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THE EFFECT OF THE ACTIVITY OF *QURECUS PERSICA* OAK PLANT SECONDARY COMPOUND EXTRACTS ON THE VIABILITY OF PROTOSCOLICES FOR *ECHINOCOCCUS GRANULOSUS* PARASITE *IN VITRO*

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Abstract

This work was conduct to account the effects of cold aqueous, alkaloid and phenolic extract for the stem peel of an *Qurecus persica* plant on the protoscolices a viability of *Echinococcus granulosus* parasite *in vitro*. Three different concentrations (50, 100, 200 mg / mL) were used for each extract and compare with 800 mg/ml concentrations of albendazole drug. Albendazole drug reveals a high activity, it is finished the viability of all protoscolices at the third day. phenolics with a concentration of (15% and 10% mg / ml) was finished the viability of all protoscolices at an fifth day. Alkaloids has third stage in effect, it is ending viability of all protoscolices in eighth, ninth and tenth day for (15%, 10% and 5% mg/ml) respectively. Cold aqueous extract ending viability of all protoscolices in ninth, tenth and eleventh day for (15%, 10% and 5% mg/ml) respectively. The Krebs-Ringer solution was a better preservative solution, that protect protoscolices viable for 15 days, all of these results with significant differences.

Keywords: *Qurecus persica* (oak plant), *in vitro, Echinococcus granulosus*

Introduction

The hydatid cyst (Hc) to appear due to infection with the *Echinococcus* genus, it is a worldwide threat to public health (Gosttein, 1992). The genus *Echinococcus* has four types of medical and health importance these includes *E. granulosus, E. multilocularis, E. oligarthus* and *E. vogeli,* these four species are widely prevalent and may cause severe disease in patients (Al-dabk and Al-Janabi, 1990). Unilocular (HC) is an illness occur by *Echinococcus granulosus* which has a global distribution and can happen high morbidity and mortality (Doty and Tompkins, 1989). The large presence of disease in the human and animal walmdaev was concentrate in sheep-breeding countries, including the middle east, South America, India, north China and Iraq, (Safioleas *et al*., 2000; Al-aubaidi, 2010). Human is an accidental intermediate host through his touch each other with the final host (usually dog) or ingestion of food and water contaminated with eggs of helminths (Thompson and McManus, 2001).

Hydatid Cyst is the most widely in the liver and lung, also it can exist in other organ's including muscles, eye, spleen, brain, orbit, lymph nodes, kidney, myocardium, tonsils, pancreas, ovaries, an uterus skin and parotid glands (Langer *et al.*, 1984). The illness is an animal infection occurs by the mature or immature stages of the *E. granulosus* (Thompson and McManus, 2001). The adult worm of *E. granulosus* is present in the dog's fasting, and the Ova are digested by (sheep, cows, mice, deer, and humans) which are intermediate hosts that free our fetus that pass through the mucous layer of the intestine into portal circulation , most of these fetus are confined to the liver, and the remainder passes from the liver and go to other members then develops into the hydatid sacs (Al-aubaidi, 2010; Gomez *et al.*, 1988). Surgical treatment of the sac are the perfect way to get rid of this disease, however, this should be without or Minimal harm to the orbital and visual fabric Function with using chemotherapy (Gomez *et al.,* 1988).

There are also a few attempts to parasite treatment without need for Surgery by using pharmaceuticals as Benzimidazole like mebendazole and albendazole to end disease and eliminates an parasite (Edan, and Ardalan, 2009). Currently, medicinal plants are used to treatment and remove these diseases and the preservation of public health are widely globally spread (Veiga *et al.,* 2005). Natural herbal products can fuse with drugs this are aimed to improve the effectiveness of medications because chemical drugs are more expensive and have several disadvantages, therefor herbal products reduce drug side effects (Arun and Asha, 2008).

