In vitro, Effects of Praziquantel, Alcoholic and Aqueous Crude Extracts of *Citrullus Colocynthis* and *Eucalyptus Globulus* on the Viability of Protoscoleces of *Echinococcus Granulosus*

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ABSTRACT

Iraqi flora is rich in plants and the possibility of finding new antimicrobial agents still widely ahead, therefore, the present work evaluated the effects of alcoholic and aqueous crude extracts of *Citrullus colocynthis*; *Eucalyptus globulus* leaves and Praziquantel drug (as scolicidal agents) on the viability of *Echinococcus granulosus* protoscoleces *in vitro.* Live protoscoleces obtained from the liver of naturally infected sheep. The viability of protoscoleces was determined by their motility and flame cells activity and staining method using 0.1% eosin solution. The number of viable protoscoleces was counted and distributed into test tubes (4 tubes for each treatment of different concentrations) and 2 tubes were kept as control without treatments. The percentage viability of protoscoleces was ranged between 93.7 to 95.3. Praziquantel drug reduced the survival of protoscoleces during seven sequenced days post incubation. Drug effects showed more influence in both concentrations of 450 µg/ml from day 4 and 600 µg/ml from day 3 post incubation, respectively. The methanolic extract of *C. colocynthis* reduced the viability of protoscoleces during eight sequenced days post incubation from 96% to zero%. The effects were marked highly in the concentrations of 300 µg/ml from day 3 post incubation. Aqueous fruit extracted decreased in the viability of protoscoleces. In the minimum used concentration (75µg/ml), the viability of protoscoleces reduced from 96% to reach zero% at day eight post incubation, whereas in the maximum concentration used (300 µg/ml), the viability reached to zero% after four days. All concentrations of leaves methanolic and aqueous extracts of *E. globulus* have an antiparasitic activity against the protoscoleces viability, but the greater effect was to 600 µg/ml that kill all protoscoleces in 6 days after treatment.

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1. Introduction

Human Cystic Echinococcosis (CE), caused by infection with the taeniidae metacestode (protoscoleces) as the larval stage of *Echinococcus granulosus*, is a cosmopolitan parasitic zoonosis that has a considerable impact on public health and animal production worldwide [1]. Hydatid cyst spreads throughout most of the world, especially in areas where sheep are found in high rate and is endemic in Asia, North Africa, South and Central Americas, North America, Canada, and the Mediterranean region. In many countries, hydatid disease is more prevalent in rural areas where there is close contact between people, dogs, and various domestic animals which act as intermediate hosts [2]. Currently, there are more than 3 million echinococcosis patients [3], and the disease causes over US$ 3 billion in economic losses each year[4]. CE is characterized by unilocular, fluid-filled cysts mainly located in the liver and lungs of patients [5]. Echinococcus cysts can survive in patients for over 50 years without apparently causing pathological damage in host tissues surrounding the cysts [6], indicating that *E. granulosus* has evolved protective mechanisms which underpin this long-term survival.

CE is difficult to treat due to the lack of an effective drug [7, 8]. Consequently, there is an urgent need for a new chemotherapeutic agent for the treatment of CE. Praziquantel (PZQ) is one of the anthelminthic used in the treatment of hydatid cysts; it is a component of Isoquinoline has an activity against the scolices *in vitro*. Presently, numerous scolicidal chemical agents have been administrated for inactivation of the hydatid cyst contents. Because of increasing resistance and adverse effects of medications include abnormalities of liver function, abdominal pain, diarrhea, nausea, vomiting, dizziness, and headache; there is a need to find alternative therapies either with the least or without side effects. Recently, there is a growing interest of research on medicinal plants due to their potential to cure many diseases, because of low prices and lower incidence of side effects when compared to synthetic drugs [9]. It is known that medicinal plants are the most important alternative to chemical drug resources, especially for antimicrobial and parasite targets and the treatment of various diseases. A total of 52 plant species were reported to be pharmacologically evaluated for their scolicidal activity against protoscoleces of *E. granulosus*, most of which belong to the families Lamiaceae; Apiaceae; Anacardiaceae; Myrtaceae, and Euphorbiaceae [10]. *Citrullus colocynthis* is known as it bitter apples and bitter cucumber, it is a small plant belonging to the Cucurbitaceae family, it is home in the Mediterranean basin and Asia especially Turkey and the desert region of India and Pakistan. The

C. colocynthis contains carbohydrates, proteins, amino acids, tannins, saponins, phenolics, flavanoids, flavone glucosides, terpenoids, alkaloids, anthranol and steroids [11]. The fruit extracts of *C. colocynthis* showed an antimicrobial effect such as Pseudomonas, Staphylococcus and Candida [12] and antifungal drugs such as *Aspergillus flavus* [13] and the effect of hypoglycemia on patients with type 2 diabetes [14] and rats [15]. In addition, *C. colocynthis* extract was found to be anti-*leishmania major* and against molluscs *Biomphalaria arebica* and anthelmintic, *Haemonchus contortus* [16,17]. *Eucalyptus globulus* (Family Myrtaceae) is a widely used tree that is cultivated in nearly20 million hectares of agricultural lands of the world [18,19]. The leaves essential oil of *E. globulus* has a number of biological properties that is a valuable source for traditional medicines [20]. As the Iraqi flora is rich in plants the possibility of finding new antimicrobial agents still widely ahead, therefore, the present study aimed to: Estimating the effect of alcoholic and aqueous crude extracts of *Citrullus colocynthis* (fruits plus seeds); *Eucalyptus globulus* leaves and Praziquantel drug (as scolicidal agents) *in vitro*, with determining the exactly effects of these compounds on the viability of protoscoleces of *Echinococcus granulosus* and in a comparison with the control groups.

