemical and surgical methods to humans. One of these plants is seeds of pumpkin which have great medical benefits, where the extracts are safe for the patient and affect the parasite at the same time [8,9].

Cucurbita maxima (pumpkin, family Cucurbitaceae) commonly known as Squash is widely used as

vegetable and a source of vitamin A, iron, phosphorus and calcium [10]. *C. maxima* seeds with considerable safety margin versus commonly consumed chemical drugs [11], is a very rich in terms of amino acids that are building blocks of proteins, especially the essential amino acids such as phenylalanine and methionine which are not produced in the human body [12]. Investigation on *C. maxima* reported that spinasterol isolated from the flowers of *C. maxima* potentially showed anti-carcinogenic, anti-genotoxic [13], and anti-mutagenic activity [14]. The *C. maxima* seeds were used in the treatment of digestive disorders [15], as well as essential oil of *C. maxima* seeds exhibited significant anthelmintic activity [10,12,16]. Recently, an *in vitro* study on hydatid cyst protoscolices revealed that 50 mg/ml of *C. maxima* seeds chloroformic extracts showed scolicidal activity [17].

The aim of the research was stating the impact of aqueous pumpkin seeds extracts for hydatid vitality and evaluating the effect of pumpkin seeds extract and then determining the concentrations lethal to all primary hydatid cysts.

Materials and Methods

Samples have collected of infected sheep livers from Tikrit in Salahuddin province, Iraq. They have placed inside a clean nylon bags and placed inside a clean container that contains ice inside of it. After that, they transferred to the lab to be conducted by the initialization parameters and counting within two hours. The current study adopted method of [18] in preparing initial.

The process of initialization is done by taking the initial primates that are found in the preserver middle and put them in test tubes and metallic mediated centrifuge for 5 minutes and 1000 rpm. Moreover, to get rid of the leaky cauldron and operation. Then applying the process of washing the physiological primates with salt solution. Shaking well the pipes and laid back in the centrifuge with the same duration and speed as well as get rid of the leaky cauldron, wash primates second time using physiological salt and shake the pipes and then returned to the centrifuge that is the same length and speed. It also gets rid of leaky and wash primates with physiological salt with one last time and make the process of count primates by withdrawing 10 microliter by microsucker micropipette of solution after jerk well then put it on a glass slide and add to

it the same amount of aqueous eosin stain concentration of 0.1%. For the purpose of knowing the vitality of initial primates at the very beginning or in the time zero before exposure to plant extracts. It shows that initial primates that are alive become bright green while the dead ones are red as a result of the entry into force of the eosin stain across the wall of primates [19]. The vitality of initial primates is accounted by crossing the number of alive primates and the total number of primates \times 100 and take the range of three results. Finally, utilize these ranges in the present study of the initial primates vitality < 93%.

The seeds of the pumpkins plants were gotten from Al-Alam, Iraq because they are found there in a frequent amount. The seeds of the pumpkins have washed thoroughly before using. Then, remove the dust and then placed in a well-ventilated room for six days until it is dried. After that, they have cut and crunched by small electric grinders and stored until use.

The extract aqueous of the experimental plant of pumpkin seed has prepared by mixing 50 grams of plant powder with 500 ml distilled water. Then, putting the mixture on magnetic stirrer for 24 hours and temperature of the 30°C, using the centrifuge speeds of 3000 rpm for 15 minutes for the purpose of separating the leaky cauldron on deposit, then focus using leaky evaporator rotor. After that, putting the cauldron in Petri dishes and put in electric oven temperature 40 m for two days for drought. Keeping that in a refrigerator [20–22]. Stock solution is prepared for aqueous extract of pumpkin seeds by thawed 0.25, 0.50, 0.75, 1.00 g of the extract in 100 ml of distilled water and concentrations are prepared from 0.25, 0.50, 0.75, 1.00 g/ml of extract and save in the refrigerator until use in vital tests the initial primates outside the living body.

Protoscolices are collected and counted their vitality so that the number of protoscolices in every pipe 2000 protoscolices/ml. In the beginning of the experiment the percentage of vitality is collected. Shaking protoscolices well to regularly distribute the primates in stuck. Transfer 2 ml of the primates stuck to each tube of glass pipe with an arbitrator cover that is consist of 99 tubes treated. The pipes that are contained of 0.25 water extract of pumpkin plant (seeds) 0.50, 0.75, 1.00 g/ml of extract watery pumpkins (seeds) of times 0, 6, 12, 24 hour and then calculating the average arithmetic mean of vitality at zero time after treatment with manual entry eosin

Table 1. Chemical detection of some active ingredients in plants

Extract plants	Tests				
	alkaloids	resins	saponins	glycosides	tannins
Pumpkin seeds	+	-	+	+	+

stain. Then, the pipes are stored in aerobic conditions temperature 10°C. And then calculating the average arithmetic of vitality of initial primates of 0, 6, 12, 24 hour, instead of running out the eosin stain. CRD tables are designed according to the test of Duncan's method.