Albendazole and mebendazole, praziquantel and amphotericin B are Benzimidazole carbamate derivatives which are regard as the preference drugs for *Echinococcosis* treatment (Reuter *et al.,* 2012), albendazole sulfoxide which effective as a scolicidal factor in the hydatid cystic treatment (Deger *et al.,* 2000), most of Commen drugs have percent of toxicity, permanent use of these treatments, especially for patients treatment, which lead to dangerous of these drugs on the body (Villarreal, 2003). *Qurecus* is the medical plant that represent the most popular genus for Fagaceae family (Panahi *et al.,* 2012), found in Turkey, Syria, Iraq , Persia, Cyprus, Greece (Evans, 2009), *Qurecus persica* oak is widely distributed in the Kurdistan of Iraq. Herbal extracts have a high potential activity and low cellular toxicity, probably due to the activity of phenolic compounds, especially its tannin compounds, therefore, it will be a promising candidate to introduces a new agent without or with minimal cellular toxicity effect, Tannins, flavonoids, and phenolic compounds were found in *Q. persica* L (Karimi *et al.*, 2013).

The advantage of herbals materials return to their phenolic components and antioxidant capacities (Rabindran *et al.,* 2003), which due to a high level amount of phenolic compounds, especially flavonoids and tannins in *Q. persica* extract which give it the potential effects as an antioxidant activity, Anti-virus antibacterial (Muliawan *et al.,* 2006; Chang *et al.,* 2002).

Alkaloids are organic compounds containing cyclic nitrogen, such as: atropine, scopolamine and hyoscyamine (Zulak *et al.,* 2006), that are used for treatment in medicinal sides (Goldman, 2001), Contributed among toxins, neurotoxins and social drugs, alkaloids act primarily with the prevention of feeding and poisons for insects and other herbivores (Harborne, 1993), They act as catalysts for neural transport systems (Wink, 2000), different components as caffeine, nicotine, opiates and cocaine are consumed by humans (Zenk and Juenger, 2007).

Phenolic have one aromatic hydrocarbon ring containing one or more hydroxyl Groups of precursors phenylpropanoid pathway were attached, They range from simple components such as phenylpropanoids, coumarin and benzoic acid to more complex components as tannins, stilbenes, and flavanoids (David *et al.,* 2011), phenolics include components which represent toxicant materials for insects (Diaz *et al,* 2019; Treutter, 2006), phenolics have defense ability against bacterial or fungal attack and also have roles in the absorption of UV ray (David *et al.,* 2011; Diaz *et al.,* 2019; Treutter, 2006).

Methods and Materials

Plant Materials Collect

The stem peel of *Qurecus persica* plant were collected from the Al-Heikma herbarium at September 2017 , plant was diagnosed by the botanist in the plant department in life sciences at the University of Babylon, Babil, Iraq. A sample of plant materials was desiccants in the shade position and then grinding by electric grinder to get on an soft powder which kept in plastic plate and then save in the Herbarium lab. of the Pharmacognosy department, College of Pharmacy.

(i) Preparation of an cold aqueous extract for *Qurecus persica* plant :

Cold aqueous extract was attended by take 10 grams from the stem peel powder of the *Qurecus persica* plant and put it with 200 ml of distilled water in the flask 400 ml in size with using a mixer for 30 minute, then put in the test tube in the centrifuge for 10 minutes at 3000 rolls / minute, extract was dried by putting it in the oven at 45 C to obtain on dried extract, keep it in the fridge until use (Hernandez *et al*., 1994).

(ii) Extraction of secondary Plant component

(a) Extraction of crude alkaloids :

10 g of dehydrated soft powder is extracted by putting it in the filter papers which fixed on thimbles, then adding 200 ml from ethanol alcohol (%99) for 24 hours by soxhlets apparatus. New products were concentrated with rotary evaporator apparatus. It dissolved in 5 ml of ethanol, with added 30 ml of Sulfuric acid (2%), then using rotary evaporator apparatus to remove an ethanol alcohol. Mayer assay gives white product to ensure an present of alkaloids. Hydroxide ammonium (%10) was putting in separating funnel.

With putting (10) ml from chloroform, mixing of product was separating into two layers, selected the bottom layer because it contains alkaloids, it was concentrated with rotary evaporator, a new dry product kept in icebox (Al-Samaraei, 1983) methods.

Fig. 1 : Separating funnel apparatus for alkaloids.

(ii) Extraction of crude phenolics:

Method of (Ribereaun, 1972) was used to extracts phenolics, 20 gm of dried extract put in a glass flask with 400 ml of (2%) acetic acid by using the reflex condenser in (70) degree centigrade water bath for 8 hours. New suspension nominated and put it with N-propanol and sodium chloride substances in the Suppression of separation , been taking the top layer containing phenolic substances, then it was focused with evaporator rotor and dry product keeping in the fridge.