2. Experimental

2.1 Material and Methods

2.1.1 Hydatid cyst samples:

Human hydatid cysts (Fig.1) were obtained from infected individuals by the aid of surgical physician, and transported immediately to laboratory for preparing and preserving processes for intended purposes. Sheep hydatid cysts (Fig.2) were obtained from sheep slaughtered in Hilla abattoir station, and immediately transported to the laboratory in insulated containers at about 4˚C.

Figure1: Human hydatid cyst

Figure2: Sheep hydatid cysts

The viability of protoscolices was determined by their motility and flame cells activity and staining method using 0.1% eosin solution. Then examined under light microscope (10x), the dead scolices stained pink color while the live one remained unstained. The numbers of protoscolices were counted using a Neubauer haemocytometer employed for enumerating white blood cells. Around 2500 viable protoscolices were added into flask containing Krebs - Ringer solution plus hydatid cyst fluid in a proportion 4:1 (KRS + HCF, 4:1).The number of viable protoscoleces was counted, and distributed into test tubes (4 tubes for each treatment of different concentrations) and 2 tubes were kept as control without treatments. The test tubes were kept in incubator at temperature range of 28-33˚C [21].

2.1.2 Study population:

Seventy- six individuals: 61 radiology confirmed hydatid cyst infected individual and 15 Casoni test confirmed hydatid cyst free individual, 15 confirmed hydatid cyst infested sheeps, five confirmed hydatid cyst free sheeps. The admitted hospital based study was carried out in: Government Hilla Teaching Hospital; Private Hospitals and private Clinics, on all outpatients with inpatients and follow up patients whom were confirmed as a hydatid cysts infested individuals. All hydatid cyst infected individuals were chosen randomly from both sexes and from different ages too, and all hydatid cyst infested sheeps were selected (especially oldest ones) after slaughtering it in Hilla abattoir station.

2.1.3 Calculation of protoscoleces number

The number of protoscoleces was counted as a mean of total viable protoscoleces in a defined volume (10 µl) of four samples for five days as follows: use suitable micropipette (10 µl size),

take 10 µl from the suspension of protoscolices together with sterilized phosphate buffer saline solution (after shaking suspension), then count protoscolices number in one milliliter, [22] as follows: mean number of protoscolices used in the constant volume (10 µl) is equal to 30 protoscolices, as shown in row:D2 of table (1) , hence the number of protoscolices in one milliliter is equal to 30 x 100 = 3000 protoscolices (2500 viable-greenish colored protoscoleces and 500 dead reddish-colored protoscoleces), and so for other samples in the same table [22].

2.1.4 Calculation of percentage viable protoscoleces:

The mean viability of protoscoleces was estimated by mixing defined volume of protoscoleces suspension with similar volume of aqueous eosin stain solution (0.01%), after shaking solution, take one drop by fine micropipette, directly view under microscopic field, count greenish-colored viable protoscoleces, and compare with reddish-colored dead protoscoleces for four frequencies [2,123].

2.1.5 Praziquantel drug stock solution:

It was prepared by dissolving 6000 mg of the drug in defined volume of distilled water (100 ml.), then kept in refrigerator at 4˚C until used at desired concentration.

2.1.6 Praziquantel effect *in vitro* study

The test tubes were treated with different concentration of Praziquantel drug (150, 300, 450, 600 µg /ml). Viability of protoscolices was estimated for each tube and was counted in subsequent days following incubation [24].

2.1.7 *Citrullus colocynthis* (fruits plus seeds)

Fresh fruits of plant were collected , left in room temperature to be dried for a period of weeks (Fig.3: A , B), then powdered to be used later for preparing crude methanolic and aqueous extracts from that plant.

Figure3: (A) General form of *Citrullus colocythis* (Wasfi, 1994), and (B) Riped fruit of *Citrullus colocythis* (Hamouds et al. 2000)

Figure4: General form of *Eucalyptus globulus* (Himal et al. 2008)

2.1.8 Preparation of methanolic extract:

Methanolic extract was prepared by adding 500ml of absolute methanol (99%) on weighting 50gm of dried plant powder. The mixture was extracted by magnetic stirrer for 72 hrs. in discontinuous period times – at room temperature and heated for 12 hrs. at 50-55˚C with stirring, then left to be cooled. The extract was pre-filtered on different layers of gauze. Then filtered by Buchner funnel using filter paper whatman No.2 with evacuation, Centrifugation of filtered extract was carried out using centrifuge at 3000 xg for 1/2 hr. The solution was evaporated using electric oven at 40-50 ˚C to dry. The dried powder was collected in clean glass bottle, kept in refrigerator at 4˚C until used [25, 26]. From the stock solution of methanolic plant extract, different concentrations were prepared: 75µg/ml,150 µg/ml, 225 µg/ml, 300 µg/ml to estimate its effects on the viability of protoscolices *in vitro.*

2.1.9 Preparation of aqueous extract

The aqueous extract was prepared by the same procedure use for methanolic extract with the exception of adding 500ml of distilled water on weighting 50gm of dried plant powder at boiling temperature.