Results and Discussion

The results of chemical detection indicate that in Table 1, a number of components and the active substances with referring to the most important pumpkin seeds such as, alkaloids, resins, saponins, tannins.

The present study includes the using water extract of the pumpkin plant seeds to demonstrate its effect on vitality of initial primates. However, water is considered as a solvent of suspect for cargo and its ability to dissolve many active compounds like tannins, saponins, terpenoids, polypeptides, some alkaloids and glycosides that have impact inhibitive on microbiology [23,24]. Therefore, it can contain the extract of water for plant of the most effective compounds. According to this study there are four

concentration experiments of plants (0.25, 0.50, 0.75 and 1.0 g/ml). Concerning the water extract and indicate the impact of the water extract on the initial primates energetic during specific periods of time while accessing to effective focus by killing the primates during the shortest period of time. Then, initial primates have been isolated from hydatid bags in the livers of sheep. protoscolices are selected out of the sheep because they show a high incidence of disease [25] as typical isolation to impact this extract on protoscolices.

The results state that the moral differences between the applied concentrations in percentage of loss of initial protoscolices (Table 2) that increase the focus has led to an increase in loss of protoscolices and comparing the treatment of control where the table shows that the focus 1.00 g/ml of seeds powder gave the highest general average loss and was 74.00% while the focus 0.25 g/ml 24.13% loss rate which diverged about moral concentrations 0.75 g/ml, 1.00 g/ml, it shows that all the applicable concentrations of plant leaf pumpkins moral differed from treatment of protoscolices loss ratio control, control treatment gave the lowest rate

Table 2. Effect of aqueous extract for pumpkin seeds in the percentage of loss of protoscolices

Time an hour/ concentration g/ml	1	6	12	24	Rate of concentration
0.25	3.00±0.577 O	18.17±0.441 L	34.33±1.20 J	41.00±0.577 I	24.13±4.46 C
0.50	9.03±0.088 M	28.17±0.167 K	41.33±0.726 H	66.67±0.88 D	36.30±6.32 BC
0.75	32.67±2.33 M	45.67±2.05 H	58.77±2.28 F	92.67±1.30 B	55.19±7.59 BC
1.00	41.00±0.289 I	67.50±0.289 F	87.50±0.500 C	98.84±0.00 A	74.00±6.73 A
Control (0.09%)	2.81±0.577 P	5.90±0.318 O	7.65±0.376 O	10.33±0.203 N	6.67±0.368 D
The rate of time	15.70±3.10 C	37.83±5.32 BC	52.33±6.70 AB	71.04±8.36 A	

The different letters mean the presence of significant differences: whether for different concentrations simultaneously (vertically: O, M, M, I, P for a time of 1 hour), or at different times for one concentration (horizontally: O, L, J, I for a concentration of 0.25)

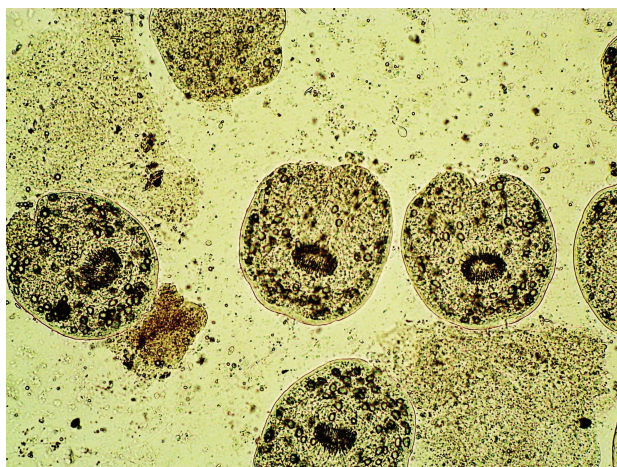


Figure 1. Protoscolices before staining with eosin stain

of 6.67% loss. As for the effect of time is evident from Table 2 that the proportion of the losses the protoscolices increase the time where it was best at the time 24 hours which was no different morally from the time 12 hours while the losses ratio was less valuable effect of protoscolices is when time 1 hour. Table 2 noted the effect of overlap between time and concentration, so that the highest impact was for transactions that use focus 1.00 g/ml with 24-hour time amounting to losses ratio of protoscolices 98.84%, while the lowest control treatment given in primates only Less with the time in 24-hour time 10.33%.