Fig. 2 : Separating funnel apparatus for phenolics.

Collection of hydatid fluid

The hydatid fluid was summation from hydatid cyst that found in infected sheep livers in Hilla abattoir, then the hydatid cyst eliminated from livers of slaughtered sheep, it is put in ice containers. Then it is transmitted into the microbiology laboratory in pharmacy college of university of Babylon. Sterilization of the sac surface by ethyl alcohol 70% was performed then hydatid cyst fluid aspirated by plastic medicinal syringe (10) ml, and it is put in the glass container (500) ml capacity, hydatid cyst opened by scissors and germinal layer aspirated by forceps in the Sterile container with phosphate buffer saline (PBS), then germinal layer washed by bottle washing that containing (PBS) a solution for large numbers of protoscolices. Collect, the solution in sterile test tubes to deposit by centrifuged three times 3000 roll / min for a period of 15 minutes for each deposition, procaine penicillin (2000 units / ml) and streptomycin at 1 g / l added to this solution before the

second wash, then filtration fluid was eliminated and added of(PBS). The final fluid was collected in sterile bottles and counting the number of protoscolices (Smyth, 1985).

Fig. 3 : sheep livers with hydatid cyst.

Fig. 4 : Germinal layer of hydatid cyst .

(a) Preparation of protoscolices

Protoscolices preserved in sterile maintained solution made of patchwork of hydatid cyst fluid and Krebs-Ringer solution (4:1) (Kadir *et al.,* 2004).

(b) The Estimation of protoscolices viability

Vitality calculated before experience by mixing a 0.01 ml of protoscolices solution with 0.01 ml of a 0.1% water eosin dye by using micro pipette and then check the vital by minimum force microscope for three repeaters. Non-dyed protoscolices are alive and deemed suitable for testing while painted protoscolices are dead and are not suitable for testing (Smyth and Barrett, 1980).

(c) The Effects of plant extracts on protoscolices in vitro

Preparation of stock solutions of both extracts of the *Qurecus persica* plant which brings the melt (20) g of dry extract in (100) ml of in sterile water, therefore, stock solution become (20%) mg/ml. Concentrations (5, 10, and 15) mg/ml are prepared from stock solutions, control is prepared from distal water only . The efficacy of both extracts was compared with 10 mg / ml of Albendazole drug.

Protoscolices were aggregated and then calculated their numbers, viability and the present ratio in the first of experiment. One milliliter (approximately 2000 viable protoscolices) from Protoscolices suspension (Kerb's ringer's + Hydatid cyst fluid 4-1) were transported to every tube of essay tests, in every essay tests put one ml of (5, 10, and 15) mg/ml from each extract and put one ml of distal water in the negative control tube and a normal saline solution tube which is considered as a positive control, incubated at 30 ºC without conservative solution changes. Calculation of viability average take tube 1, 3, 24, 48, 72, 96, 120, 144, 168, 192,216 and 240- hour Data was derived from the mean value of 3 replicas(Doaa *et al,* 2011).

Results

Table 1 : The Percent ratio of protoscolices vitality in 30 microliter.

Viability of protoscolices	Mean
Total number Average of protoscolices	83
Live	79
Dead	4
%	95.95