2.1.10 *Eucalyptus globulus* (leaves)

Eucalyptus plant leaves were cut and collected from Eucalyptus trees of Babylon University (Fig.4) and left to be dried at room temperature, ground into a fine powder. The methanolic and aqueous crude extracts of *Eucalyptus globulus* leaves were extracted by the same procedures [25, 26] used for *Citrullus colocynthis.* The prepared concentrations of crude extracts were: 100, 200,400 , 600 µg/ml, to study the influence of *E. globulus* on the viability of protoscolices *in vitro* study.

2.1.11 Effect of plants methanolic extract *in vitro* study

To show the efficiency of the methanolic and water extracts of *Citrullus colocynthis* and *Eucalyptus globulus* on the viability of protoscoleces of *E. granulosus,* different concentrations were prepared extracts randomly: (75 µg /ml, 150 µg /ml, 225 µg /ml , 300 µg /ml of *Citrullus colocynthis* and 100 µg/ml, 200 µg /ml , 400 µg /ml , 600 µg /ml of *Eucalyptus globulus* , respectively), because for our knowledge no previous studies were performed on these two plant extracts" in our countryside" to fix their doses.

2.1.12 Statistical analysis

The obtained results were analyzed statistically as percentages; means ; standard deviation ; odd ratio ; and t- test was done to compare between each two groups. The difference at $p<0.05$, $p<0.01$ was regarded significant [27].

3. Results and Discussions

3.1 Counting of protoscoleces number

The obtained results showed the total number of protoscoleces in 10 µl within four samples (Table: 1 and Fig.5: A, B), and depicted as a mean and standard deviation .Statistically, there was a significant difference between the total number of protoscoleces in the day of sample collection (D0) and between that of post four days (D4) incubation in preservative solution (34±

4.32) and (27.5±1.29) respectively, at $p<0.05$. Whereas, there were no significant differences between D0 and D1, D2, and D3, respectively.

Table 1: Estimation of mean total number of protoscoleces in 10 µl in four samples by use of constant volume during different days within preservative solution .

Time(day)	Number of calculated protoscoleces in four samples				Mean ± Standard deviation (X±SD)
	S1	S2	S3	S4	
˙D0	38	34	28	36	34±4.32
D1 ns	35	31	30	32	32±2.16
D2 ns	30	30	31	29	30±0.81
D3 ns	30	28	30	28	29±1.15
D4*	28	27	29	26	27.5±1.29

*There is no significant differences between all groups, except group Do and group D4, at $p<0.05$. ˙ D0 = refers to the first day of sample collection (before treatment).

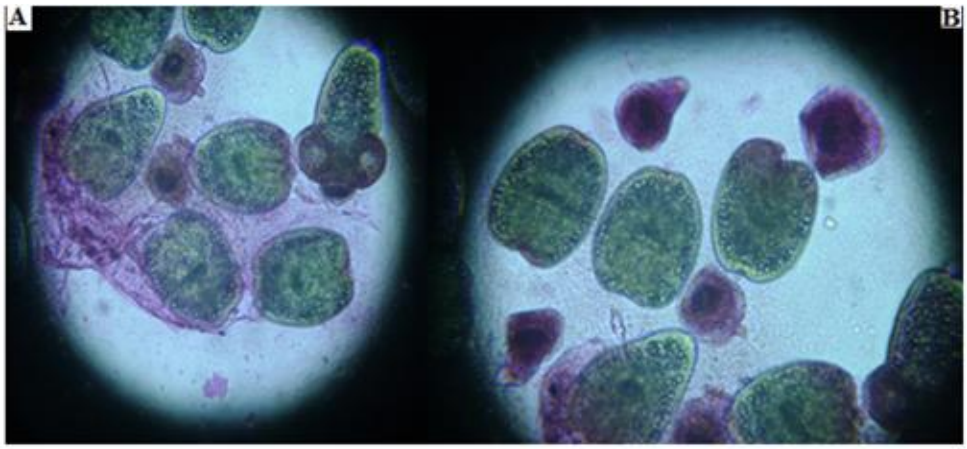

Figure5: Greenish-viable protoscoleces (A and B) viewed under 40x of microscopic field (syringed from human being infested liver)

