WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 8, 157-179. Research Article ISSN 2277– 7105

EFFICACY OF *CYPERUS ROTUNDUS* RHIZOMES-TUBERS EXTRACTS AGAINST PROTOSCOLECES OF *ECHINOCOCCUS GRANULOSUS*

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Article Received on
06 June 2017,
Revised on 26 June 2017,
Accepted on 16 July 2017
DOI: 10.20959/wjpr20178-9053

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ABSTRACT

Background: Cystic echinococcosis is a zoonotic disease with increasing public health concern. Dissemination of protoscoleces –rich fluid during surgery is a major cause of recurrence, so injection of scolicidal agent into hydatid cyst to reduce the risk of spillage of viable protoscoleces is an integral part of the surgical operation. **Objectives:** To determine the in vitro protoscolicidal effect of *Cyperus rotundus* rhizomes-tubers extracts against *Echinococcus granulosus*. **Materials and methods:** *C.rotundus* rhizomes-tubers were collected from Bardbar village, Erbil, Kurdistan, Iraq. Freshly isolated protoscoleces were incubated with (500, 100,20,4 mg/ml) of aqueous and alcoholic extracts. Protoscoleces were tested with 0.1%eosin stain; also treated protoscoleces were processed for scanning electron microscopy compared to control one. **Results**: The effect on protoscoleces was investigated by eosin staining, light microscopy, and electron microscopy. The maximum effect was found in concentration of 500 mg/ ml of both extracts, mortality reached 100% after 2 hrs. post- incubation. The effect was seemed to be dose and time dependent with little differences between the two types of extracts. Results of viability tests were consistent with protoscoleces alteration and damage observed under both light and scanning electron microscopy. The first site of damage was the tegument of the parasite and the alteration included rostellar disorganization, loss of hooks and calcareous corpuscles, loss of turgidity of the outer membrane, and formation of the blebs on the tegument. **Conclusion:** The data recorded in this article demonstrate for the first time a clear in vitro scolicidal effect of *C.rotundus* rhizomes-tubers extracts against *E.granulosus* protoscoleces, however, in vivo effect of these extracts need to be examined.

KEYWORDS: *Cyperus rotundus*, Protoscolicidal, *Echinococcus granulosus*, Ultrastructure.

INTRODUCTION

Cystic echinococcosis is a zoonotic infection caused by the metacestode stage of various strains of *Echinococcus granulosus*[1] and continues to be a substantial cause of morbidity and mortality in many parts of the world. The parasite life cycle involve dogs and other canids as definitive hosts for the intestinal tapeworm, as well as domestic and wild ungulates as intermediate hosts for the tissue invading larval stage.[2]

Unilocular hydatids develop as single cysts in the liver secondarily in the lungs and other locations. The cysts are filled with clear fluid and may contain smaller "daughter" cysts. Within the parent and daughter cysts are brood capsules and protoscoleces, which can number in thousands.[3]

The protoscolex plays a key role in the life cycle of the parasite being the only infective form to canine and other carnivores.[4] In the definitive host the adult worms attach to the intestinal epithelium and released eggs which are shed into environment with feces which represent the infective stage for humans and others suitable intermediate hosts.[5]

Several studies classified the disease as with an increasing public health concern and that can be regarded as emerging or re-emerging disease in many countries.[2,6] Echinococcal infection has been designated by the WHO as a Neglected Tropical Disease because it principally affects economically poor populations in medically underserviced settings

Moreover the disease causes significant morbidity and considerable socioeconomic impact in highly endemic regions which lead to economic losses derived from decreased productivity and viscera condemnation in livestock species[7,8] Torgerson[9] referred that livestock cystic echinococcosis is wide spread through many regions of Middle East, including Iraq.

*E.*granulosus has a worldwide geographical distribution in Iraq including Kurdistan the disease is considered as hyper endemic and one of the countries' most important parasitic disease with significant socio-economic effect ,since both human and their livestock are infected.[10,11] Also Sajjadi[12] mentioned that the situation of echinococcosis is highly prevalent in Middle East and Arabic North Africa including Iraq.

Surgical management of echinococcal cystic disease was the primary therapeutic modality

and continues to be the gold standard treatment of large complex cysts.[13] Despite progress in surgical technique, secondary echinococcosis owing to spillage of viable parasite material during the intervention may occur. Prevalence of long-term recurrence is in range of 2%-25%.[14] Also, anaphylactic reaction represent further risk on rare occasions, so protoscolicidal substances are often applied, since there is a risk of spillage of cyst fluid.[15]

Puncture, aspiration, injection, re-aspiration(PAIR) is a technique that was introduced in the mid-eighties and includes percutaneous puncture of the cyst under ultrasonic guidance, aspiration of substantial amounts of cyst fluid, injection of protoscolicidal substance (eg.95% ethanol)and re-aspiration of fluid after 5-20 min.[16]

Treatment of cystic echinococcosis has been developed after a series of animal studies, both albendazole and mebendazole are considered to be equally effective[5] but other researcher concluded that albendazole is more effective than mebendazole in the treatment of hydatid cyst caused by *E.granulosus* and that both the intensity and the frequency of the usually mild adverse effects are comparable which including raised transaminases, abdominal pain, headache, vertigo, urticaria, jaundice.[17]

Furthermore, Horton[18] mentioned that albendazole has a relatively benign safety profile with three principle areas affected (liver functions, bone marrow and hair). Horton recorded that liver function abnormalities occurred in about20% also other study confirmed that 10% of patients develop rises in transaminase and thus due to drug parasite interaction[20] other adverse effect included haematuria, leukopenia and it is teratogenic in rats and should not be given to pregnant women.[21]

Some studies concluded that 20%-40% of cases with hydatid cyst do not respond favorably to chemotherapy with these recommended drug[22] so the main concern in chemotherapeutic treatment of cystic echinococcosis remains unsolved since no currently available drug are totally effective against hydatid cyst and without adverse side effect.[23] Perez-serrono *etal.*,[24] recorded that the protoscolicidal action of albendazole and albendazole sulphoxide is very slow and this idea agree with Kuster etal[25] whom mentioned that the current chemotherapeutic treatment by albendazole and mebendazole is high cost, lifelong consumption parasitostatic rather than parasiticidal with high recurrence rates after treatment and an elevated risk of adverse effects.