P_{value}=0.0006

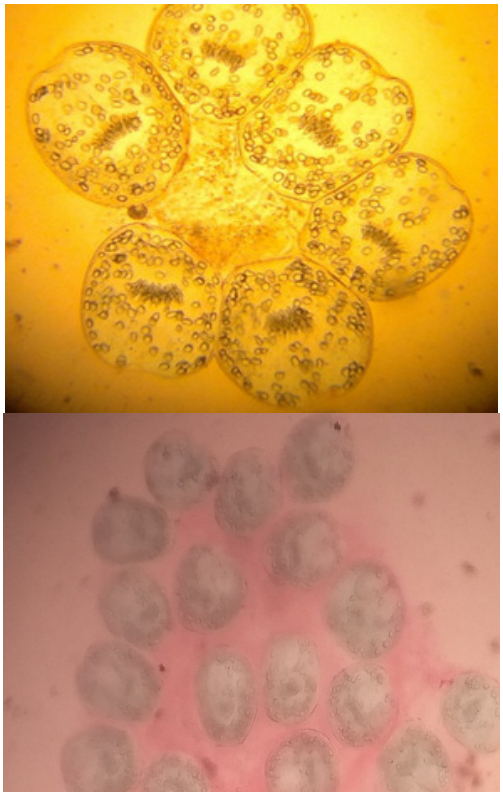

Fig. 5 : Viable protoscolices before treatment

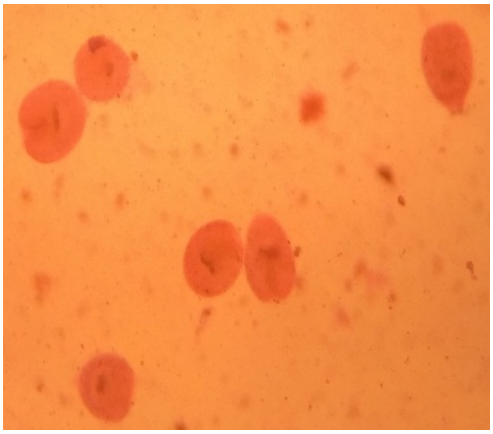

Fig. 6 : Dead protoscolices before treatment.

Table 2 : The effect of extracts concentrations on vitality of protoscolices *in vitro*.

Time Conc.	0	1hour	1day	2 day	3 day	4 day	5 day	6 day	7 day	8 day	9 day	10 day	11 day	12 day	13 day	14 day	15	16
	Control group																	
	95.9	85.0	92.1	89.2	85	81.1	76.2	71	66.7	60.1	52	47	41.13	37	29.5	20.2	11.3	0
	Albendazole drug																	
10%	95.6	72	30.43	0														
	Cold aqueous extract																	
5%	95.9	93.4	88	80.4	71.2	53.5	41.8	34.6	21.17	14.5	8.13	3.13	0					
10%	95.9	93.2	87.4	80.03	70.53	53.80	41.33	34.20	21.60	15.03	8.07	0	0					
15%	95.9	93.1	87.1	79.03	68	53.07	40.03	33.17	21.17	14.03	0	0	0					
	Alkaloids extract																	
5%	95.9	94.7	85.30	73	61.67	55.17	44.10	33.20	25.20	15.33	6.30	0						
10%	95.9	94.43	85.70	72.03	63.10	54.07	43.70	34.27	24.20	16.20	0	0						
15%	95.9	94.3	85	70.33	60.83	52.33	43.1	31.20	19.33	0	0	0						
	phenolics extract																	
5%	95.9	93.53	84.10	69.40	40.27	29.30	17.10	0										
10%	95.9	93	85.20	66.30	37.40	20.50	0	0										
15%	95.9	92.1	79.2	56.4	34	16.7	0	0										

L.S.D. (0.05)=1.443

Statistical analysis

Analyze of data take place by using ANOVA test by using the statistics system (SPSS). P <0.05 statistical values were of a moral difference.

Discussion

Hydatidosis is a zoonotic sickness, endemic dramatically and cosmopolitan, caused by *Echinococcus granulosus* larval phase in sheep, cows and the human which acts as intermediate hosts while adult helminth found in a dog ,wolfs, foxes and other carnivores which acts as the final host, it's the important and commonly parasitic illness present with the greatest numbers in indigent countries, especially rural regions, where pupils contact with the dogs and domestics animals (Nepalia *et al.,* 2006; Fadhil and Baiee, 2018).

In this present study we got on that preservative solution was the better medium for protoscolices still valid for 15 days after being, putting in sterile preservative solution for incubated at 31˚C, these results agreement with formerly studies outcomes that perform in vitro with (Kadir *et al.,* 2004; Rassan, 1994) they were record that the Krebs-Ringer solution with the fluid of hydatid cyst was best kept in percent 4:1 for protoscolices still live in vitro , also this result compatible with the result of (Doaa *et al.,* 2011) they were reported that the preservative solution was a good medium which to protect the protoscolices, active and viable for 15 days, these results or outcomes may be return to the real that Krebs-Ringer solution has organic and inorganic materials as nutritional factors and a number of minerals (magnesium chloride, glucose, sodium chloride, potassium chloride, sodium phosphate monobasic, and sodium phosphate dibasic) that give protoscolices with the little requirements to protect them alive for a long interim of time (Zhang and McManus, 2003).