The present study has estimated the total number of protoscoleces within four samples and depicted as a mean and standard deviation. Statistically, there was significant difference between the total number of protoscoleces in the day of sample collection (D0) and between that of post four days (D4) incubation in preservative solution, at $p<0.05$. Whereas, there were no significant differences between D0 and D1, D2 and D3, respectively. Interestingly, other studies noted

similar results [21, 22, 28, 29]. The obtained results might give us an index about the nature of the preservative solution (Krebs - Ringer solution) that be used to preserve the studied parasite *in vitro* conditions for extended and during desired period to be survived well [22, 23]. Furthermore, the chemical composition (fats, proteins, carbohydrates, calcium, glucose, phosphates and other nutritive compounds), for Krebs-Ringer solution was stated and determined by many studies [5, 13, 22, 30]. So that and accordingly, we have used the Krebs - Ringer solution with hydatid cyst fluid in a proportion of 4: 1 " as preservative and nutritive medium " *in vitro* conditions for protoscoleces suspension during the period of the study. The study results is in agreement with the results recorded by other studies [23, 28]. It is noteworthy, that the examined hydatid cyst in this study was sampled from infested sheep's liver and the total viability of protoscoleces within four replications was estimated as a mean and standard deviation (143±7.87),this result is similar to the results that mentioned by other studies [21,24, 28, 29, 31]. Many studies had noticed that the fertility of hydatid cyst in infested sheep is higher and more than that present in human and in other intermediate hosts [23, 28, 31]. Thus and accordingly, we have employed the hydatid cysts from infested sheep for completion the *in vitro* experiments of study.

3.2 Estimating the viability of protoscolices *in vitro* study

The obtained results of the present study showed that the percentage of viability of protoscoleces was ranged between 93.7 to 95.3 .The examined hydatid cyst here was sampled from infested sheep's liver and the total viability of protoscoleces within four replications was estimated as a mean and standard deviation = 143±7.87 (Table:2).

Table 2: Counting of viable percentage of protoscoleces in 40 µl within four replications.

Counts of protoscoleces	Replications (R)				∑ Sum.	Mean ± Standard deviation (X±SD)
	R1	R2	R3	R4		
Total number	155	160	150	140	605	151.25±8.53
Viable number	147	150	143	132	572	143±7.87
Percentage of viability (%)	94.8	93.7	95.3	94.2	94.5	

3.3 Effect of Praziquantel drug as protoscolicidal agent *in vitro* study

The obtained results showed that the PZQ drug reduced the viability of protoscoleces during seven sequenced days post incubation. Its effects were marked with increasing its concentration and period of incubation. Drug effects were more influence in both concentrations of 450 µg/ml from day 4 and 600 µg/ml from day 3 post incubation respectively (Table3 and Fig. 6).

Table 3: Effect of praziquantel drug, as scolicidal agent, on the viability of protoscoleces in *vitro*

Incubation in days / Conc. (µg/ml)	Percentage of protoscoleces viability (%)							
	D0	D1	D2	D3	D4	D5	D6	D7
150	94	90	81	73	52	34	12	0.0
300	94	85	77	60	31	11	0.0	0.0
450	94	79	48	26	0.0	0.0	0.0	0.0
600	94	74	35	0.0	0.0	0.0	0.0	0.0
Control Untreated	94	93	91	89	90	86	84	82

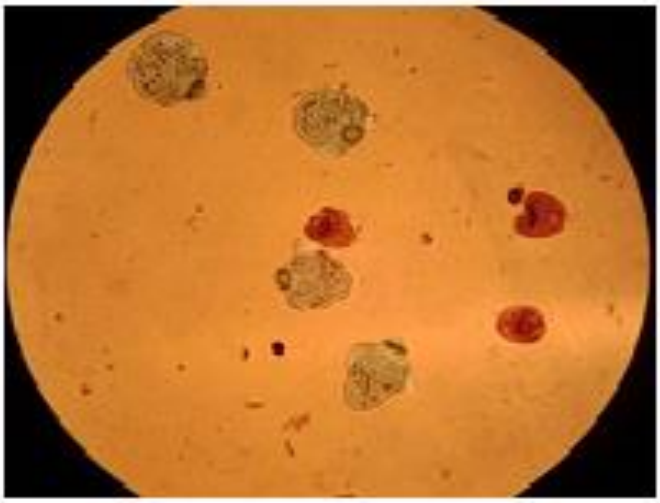

Figure6: Parziquantel drug treated protoscolces *in vitro* conditions yielded in greenish-viable & reddish-deal protoscoleces viewed under 10x microscopic field

The results of present study are in consistent with the results of other studies [24, 32]. Furthermore, some studies noted the PZQ as a drug of choice with marked effects against both juvenile and adult echinococcus parasites [24, 33]. PZQ is a synthetic heterocyclic broad-spectrum anthelminthic agent effective against parasitic Schistosome species as well as most other trematodes and adult cestodes. Whereas it is very effective against adult tapeworms, as well as against *E. granulosus* protoscoleces in vitro, in vivo studies on *E. granulosus* metacestode infections have been inconclusive [34]. Recently, it was suggested that PZQ exerts a substantial effect against metacestodes and protoscoleces when applied in combination with albendazole. Jelowdar (2017), has mentioned that the PZQ chemoprophylaxis treatment reduced the wet weight and size of developed cysts of *E. granulosus* 79%. PZQ is known to have a disruptive effect on the tegument of both the adult and protoscolex stage of *E. granulosus*. Many studies, had noticed PZQ as an isoquinoline that is active *in vitro* against protoscoleces of *E. granulosus* at concentrations as low as 20 ρg 1-1 [24], and in only 3 days [32]. It also has anti hydatid activity in animal models [21, 35] and reported both animal experiments and clinical trials with PZQ for the treatment of abdominal hydatidosis due to *E. granulosus*. One hundred and one CHD patients were treated with PZQ at a dose of 25 mg kg-' day-1 for 10 days resulting in significant efficacy as assessed by histopathological examination and clinical follow-up [21]. A combination of albendazole and PZQ was no more effective than either agent alone. For human hydatid disease, 14 cases were treated by surgery and PZQ (25-50 mg kg-' day-' for 20-30 days) for 2-3 courses [33, 36]. Overall these data, listed above, we might explain why such