Cyperus rotundus Linn. (Family - *Cyperaceae*), commonly known as Al Saad in Iraq is a

common perennial weed with slender, scaly creeping rhizomes, bulbous at the base and arising singly from the tubers which are about 1-3 cm long. The tubers are externally blackish in colour and reddish white inside, with a characteristic odour.[26]

Despite the allelopathic effects of the plant, the tubers have been used as an occasional nutrient source and have a long history in traditional medicine[27] and rhizomes consider as edible parts of the plant.[28]

Scientists have reported that rhizomes and tubers of *C. rotundus* possess antidiarrheal, antioxidant, anti-inflammatory, anti-mutagenic, antiperiodic, anticonvulsant, anti-saturative, anti-pyretic, antifungal, antidiabetic, antimalarial, anti lipidemic, anti- bacterial, antiviral, anti-tumour, cardio protective and wound- healing properties.[29]

Recently, Xu *et al*.,[30] revealed the anti-hepatitis B virus as bioactivity of *C.rotundus* rhizomes. In addition to, the work of Zhou *et al*.,[31] whom purified two new cycloartane glycosides from rhizomes of *C. rotundus* and evaluated their anti-depressant activity in mice models.

Moreover, Johari *et al*.,[32] concluded that *C. rotundus* can be considered as novel, safe, effective and economical alternative for the treatment of inflammatory bowel disease. Other phytochemical study on *C.rotundus* showed that the extracts exhibited antioxidant and an apoptosis inducer.[33]

In addition, the tubers and rhizomes of *C. rotundus* have been used in perfumes, spices and Ayurvedic remedies in Arab countries, Africa, China and India for centuries. Its tubers have long been used as a natural remedy to cure spasms, diarrhoea, dysmenorrhea and menstrual irregularities.[34]

Regarding the phytochemical investigations, Previous studies identified many chemical constituents such as alkaloids, cyperol, flavonoids, fatty oils, furochromones, glycerol, linolenic acid, myristicacid, nootkatone, starch, saponins, sesqui- terpenes, sitosterol, stearic acid, terpenoids, polyphenol and novel sesquiterpenoids in the tubers and rhizomes of *C. rotundus*[28,29] These chemicals are responsible for several therapeutic, pesticidal, fungicidal, and insecticidal properties of *C.rotundus.*

Locally, *C.rotundus* used in traditional medicine in Erbil-Kurdistan, Iraq as decoction for

flatulence, nausea, vomiting, regulating hormones (prolactin), tonic hypoglycaemic and diuretic.[35] The tuber part of *C. rotundus* is one of the oldest known medicinal plants used for the treatment of dysmenorrhoeal and menstrual irregularities.[36]

The present study was designed to determine the *in vitro* efficacy of *C. rotundus* rhizomes-tubers extracts against viability of *E.granulosus* protoscoleces and to investigate the morphological alteration that induced by this plants extracts using light and scanning electron microscope.

MATERIALS AND METHODS

2-1- Experimental design

The present research was started with the collection of Rhizomes-tubers of *C.rotundus*, then aqueous and alcoholic extracts were prepared, followed by collection of protoscoleces from sheep infected with *E. granulosus* hydatid cyst which divided into two groups treated and non-treated group.

Then scolicidal effect of both extracts at different concentrations and for various exposure times was assessed by light and scanning electron microscopy and the viability percentage was estimated by eosin exclusion test as vital stain. Each experiment was achieved in duplicate.

2-2- Plant materials

C.rotundus rhizomes-tubers were collected from Bardbar village, Degala, Erbil, Kurdistan, Iraq. The botanical identification was carried out by M. Shorish Gorony (Biology Department, Science Faculty, Soran University). The plant was dried in shade and grounded into fine powder by electrical grinder.

Two 50gm of rhizome powder where extracted separately, one of them with 250 ml of 70% ethanol and the other with 250ml distilled water, each mixture was agitated for 2hrs. with magnetic stirring, then kept at rest form 24hrs. the extract were filtered and dried and weighted to determine the concentration for preparing the stock solution which was 500 mg/ml.

2-3- Collection of protoscoleces and viability test

Hydatid cyst of *E.granulosus* from naturally infected sheep livers were obtained from municipal abattoir of Soran city, Erbil.

The hydatid fluids were aspirated aseptically by 50 ml syringes after sterilizing the outer surfaces of the cysts with 70% ethanol, then the cysts were cut open to separate the remaining protoscoleces from the cyst tissues[37], after that the samples where left for 15 minutes to settle down the protoscoleces. Dead protoscoleces were eliminated by repeated washing with hydatid fluid, followed by discarding the supernatant after 5mins. Then the yielded protoscoleces were washed with normal saline and hydatid fluid.

Viability of protoscoleces was assessed by muscular movements, morphological perfectness of the whole body, motility of flame cells and ease of 0.1% aqueous eosin[38] colorless protoscoleces were considered as viable whereas the red stained protoscoleces were considered as dead. When the viability percentage of protoscoleces in the sediment was more than 97% the sample was considered to be appropriate for further experiments directly. The percentage of viability was calculated by counting 100 protoscoleces and estimated as the percentage of viable protoscoleces to total protoscoleces.

2-4- *In vitro* scolicidal effect of *C.rotundus* rhizomes –tubers extracts

To investigate the scolicidal effects of *C.rotundus* rhizomes extracts against protoscoleces of *E.granulosus*, four serial concentrations of both plant extracts were prepared (500,100,20,4 mg/ml) and tested within different exposure times (15min., 30 min., 1hr., 1.5hr., 2hr., 3hr., 4hr. and 5hr.) aliquot of 0.5ml of protoscoleces solution ($2x10^3$ml) was placed in sterile glass vial, then 0.5ml of various concentration of the extracts was added. The contents were gently mixed and incubated at 37c. at the end of each incubation exposure time the upper phase was carefully removed in order to not disturb the protoscoleces. Then 0.5ml of 0.1% aqueous eosin stain was added and mixed gently, followed by discarding the upper part of solution after 10 min. finally the pellet of protoscoleces was smeared on glass slide and cover with cover slide and examined under light microscope. The percentage of mortality was determined by counting 200 protoscoleces and estimated as the percentage of dead protoscoleces to total number. Non treated protoscoleces which incubated in hydatid fluid and normal saline (2:1) for each time intervals were considered as control group.[39] The viability was assessed by 0.1% eosin staining (0.1gm in 100ml distilled water) under light microscope.