Statical analysis appears that the viable percentage for protoscolices was (95.95%) in table one, this result is consistency to result (Doaa *et al.,* 2011) they were reported that vitality percent of protoscolices was (97.9%), as well as accord with (Khalaf *et al.,* 2011) they were record viability percent of protoscolices (95.33%), also similar with (AL-Quraishi1 *et al.*, 2015) in Iraq, they were reported that viability percent of protoscolices was (94.83%), this result may be attributed to that hydatid cyst that found in sheep have a high fertility from the other hydatid cyst that are reside in cows, buffalo and goats because the sheep are the main intermediate host for this parasite (John and Petri, 2006).

Table 2 appear that Albendazole drug has a high efficacy by killing protoscolices during two days only after treatment, this result consistency with the result of (Ma *et al.,* 2004) in China, they were scored high efficiency for Albendazole at 25 mg /kg/day in conjunction with alkaloids at 25 mg/kg/day, that led to high reduced growth rate of hydatid cyst and a weight of hydatid cyst into (18.3 ± 4.6 mg) compare with the control group (76.6±12.0), as well as compatible with (Rafiei *et al.,* 2009) in Iran, they were reported big activity for Albendazole 50mg/kg/day plus 600mg/kg/day of Praziquantel that reduced the number, weight and cyst sizes into 91.70%, 90% and 80.3%, respectively, with statistically significant in mice, as consistency with (Doaa *et al.*, 2011) in Egypt, they were scored significant effect for Albendazole (800 µg/ml) in vitro on the viability of protoscolices which died at 10 day, also agreement with (Khalaf *et al,* 2011) they were reported a high activity of Albendazole at 1000 µg/ml after 3 days – post treatment, activity of Albendazole may be attributed to that Albendazole could be a toxic in some cases or subjects (Pawlowski, 1997) and are related with severe hepatobiliary problems (Topcu *et al.,* 2006).

Natural scolicidal factors provide a safe alternative without the influence of associated (Gholami *et al.*, 2013), Albendazole are currently preferred drugs for cystic echinococcosis disease (Reuter *et al.*, 2003).

The results of this study indicate that the phenolic extract of the *Qurecus persica oak* plant discouraged big effect in reducing the number of viable protoscolices with a high percentage where all these protoscolices and the vitality percentage come to 0% at the fifth day after treatment at a concentration of 15% and 10%, this study agreed with other studies that conducted in Iraq and other country, like the study that conducted in China with (Ma *et al.,* 2007) who

explained that *S. moorcroftiana* plant alkaloids extract have protoscolicidal effects and the combination between alkaloids and Albendazole has an extra moral influence, as consistency with (AL-Quraishi1 *et al.,* 2015) which showed that a significant decrease in sizes and weights of hydatidosis when using alkaloids and phenolics at concentration (270, 280) g/ml respectively compared to the size of hydatidosis for positive control animals, as well as compatible with (Mahmoudvand *et al.,* 2016) in Iran, he was found to be extracted at the concentrations of 500 and 250 mg/mL led to kill protoscoleces 100% after 10, 20 minutes of exposure, also accord with (Larki *et al.,* 2017) in Iran, they noted that the use of a Gallic acid as an effective component of some herbal scolicidal factors as possible that is responsible for killing the protoscolices, Phenolic are one of the most extensive existing group that plays an important role in the growth and reproduction of plants and also it is act as a mechanisms of protection against microorganisms such as parasites and bacteria (Baidez *et al*., 2007). There are many vital activities for phenolics which have some curative properties such as anti-inflammatory, anticancer, antiviral ,antibacterial and antioxidant activities (Baidez *et al.,* 2007; Han *et al.,* 2007; Veeriah *et al.,* 2006; Owen *et al.,* 2000).