differences existed in the present study when compared with those results recorded in other studies. Hence, such resulted differences might be due to the following items: the difference in the concentration of the PZQ drug used in each experimental study, dictated length of incubation period, type with efficiency of the preservative media , the nature of the study conditions (in vitro or in vivo), the number of the samples used, the origin of hydatid cysts (from human being, sheep, cows, horses , etc.) , the route of drug administration, the strain of the studied *E. granulosus* parasite, the developmental stage of the parasite, the skillful of the researcher, or may be related to other reasons undetermined yet.

3.4 Effect of methanolic extract of *Citrullus colocynthis* on the viability of protoscoleces *in vitro* study

The present study showed that the methanolic extract of *C. colocynthis* reduced the viability of protoscoleces during eight sequenced days post incubation from 96% to zero%, respectively (Table:4 , Fig. 7). These effects increased with increasing the concentration and incubation period. The effects were marked highly in the concentration of 300 µg/ml from day 3 post incubation, with viability of incubated protoscoleces reached to zero.

Table 4: Effect of methanolic extract of *Citrullus colocynthis* (fruits plus seeds) on the viability of protoscoleces *in vitro*.

Incubation in day / Conc.(µg/ml)	Percentage of protoscoleces viability (%)								
	D0	D1	D2	D3	D4	D5	D6	D7	D8
75	96	88	76	64	49	36	18	5.0	0.0
150	96	80	66	51	32	10	0.0	0.0	0.0
225	96	72	58	30	8.0	0.0	0.0	0.0	0.0
300	96	60	29	0.0	0.0	0.0	0.0	0.0	0.0
Control untreated	96	94	91	90	88	88	86	84	83

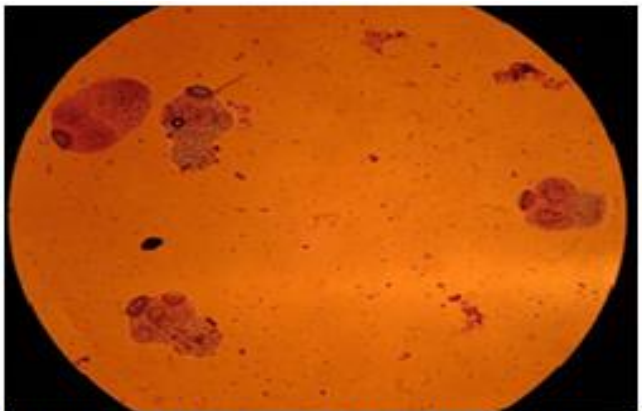

Figure7: Prodoscoleces treated by methanolic extract of C colocynthis in vitro conditions viewed under 10x of microscopic field

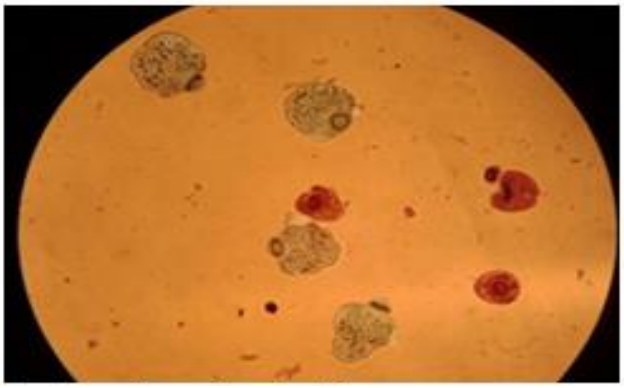

Figure8: Prodoscoleces treated by aquesus extract of C colocynthis in vitro conditions viewed under 10x of microscopic field

The result of the present study is in agreement with the results of other studies [16, 26]. As a comparison with that of control group, all the concentrations used in the present study were exhibited the protoscolicidal activity, the reducing and/or stopping protoscoleces viability, but in different degrees and at different periods too. Indeed, there are many studies that have analyzed the methanolic extract of the *C. colocynthis* compositionally and found it to be composed of various biochemical and reactive compounds such as: citrullol BB, elaterin, elatericin B , dihydro-elatericin B , colocynthin , colocynthidin , and other glycosides , terpins , saponines , lectins , and others [18 , 21]. Interestingly, the *C. colocynthis* lies in some chemical substances that produce a definite physiologic action on the human body. The most important of these bioactive compounds of the plant are alkaloids, flavonoids, glycosides, terpenoids, tannins and phenolic compounds [15]. Studies have shown the significance of oxidative stress, mitochondrial dysfunction and free radicals in aging and in pathogenesis of many diseases [11, 37]. Thus, we might keep in our minds that the obtained results can be considered as a result of various actions