2-5- Light microscopy examination of protoscoleces

Aliquot of 100 microliter of pooled protoscoleces was transferred over a slide and mixed with same quantity of 0.1% eosin. After ten minutes, it examined, the viable protoscoleces remained colorless while the dead one were stained red. The viability test was carried out from 15 min till 5 hrs. for treated and control protoscoleces.

2-6- Scanning electron microscope examination of protoscoleces

According to previous rsearchers[40-42] samples were fixed with 2.5 glutaraldehyde in 0.1 M sodium cacodylate buffer pH7.4 for 24 hr. at 4 C. Then two washes in the same buffer were made each for 15 min. After that the specimens were dehydrated by sequential concentration of ethanol 30% to 90% followed by a dip in absolute ethanol. Finally the protoscoleces were sputter –coated with gold and inspected on scanning electron microscopy model FEI Quanta 450 in the research center of Soran University.

3. RESULTS

3-1- Morphological effect of *C. rotundus* extracts on *E.granulosus* protoscoleces by light microscopy

The present study revealed that some protoscoleces after incubation with different concentration of both extracts of *C. rotundus* rhizomes –tubers absorbed the eosin stain and colored red which indicated their death (Fig.1C). Tegument is the first site of affected (Fig.1D), most of these affected protoscoleces were invaginated shape and displayed different morphological alterations like tegumental changes and damage (Fig.1F,2B,3B), disorganization of rostellar hooks, loss of hooks and calcareous corpuscles and loss of rostellar cone.(Fig.2,D,A,E,3A).

On the other hand protoscoleces of control group seemed to be colorless, viable, motile and exhibited no morphological changes throughout the experimental period (Fig.1A) with arranged hooks and normal bright calcareous corpuscles, seemed to be morphologically intact.

Initially the effects were detected rapidly in protoscoleces that incubated with highest concentration500 mg/ml of both extract, also for other concentration, the same alteration was observed but later. The result showed that, no vital protoscoleces were detected after 2 hr. post incubation with 500mg/ml compared with the lowest concentration which killed all the protoscoleces after 5 hr. post incubation and for both aqueous and alcoholic extracts (Fig.1E, 2C).

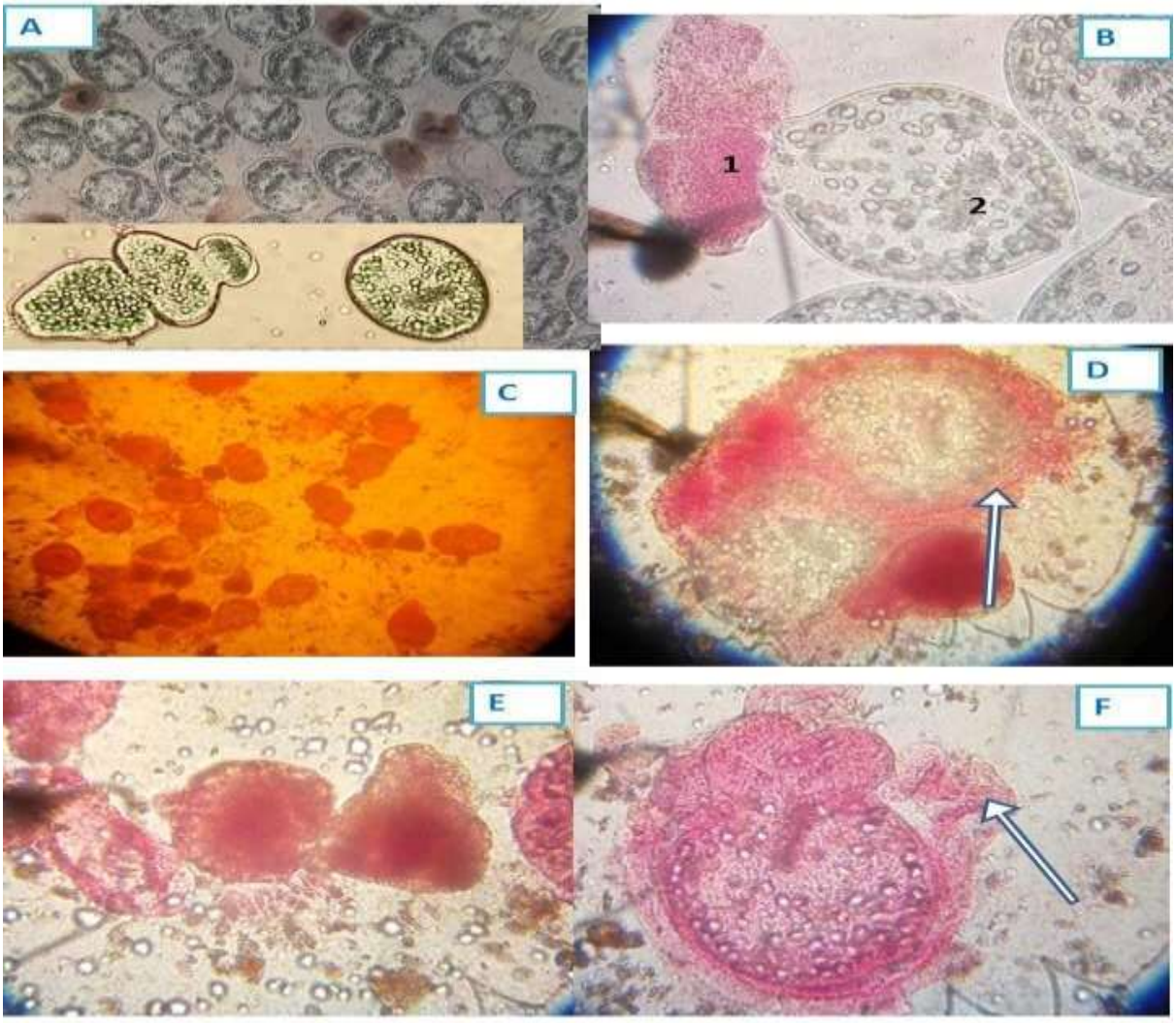

Figure 1. Light microscopy of *E.granulosus* protoscoleces. A –Invaginated and evaginated control protoscoleces. B -Protoscoleces after half hr. of incubation with rhizomes-tubers alcoholic extract, 1-Dead evaginated protoscolex, 2-Live invaginated protoscolex. C-Dead protoscoleces after 1 hr. post incubation with aqueous extract. D - Tegument is the first site of affected (arrow). E- Death and damage of protoscoleces after 2hr. F-Dead protoscolex with tegumental alteration and destruction after 2hr.of treatment.