Many studies confirmed that phenolcs are capable of killing protoscolices, then decrease of viability percentage (Delorenzi *et al*., 2000), also it was noticed that the number of alive protoscolices decreased with the increase of time (Al-Maliki, 2008), decreases the viability of protoscolices of *Echinococcus granulosus* may be because of the ability of these phenolic compounds to object respiratory process in mitochondria, then inhibition of carbohydrates, fats and intermediate the reactions of metabolism of proteins which are important for protoscolices viability or because of the ability of chemical compounds to destruct the cell membrane contain in fats and proteins, therefore the process leads to death of *Echinococcus granulosus* parasite (Delorenzi *et al.,* 2000; Topcu *et al.,* 2006), other studies appeared that phenolic extract inhibits respiratory chain enzymes including thiol group (-SH) which is replaced by the carbonyl group existent in phenolics after oxidation of hydroxyl group (-OH) by molecular O and removal of a hydrogen molecule (Houghton *et al.,* 1995; Hailat *et al.,* 1998).

The researcher (Bajalan *et al.,* 2014) found that *Qurecus persica* contain phenolics by using the High performance liquid chromatography (HPLC) technique. The researcher results (Ma *et al.*, 2007) indicate the presence of P-comaric acid, Syringic acid and Vanilic acid in the Oak leaf and also he was found that tannin and Gallic acid are the main components of the oak tree.

There was a noticed that a concentration of (15%) has a greater effect than (10% and 5%) concentrations because of they possess the high antiparasite activity to kill all protoscolices in less time. The mechanism is explained that phenolics lead to shattering or disorder in respiratory process in mitochondria, then also discouraged the metabolism of carbohydrates, fat and proteins which lead to death of *Echinococcus granulosus* protoscolices (Delorenzi *et al.,* 2000). Some studies ensured that phenolics extract inhibits respiratory chain enzymes containing a thiol group (-SH) which is substituted by carbonyl group present in phenolics after oxidation of hydroxyl group (-OH) by molecular oxygen and elimination of a hydrogen molecule (Houghton *et al.,* 1995; Hailat *et al.,* 1998). They were noticed that the extract concentration of (0.5 gm/ml) had a high effect where they are found that the number of alive protoscolices decreased in the course of time, especially at fourth, fifth, sixth and seventh days therefore protoscolices viability percentage come to 0%. This means that this concentration was more effective than the concentration of (0.25 gm/ml). The study which carried out on *Echinococcus granulosus*, ensured that the high concentration of extract was more active than low concentration (Hailat *et al*., 1998). Researchers in all region of the world began to clarify the real impact of many traditional plants using medication that has been announced yet by controlled scientific experiments.

The alkaloids had a role in access the viability percentage to 0% after eighth days at a concentration of 15%, these results agreed with (Ma *et al.,* 2007) Who noted that *S. moorcroftiana* alkaloids have fatal effect for protoscolices and meant he found that mixing of alkaloids and albendazole together with effective moral. Also (Khalaf *et al.,* 2011) showed that alkaloids extract of *Chlorophyta* and *Cyanophyta* had less effect against the hydatid disease than the crude alcoholic extract.

This study showed that a cold aqueous plant extract has lower activity compare activity of other extracts in this search , a concentration of 15% lead to final activity of protoscolices after ninth day, this result compatible with (Al-Bashir1 *et al.,* 2011), they were registered a high activity by reduce the number of cysts and it is diameters and high reduction percent for cyst when used *Myrtus communis* plant leaves aqueous extract by (6 mg /ml and 12mg /ml) for treated infected mice, as agreement with the result of (Al-Mayali and Anah, 2013) They noticed a high efficient for *Cucurbita maxima*, plant seed, cold aqueous extract with (60mg/ml) to the final viability of protoscolices 100% after one day ,also compatible with (Al-Kahlidy and Al-Hamiary, 2016) they were record that aqueous extract of *Opuntia ficus* plant has a high efficiency in killing protoscolices into 100% on the second day With concentration 10 mg/ml , this result may be return to *Q. persica* plant species that contains a big amount of tannins in both types hydrolysable and intense (Makkar, 2003; Makkar *et al.,* 1991).

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Conclusion

Phenolic compounds have the ability for killing protoscolices in little time with a few concentration and other compounds of *Qurecus persica* plant also capable for killing protoscolices, this ability can be used in the future for treatment of parasitic illness, especially hydatid cyst.

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