of these methanol extracted-plant-compounds on the parasites structurally, physiology, and evenly on the parasites viability too. Baloch(2013), has mentioned that the methanolic extract of *C. colocynthis* fruit is highly effective in the complete inhibition of the growth of the proastigotes stage of the *Leishmania major* at a concentration of 500 µg/ml. Ullah (2013) studied the effect of methanolic extract of *C. colocynthis* fruit at 100 mg/ml against *Haemonchus contortus in vitro*. All worms were found to be dead after 4 hours of exposure to the extract. [11,12] studied the effect of the alcohol extract of the *C. colocynthis* fruit in the in the adult worms of *Orthocoelium scoliocoelium in vitro*, and found that the extract with a concentration of 40 mg / ml was effective in loss of movement and paralysis after 5 hours of exposure to the extract. Hussein(2019), was mentioned that the methanolic extracts of the fruits of the *C. colocynthis* was very effective in the destruction of protoscoleces, and that the percentage of mortality increased with increasing concentration and exposure time of the extracts. At 4 mg / ml, the mortality rate was 35.6% after 30 minutes of exposure to the extracts, while the mortality rate was 37.7%, after 120 minutes of exposure to the extracts and at 16 mg / ml, the mortality rate was 83.3% after 30 minutes of exposure to the extracts, while the mortality rate was 100% after 120 minutes of exposure to the extracts. The mortality rate of the protoscoleces treated by methanolic extracts of the fruits of the *C. colocynthis* 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 protoscoleces. Then this leads to the destruction of the cell wall and its proteins and fats till the protoscoleces [11, 32]. The mortality rate of the protoscoleces can also be attributed to the tannins, which have the ability to bind to the proteins inside the organism, preventing their degradation, which in turn leads to inhibiting the metabolism of nitrogen and amino acids that are essential to the survival of the organism, it may also be attributed to the fact that the tannins destroy the cellular membrane of the organism through the effect on the fat and proteins in it and then lose parasite ability to grow, and may penetrate the cell membrane and block the active substances of some enzymes inside the cell, which may be necessary for the growth of the parasite and proliferation [2, 12]. 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. Naguleswaran (2006), has mentioned that the Phenols make the membrane lose its which result in passing of various toxic substances without regulating and this leads to the death of parasite.

3.5. Effect of aqueous extract of *Citrullus colocynthis* on the viability of protoscoleces *in vitro* study

The results of present study showed high decrease in the viability of protoscoleces within four concentration of aqueous extract. In the minimum concentration used (75µg/ml), the viability of protoscoleces (96%) was reduced to reach zero% at day eight post incubation, whereas in the maximum concentration used (300 µg/ml), the viability reached to zero% after four days of incubation (Table 5 and Fig. 8).

Table 5: Effect of aqueous extract of *citrullus colocynthis* (fruits plus seeds) on the viability of protoscoleces *in vitro*.

Incubation /day / Conc.(µg/ml)	Percentage of protoscoleces viability (%)								
	D0	D1	D2	D3	D4	D5	D6	D7	D8
75	96	91	79	66	52	38	21	10	0.0
150	96	84	70	54	38	20	6.0	0.0	0.0
225	96	73	60	33	26	5.0	0.0	0.0	0.0
300	96	66	34	10	0.0	0.0	0.0	0.0	0.0
Control untreated	96	94	92	91	8	85	83	81	80

The results of present study showed dramatic decrease in the viability of protoscoleces within four concentration of aqueous fruit extract. In the minimum concentration used (75µg/ml), the viability of protoscoleces (96%) is reduced to reach zero% at day eight post incubation, whereas in the maximum concentration used (300 µg/ml), the viability reached to zero% after four days of incubation. The present findings are in agreement with those findings reported by other studies [38, 39]. The fruit aqueous extract of *C. colocynthis* is characterized to have many important substances with vital roles against numerous microorganisms and diseases, as in hydatidosis [10]. As it is well known, that the plant methanolic extract is always being characterized with high inhibitory effects against targeted parasites than that exhibited by

aqueous ones [10, 11]. Interestingly, we have obtained and confirmed such fact through the present study. So that, when we return back to the obtained results (tables 6 , 7) and inspect it well and make comparison between both types of extract and in relation to the same concentration used in, we will be assured that the methanolic extract is the best for use than that of aqueous ones [11, 40].

3.6 sdy:

The results of the present study showed that the maximum effect was to 600 µg/ml that kill all protoscoleces in 6 days after treatment, while the lowest effect was to 100 µg/ml of the methanolic extract that decreased the viability to zero % in 8 days after treatment, and the rest of extract concentrations effects were between these two limits times(Table:6 , Fig.9).While the protoscoleces still live in control group in KRS+HCF in ratio 4:1 over than 16 days of incubation (see Fig. 10).

Table 6: Effect of methanolic extract of *Eucalyptus globulus* leaves on the viability of protoscoleces *in vitro*.