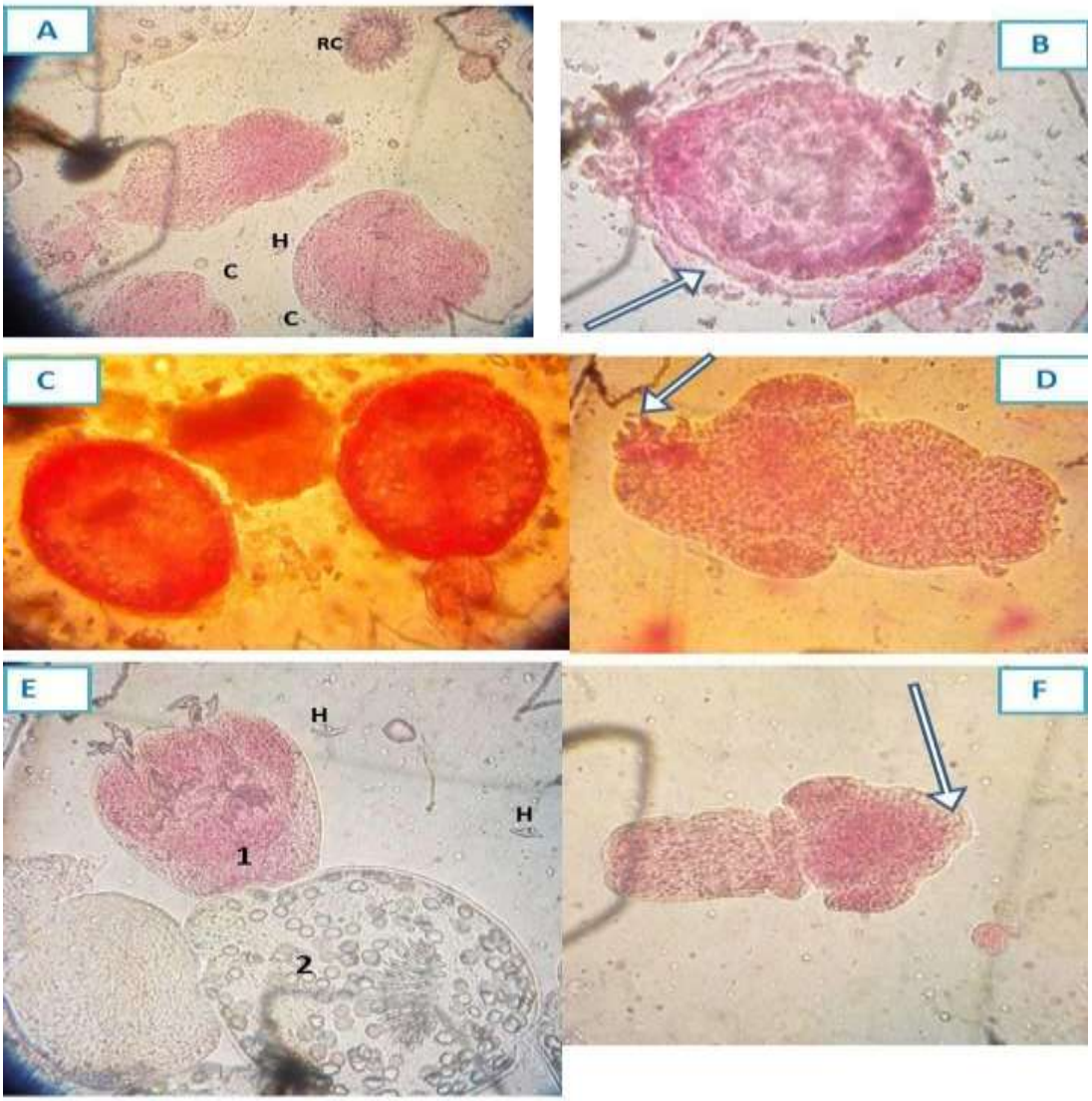

Fig. 2-Light microscopy of protoscoleces treated with rhizomes-tubers extraxts. A -Dead protoscoleces with disorganization of hooks, RC, rostellar cone, H, hook, C, calcareous corpuscle. B- Complete tegument damage (arrow) C-Dead protoscoleces. D - Dead evaginated protoscolex with disorganization of rosteller hooks. E -Loss of hooks in dead protoscolex (1) and free hook(H)compared to live protoscolex (2). F - Dead evaginated protosclex with loss of hooks (arrow).

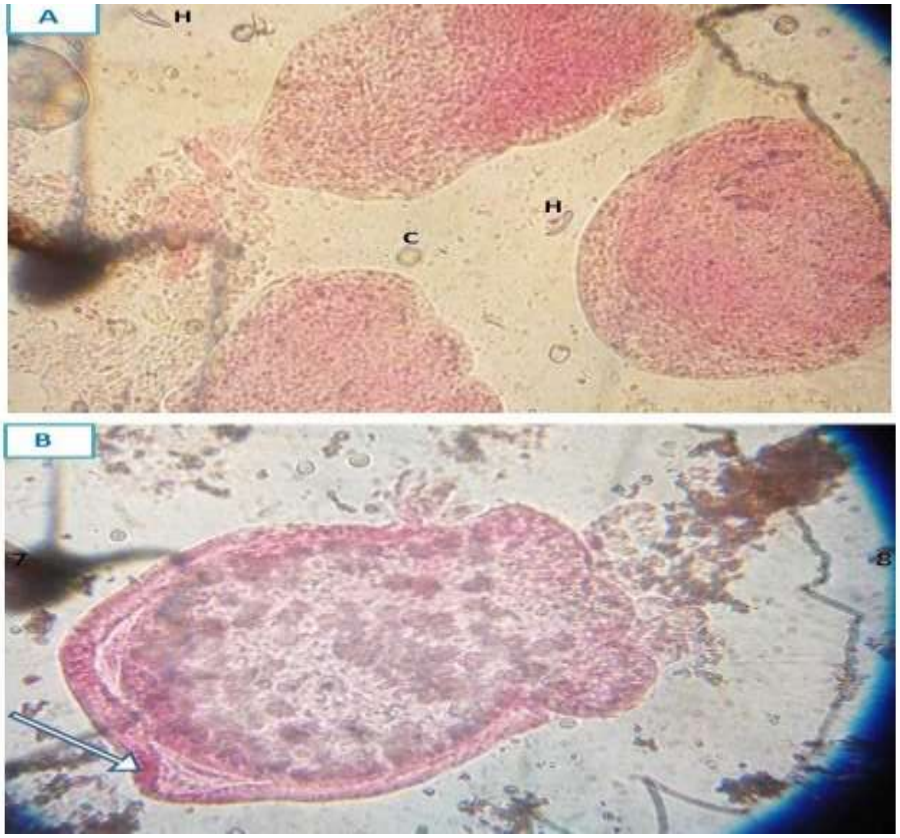

Fig.3. Light microscopy of treated protoscoleces with rhizomes –tubers alcoholic extract after 2hr. post incubation, A -Dead protoscoleces, loss of hooks, Free hook, Calcareous corpuscle. B-Dead protoscolex with altered tegument(arrow) and loss of hooks and integrity.