The treatment of the initial primates in outside of

the living body by water extract pumpkins seed with concentration (0.25, 0.50, 0.75 and 1.0 g/ml) that led to a decline in the proportion of initial primates and dynamic fit is directly related to increased focus to water extract and leaf increase exposure period.

The high efficiency for water extract of pumpkins seed in loss protoscolices may be due to the existence of tetracyclic triterpenes. It can be dissolved in water as in the single or bilateral turbines including compound limonene in volatile oils of Angelica Lemon with poison influence on the fourth stage larvae of mosquitoes alkaloids [26]. This effect may be attributed to water extract of pumpkin seeds to a tripartite seminars. It has a role in weakening the initial metabolic events as well as stop cytochrome c oxidase and transport chain. The electrons through the cell respiration process because effective aggregates that interfere with enzymes involved in agroindustry metabolism which then leads to stop of the cell and death [27,28], single turbines work on analyzing the bacterial cell membrane and then an imbalance in cellular and metabolic events that lead to cell death and Eocene tint to it.

The high rate of killing can be due to the presence of (alkaloids and glycosides). For the glycosides, their effectiveness is related to the non-diabetic part in their composition [29], as the diabetic part differs from one glycosides to another,

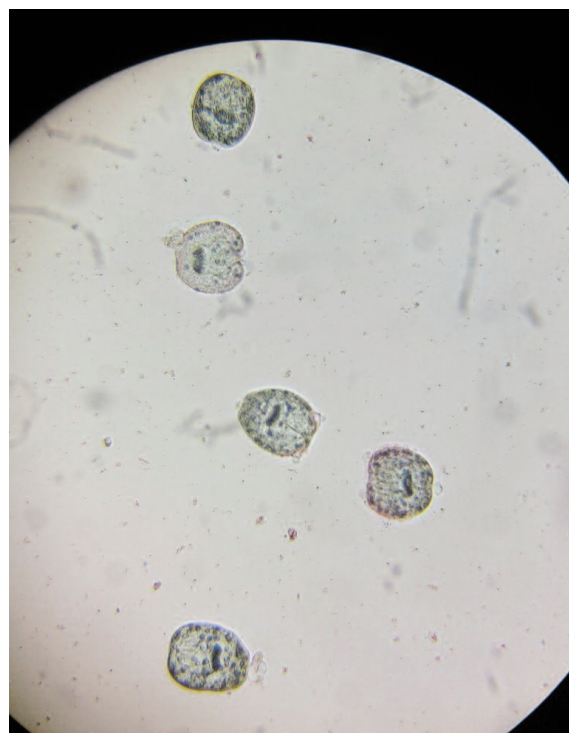
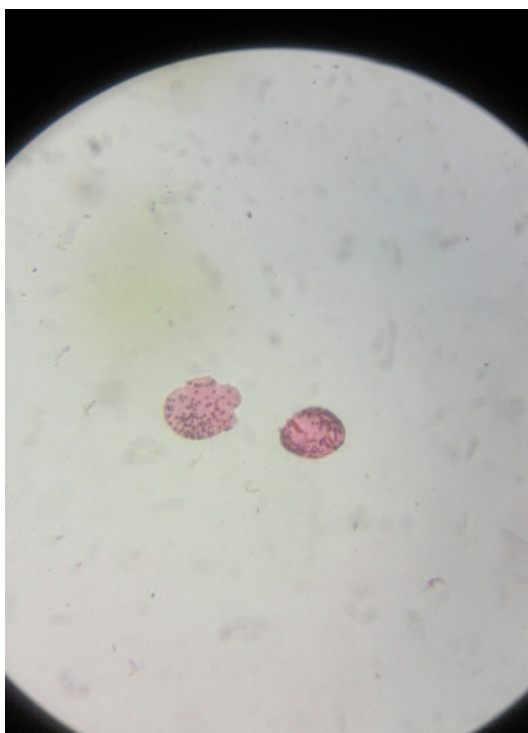


Figure 2 A,B. Protoscolices after staining with eosin stain (red color means dead but green color means a live)

which gives it a wide range of effect. The mechanism of the effect of glycosides is interfering with the functions of the cell membrane, particularly the binding with proteins and lipids of the membranes and inhibiting the effectiveness of some enzymes [30]. Quicisides may be affected in *Quercus infectoria* oak extract on the external parasite Ixodidae [31]. As for alkaloids, recent research has proved that they have anti-parasitic properties and they are not toxic to the organisms that produce them, but are toxic to alien organisms or cells and are selective [32–35]. It also releases the state enzymes, which leads to the loss of vital organelles and the destruction of the nuclei of the cells that generate the parasite and then kill it [36].

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