Incubation /day / Conc.(µg/ml)	Percentage of protoscoleces viability (%)								
	D0	D1	D2	D3	D4	D5	D6	D7	D8
100	95	91	86	75	60	48	30	10	0.0
200	95	85	78	70	58	40	17	0.0	0.0
400	95	77	68	57	42	28	9.0	0.0	0.0
600	95	71	59	47	29	8.0	0.0	0.0	0.0
Control untreated	95	93	90	90	88	87	87	81	80

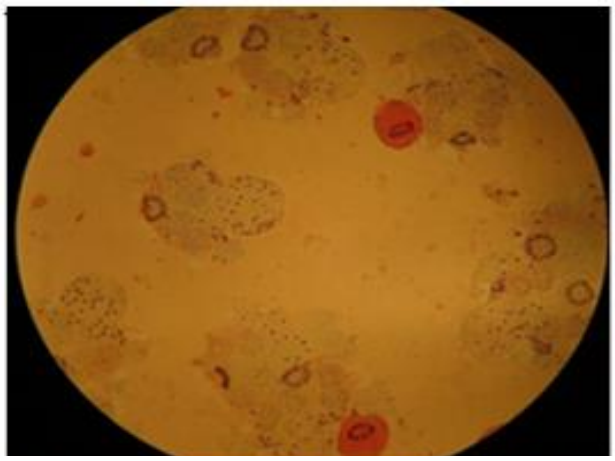

Figure9: Prodoscoleces treated by methanolic extract of Eglobulus in vitro conditions viewed under 40x of microscopic field

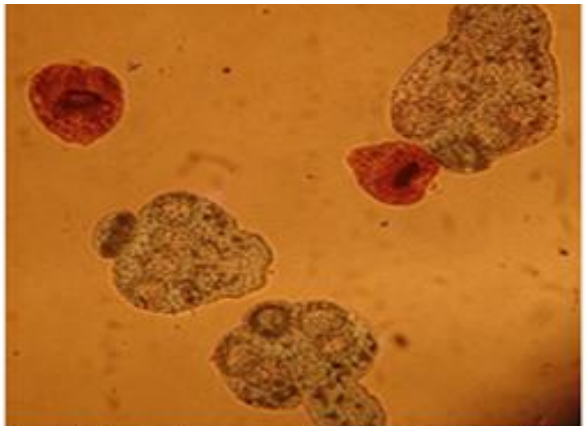

Figure10: Untreated Prodoscoleces kept in preservative medium (Kreps-ringer solution) in vitro conditions viewed under 10x of microscopic field

The results of the present study showed that all concentrations of leaves methanolic extracts of *E. globulus* have an antiparasitic activity against the protoscoleces viability, the greater effect was to 600 µg/ml that kill all protoscoleces in 6 days after treatment, while the lowest effect was to 100 µg/ml as decreased the viability to zero % in 8 days after treatment, other extract concentrations effects were between these two limit times. While the protoscoleces still live in control group in KRS+HCF in ratio 4:1 over than 16 days of incubation. These effects were compared with those of other studies, a commonly used inhibition for protoscoleces *in vitro*, [25, 40]. However, the methanolic extract of *E. globulus* demonstrated a marked antiparasitic effect against the viability of protoscoleces [39] reported that leaves contain different bioactive compounds which cause a variety of medicinal effects. The explanation for such obtained results might be due to the important role of these extracts in the breakdown of the biological activities of protoscoleces by interference with its metabolism, and may favor special target sites such as

inhibitors of protein and DNA synthesis or within the cytoplasmic components such as lactam-antibiotics [38, 41, 42].

3.7 Effect of aqueous extract of *Eucalyptus globulus* on the viability of protoscoleces *in vitro* study

The results of present study showed that all concentrations of leaves aqueous extracts of *E. globulus* have an antiparasitic activity against the protoscoleces viability, the greater effect was to 600 µg/ml that kill all protoscoleces in 6 days after treatment, while the lowest effect was to 100 µg/ml that decreased the viability to zero % in 8 days after treatment (Table: 7, Fig. 11).

Table 7: Effect of aqueous extract of *Eucalyptus globulus* leaves on the viability of protoscoleces ***in vitro.***

Incubation /day / Conc.(age/ml)	Percentage of protoscoleces viability (%)								
	D0	D1	D2	D3	D4	D5	D6	D7	D8
100	95	93	88	77	62	50	33	13	0.0
200	95	85	79	72	61	46	30	8.0	0.0
400	95	80	72	60	44	27	18	0.0	0.0
600	95	74	60	51	33	12	0.0	0.0	0.0
Control untreated	95	93	91	90	89	87	86	84	80

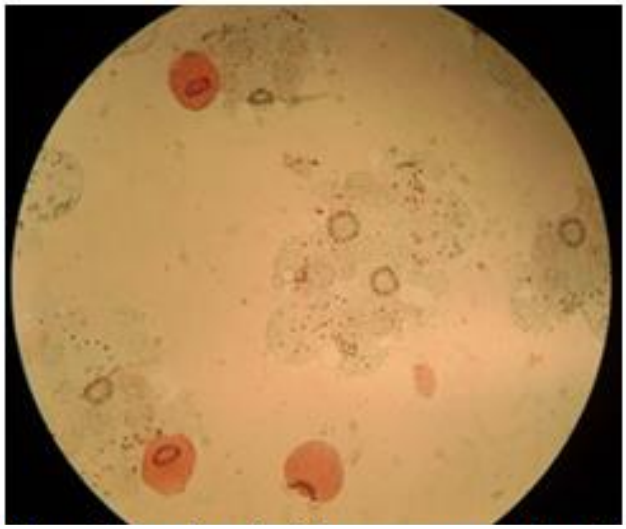

Figure11: Prodoscoleces treated by aqueous extract of E. globulus in vitro conditions viewed under 40x of microscopic field