3-2-Effect of *C.rotundus* rhizomes –tubers extracts on viability of *E.granulosus* protoscoleces

The present results proved that the protoscolicidal effect of both aqueous and alcoholic extracts were dose and time dependent The highest concentration of alcoholic extract 500 mg/ml exhibited significant growth inhibition within 2hr. post incubation in comparition with other concentrations and with control group (Fig.4).

Similarly the highest concentration of aqueous extract caused percentage of mortality as 100% within 2hr. By contrast the lowest concentration was showed low efficiency and killed all the protoscoleces after 5hr.post incubation (Fig.5).

In addition, control group showed no viability decrease and the protoscoleces remained viable during the experiment and for many days after experimental period without any structural damages (Fig.4, 5).

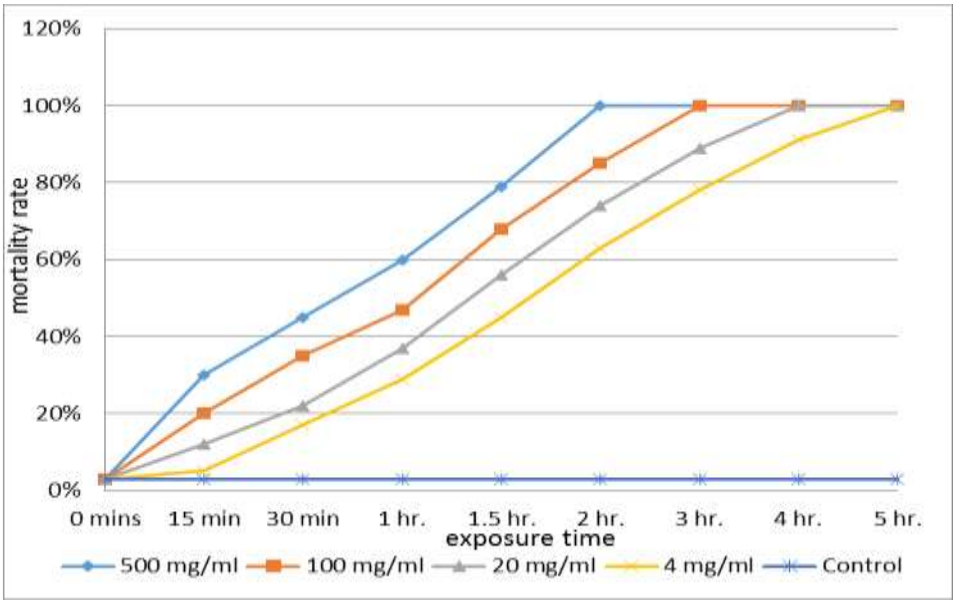

Fig.4- Mortality of *E.granulosus* protoscoleces after exposure to rhizomes-tubers alcoholic extract.

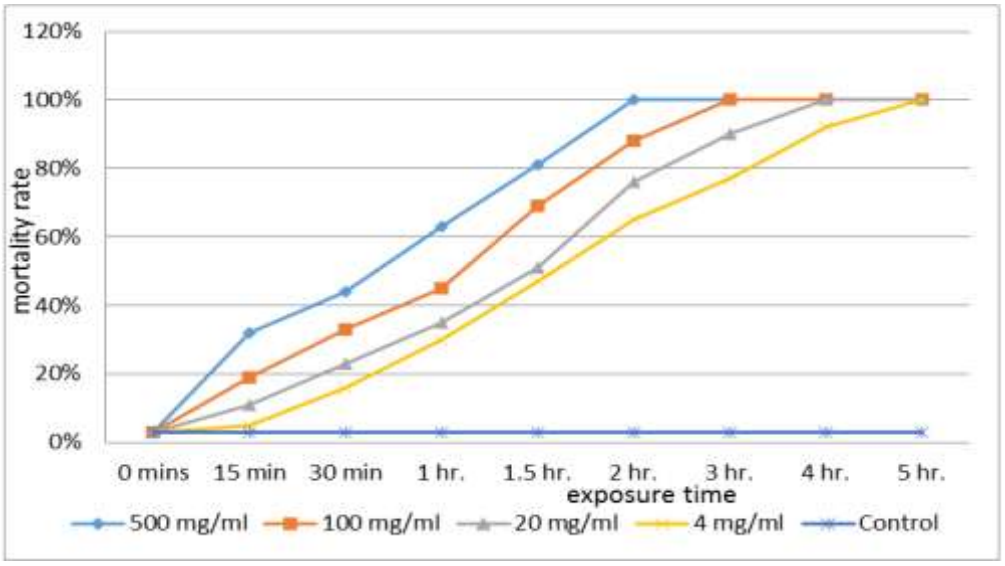

Fig.5- Mortality of *E.granulosus* protoscoleces after exposure to rhizomes-tubers aqueous extract.

3-3-Effect of *C.rotundus* rhizomes-tubers extracts against *E.granulosus* protoscoleces by scanning electron microscopy

The present finding that demonstrated by SEM was consistent with the effect of both extracts on the viability of protoscoleces.

The images of SEM indicated that the control group of protoscoleces showed no morphological or structural changes (Fig.6A, B, C and D). Many treated protoscoleces

exhibited extensive extracts induced damages with shedding of hooks and lost the normal morphology (Fig.6E&F). The tegument of treated protoscoleces with 500gm/ml of both extracts was markedly altered, with tegumental digitiform extensions, loss of the integrity and turgidity of the outer membrane, also several blebs were observed, some protoscoleces showed presence of destruction sites with disorganization of protoscoleces rostellum hooks (Fig.7A, B, C,&D, Fig. 8 A &B).