Interestingly, the result of this experiment (leaves aqueous extract), is similar to that of methanolic extract but with slight differences . It is consistent with the results that reported by other studies [10, 34, 40]. Virtually, many studies showed the chemical composition of the plant *E. globulus*, and it was found that the plant contains several important compounds such as eucalyptol (cineol), terpineol, sequiterpene, alcohol, aliphatic aldehyde, isoamyl alcohol, ethanol and terpenes [11, 20, 25, 42]. As it is well known, not all chemical substances of plants could be dissolved and extracted at the same level, and not all dissolving solutions(alcoholic and watery) function to dissolve at the same level too. So that, these differences mentioned above can partially explain why such differences existed between both alcoholic and aqueous extractions. Moazeni,(2019) have mentioned that the 0.5% oil of *E. globulus* demonstrated 97.38% and 100% scolicidal activity after 1 and 3 min respectively. The mean protoscolicidal power of 1% *E. globulus*, was 100% after one minute. Who, mentioned that the leave extracts of *E. globulus* demonstrated high scolicidal power in a short period of time. Hence, this herbal product could be considered as a potent natural scolicidal agent that could be used before and during surgery of hydatid disease.

4. Conclusions

Praziquantel drug reduced the survival of protoscoleces during seven sequenced days post incubation. Drug effects show more influence in both concentrations of 450 µg/ml from day 4 and 600 µg/ml from day 3 post incubation, respectively. Alcoholic and aqueous crude extracts of *C. colocynthis* have activity against the protoscoleces viability, but the greater effect was to 300

µg/ml, that kill all protoscoleces in day3 and day 4 post incubation respectively, while the lowest effect was to 75µg/ml /ml that decreased the viability to zero % at day eight post incubation. All concentrations of leaves methanolic and aqueous extracts of *E. globulus* have an antiparasitic activity against the protoscoleces viability, but the greater effect was to 600 µg/ml that kill all protoscoleces in 6 days after treatment. The protoscoleces still live in control group in KRS+HCF in ratio 4:1 over than 16 days of incubation.

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في المختبر، تأثير عقار البرازكوانتيل ومستخلصات الخام الكحولي والمائي لنباتي الحنظل واليوكالبتوز على حيوية الرؤيسات الأولية لطفيلي المشوكات الحبيبية

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المستخلص

ان الاستشفاء من الامراض المختلفة بواسطة استخدام المستخلصات النباتية هو معمول به مندو قرون عديدة، وحيث ان العراق يمتاز بوفرة النباتات فان إمكانية الحصول على عوامل جديده ضد الميكروبات لايزال مستمر وبكثرة. وعليه فالعمل الحالي يبين التأثيرات لمستخلصي الخام الكحولي والمائي لنباتي الحنظل واليوكالبتوز، وكذلك عقار البرازكوانتيل على حيوية الرؤيسات الأولية لطفيلي الاكياس العدريه في المختبر. تم الحصول على الرؤيسات الأولية من اكباد الأغنام المصابة، وتم تحديد حركة الرؤيسات الأولية من خلال حركتها وفعالية الخلايا المتقدة وطريقة التصبيغ باستخدام صبغة محلول الايوسين 1%. وقد اجري حساب العدد الحي للرؤيسات الأولية ووزع في انابيب اختبار (أربعة انابيب لكل معاملة للتراكيز المختلفة مع وضع مجموعة سيطرة). تراوحت النسبة المؤية لحيوية الرؤيسات الأولية من 93،7 الى 95،3 . لوحض انخفاض حيوية الرؤيسات باستخدام عقار البرازكوانتيل خلال سبعة أيام متتالية بعد الحضانة. وجد اشد تأثير للعقار في التركيزين 450 µg/ml في اليوم الرابع و600 µg/ml في اليوم الثالث بعد الحضانة، على التوالي. انخفضت نسبة الحيوية للرؤيسات بواسطة تاثير المستخلص الكحولي لنبات الحنظل ضمن الثمانية أيام المتسلسلة بعد الحضانة من 96% الى 0.00%. سجل اقوى تاثير تثبيطي باستخدام التركيز 300 µg/ml في اليوم الثالث بعد الحضانة. لوحض اعلى انخفاض في حيوية الرؤيسات ضمن الأربعة تراكيز المستخدمة للمستخلص المائي للأوراق. انخفضت حيوية الرؤيسات بعد الحضانة (96%) لتصل الى الصفر في اليوم الثامن باستخدام اقل تركيز(75µg/ml)، بينما في اعلى تركيز مستخدم (300 µg/ml) قلت الحيوية الى الصفر بعد أربعة أيام. أضهرت جميع التراكيز المستخدمه من المستخلص الكحولي والمائي لأوراق نبات اليوكالبتوز فعل تثبيطي لحيوية الروؤيسات الأولية للطفيلي، حيث وجد اشد تاثير قاتل للروؤيسات الأولية في التركيز 600 µg/ml في اليوم السادس بعد المعاملة.