There was also aggregation and adherence of the treated protoscoleces with each other (Fig.7 C), no differences were detected in the effect of both aqueous and alcoholic extracts upon the treated protoscoleces.

In addition numerous protoscoleces lost their normal morphology and seemed to be completely damaged (Fig.7A,D).

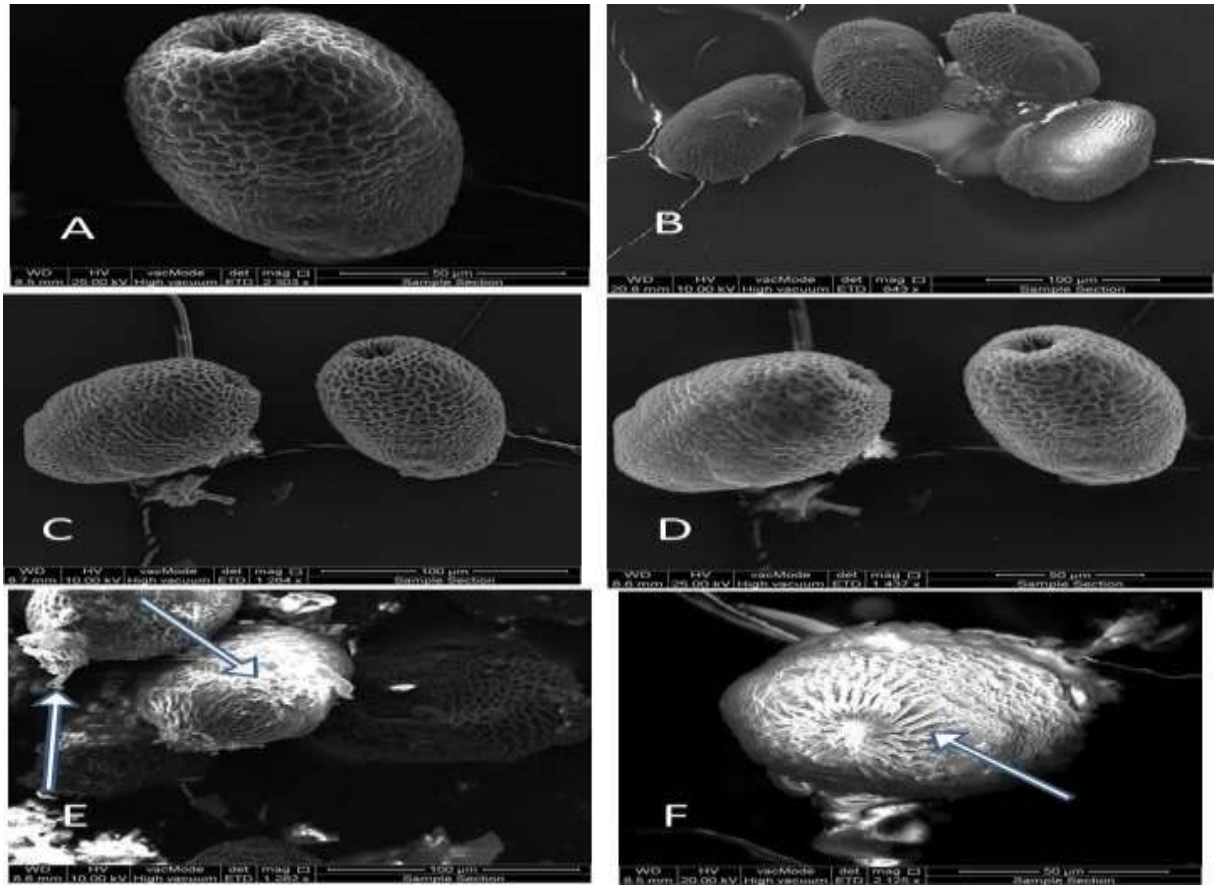

Fig. 6. Scanning electron microscopy of *E.granulosus* protoscoleces, A,B,C&D untreated control protoscoleces, E and F evaginated treated protoscoleces after 2 hr. of incubation with *C.rotundus* extracts. The tegument was markedly altered (arrow in E), with rostellum disorganization (arrow in F).

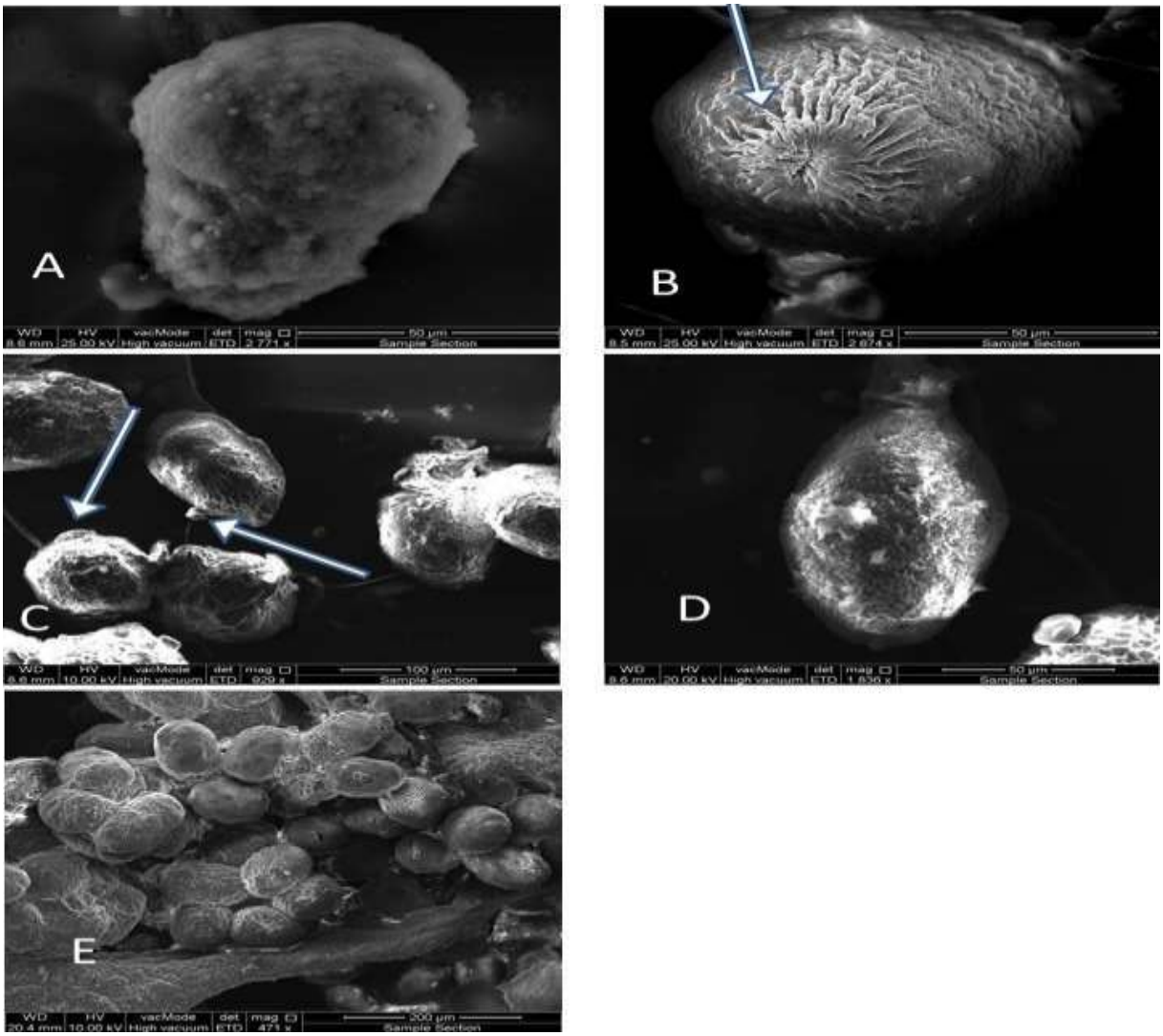

Fig.7. Scanning electron microscopy of *E.granulosus* protoscoleces incubated in vitro with *C.rotundus* extracts A-D. Note the extensive extract induced damage, loss of hooks in image B (arrow), formation of blebs in photo C (arrows) also adherence of protoscoleces together. Altered tegument of treated protoscoleces in A& D. Aggregation and adherence of the protoscoleces together in image E.

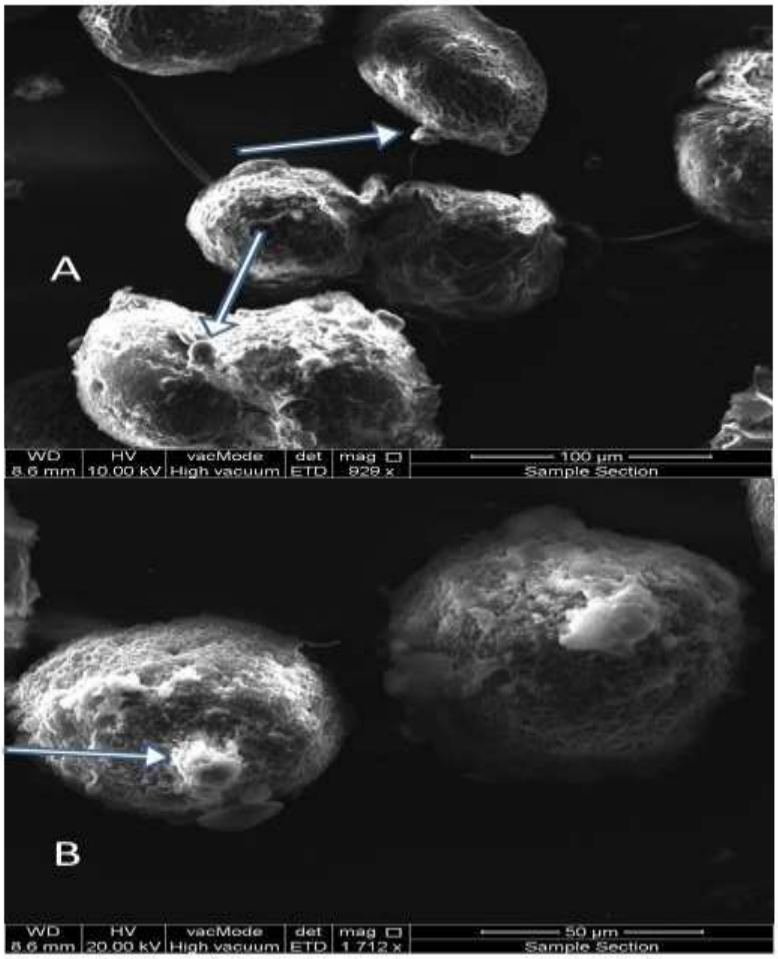

Fig. 8. Scanning electron microscopy of *E. granulosus* protoscoleces incubated with 500 mg/ml of rhizomes-tubers extract of *C.rotundus*. Note the numerous blebs formation as in A (arrows) and contracted protoscolex with shedding of hooks in image B (arrow).

4-DISCUSSION

The best choice of treatment for human hydatidosis is surgery, but spillage of protoscoleces rich infective fluid from cysts during surgical operation is the major cause of its recurrence and multiple secondary echinococcosis in addition to the risk of anaphylactic reaction which known as unique to this disease.[43,44] So the injection of protoscolicidal agents into the hydatid cyst to reduce the risk of dissemination of protoscoleces is an integral part of surgical technique.[45]

It is worthwhile to mention there is no ideal agent that is both effective and safe has been reported.[46] Therefore the development of new scolicidal agents with low side effects and more efficacies is an urgent need for surgeons.[47] Also Adas et al.,[48] concluded previously that creating a newly improved, less harmful and more effective preparation is necessary for

hydatid disease treatment.

This is the first study regarding the *in vitro* efficacy of *C. rotundus* rhizomes- tubers extracts against *E.granulosus* protoscoleces, and also represents the first contribution of ultrastructural data in this aspect.

The present results indicated that *C.rotundus* extracts exhibited protoscolicidal effect which was dose and time dependent and killed all the protoscoleces within 2 hr. post incubation at higher concentration for both of aqueous and alcoholic extracts. This effect related to the chemical compounds of rhizomes tubers which recorded by previous phytochemical studies and disclosed the presence of alkaloids, flavonoids, glycosides starch, steroids, tannins, synthetic resin compounds and plenty of novel sesquiterpenoids.[28,49]

The protoscolicidal effect of *C.rotundus* rhizomes may be related to the alkaloids compound, others workers proved the action of alkaloids in Sophora mooreroffiana seeds which showed efficiency against *E.granulosus* protoscoleces.[50]

Moreover Soumaya *et al.*,[33] showed in phytochemical study of *C.rotundus* extracts the presence of total poly phenol, flavonoids compounds and tannins, also they identified orientin as one among other flavonoids and they considered it has potential role in biological activity of the extracts and it may be influence the antioxidant and apoptotic activity specifically in the alcoholic extract.[51]

In the present study, as far as the extracts were as crude form and probably contain many compounds which may act synergistically, so it is difficult to decide which compound is responsible for the protoscolicidal effect.

Recently in Saudi Arabia, they found that the alcoholic extract of *C.rotundus* rhizomes had cytotoxic effect against three carcinoma cell lines, with antibacterial and antifungal activity.[27] And the ethanolic extract of the whole plant have potent activities against bacteria as well as fungi.[26,29,52]

In previous study in Iraq also Nima *et al.*,[36] proved that the oil of the mentioned plant showed remarkable activity against Gram positive bacteria and composed mainly of cyperol, sesquiterpene and rotundine.

The present study showed that the tegument of the protoscoleces was the primary site of damage as proved by light and scanning electron microscopy when it treated with both extracts of *C. rotundus* rhizomes –tubers.

Tegument is known to be a dynamic cellular structure that plays avital role in the physiology of cestode, being involved in nutrient absorption, defense against enzymatic and immunological attack by the host, in excretion and ionic exchange. In addition large number of important enzymes is anchored in the tegument.[53]

The change in the protoscoleces included tegumental alteration, rostellar disorganization, loss of rostellum hooks, followed by the formation of numerous blebs on the tegument, previously the same finding was observed in the protoscoleces cultured in the presence of various chemical substances Praziquantel and albendazole combination[54], flubendazole[40], thymol[22] nitazoxanide[5], sodium arsenite[55] and carvacrol.[56]

It would appear logical to assume that loss of hooks and formation of blebs are "stress response" brought about in protoscoleces by any harmful condition.[24]

Furthermore, the alteration of the tegument affect the tegumental microtrichea which probably interfere with protoscoleces nutrition, since microtrichea are directly associated with nutrition's absorption.[57]

Also Das *et al*.,[58] mentioned to the presence of glycogen in helminthes which serves as the most important energy reserves, its concentration and metabolism are known to be affected by several drugs given that treated parasites are under stress condition. In addition, stressful condition caused by drug could indicate an additional stimulation of glycolysis.[59]

The ultrastructural study permitted us to examine the induced effect; identical ultrastructural changes were induced in the treated protoscoleces with both extracts in different concentrations.

The present results agree with Xing *et al*.,[55] whom mentioned that the tegument of the protoscolecs was the primary site of damage and showed ultrastructural alterations when it treated with sodium arsenite in vitro and reasoned it as stress responses. In comparition to treatment with benzimidazole- methylcarbamate compounds which bind to parasite B-tubulin and this disrupts the tubulin microtubule dynamic equilibrium which lead to reduction in

glucose uptake and the consequent decrease in glycogen content[59] which generate metabolic and structural alteration in the parasite leading to its death.

Or the death of the protoscoleces may be due to programmed cell death or apoptosis which is the most common form of eukaryotic cell death.[60] Also high level of apoptosis has been reported in *E.granulosus* hydatid cyst.[42,61,62]

Regarding *C. rotundus* and within this hypothesis, recent studies concluded that the aerial parts extract of this plant exhibit a potential use as antioxidant and apoptosis inducer and explained that the anti-proliferative effect of these extracts is due to induction of apoptosis and suggested the regulation of apoptotic genes may be controlled by their phenolic content, orientin is one of the main flavonoids identified in alcoholic extract.[33]

Moreover, the result of Hu *et al*.,[63] confirmed the presence of apoptosis and the existence of CED-3 like apoptotic gene in protoscoleces of *E. granulosus* and proved that H2O2and hexamenthasone can induce cell apoptosis of protoscoleces in drug- induced apoptosis mechanisms.

Other study also documented the occurrence of apoptosis in the larval stage of *E. granulosus* by caspase 3 enzyme mechanism and this activity was higher in infertile cyst compared to fertile one, concluded that apoptosis may be involved in hydatid cyst infertility[61], also the involvement of caspase -3 dependent pathway in apoptosis of *E. granulosus* protoscoleces was recorded by Shi *et al*.[42]

Regarding the safety and the toxological studies of *C.rotundus* several works were done, recently Johari *et al*.,[32] showed that this plant is safe, economic and effective alternative for the treatment of patients with inflammatory bowel disease by effect the cytokine gene expression in rat model experiment and cytokine is important regulator of inflammation and tissue repair which play key role in the pathogenesis of this disease because the plant has anti –inflammatory and antioxidant activity.

The cytotoxicity of ethanolic extract of *C.rotundus* (whole plant) was detected by MTT-assay including (Vero cell line) and their result indicated the safety of it.[55] In addition, Singh *et al*.,[64] concluded that the ethanolic extract of *C.rotundus* rhizomes was safe and no mortality occurred in the Albino mice up to dose 2000mg/kg without any toxicity. Beside the confirmation that *C. rotundus* is believed safe for human use.[28]

Furthermore, in an acute toxicity test at dose of 5000mg/kg, all rats did not expressed signs of toxicity and mortality after a single oral administration of 95%ethanol extract of rhizomes of *C. rotundus*. Subacute toxicity result showed that rats administration with rhizomes extract at dose of 1000mg/ml daily over 14 days did not cause mortality or behavioral changes[65], also no mortality or morbidity was recorded up to 2000mg/kg in Wister rats.[66]

Finally, studies revealed that the rhizomes and tubers of *C. rotundus* are not toxic and hence reliably safe for use in Ayurvedic medicine.[29]

In conclusion, we here demonstrate for the first time the *in vitro* efficacy and the protoscolicidal activity of *C.rotundus* rhizomes– tubers alcoholic and aqueous extracts against *E. granulosus* protoscoleces. Further study is needed to investigate the efficacy of this plant in an animal model which may represent as an alternative treatment option against hydatid cyst disease.

ACKNOWLEDGEMENTS

The authors are grateful to M. Dlshad H. Hassan (Soran University, Science Faculty, Biology Department) for providing electron microscopy materials used in the present experimental work, also we knowledge M. Nashwan Jamal Abdulla for collecting the plants specimen.

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