Variations in the viability and macromolecules concentration of *E. granulosus* protoscolices isolated from ruminants consequence treatment with *Nigella sativa* seed's oil (*In vitro* study)

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Abstract

The aim of the present study was to investigate the scolicidal activity of *Nigella sativa* seed's oil concerning the viability *in vitro* and biomolecules content (carbohydrates, proteins and nucleic acids) of *Echinococcus granulosus* protoscolices. Protoscolices were aseptically aspirated from cysts of livers and lungs (naturally infected) which eradicated from goats and sheep that had been slaughtered at Mosul local abattoir / Nineveh / Iraq from September 2017 to May 2018. Various concentrations of *N. sativa* seed's oil (20 to 60 µl/ml) were applied on special time interval (5-40min). Viability of protoscolices was checked using vital stain (0.1% aqueous eosin). Concentration of carbohydrates, proteins and nucleic acids (DNA and RNA) were estimated after treating the protoscolices with LC_{50} of the seed's oil (40 µl/ml) comparing with untreated group. It is revealed that mortality rate of protoscolices, those were treated for 10 minutes with 40 µl/ml and 60 µl/ml of *Nigella sativa* oil, were approximately 50% and 100% respectively. Viability % of protoscolices treated with 20% hypertonic saline solution for 5 minutes was 43%, whereas, 16% of protoscolices were viable when treated with 60 µl/ml of seed's oil at the same time. The experiments detect concentration and time-dependent scolicidal effect of *N. sativa* seed's oil on the *E. granulosus* protoscolices. Mean concentrations of carbohydrates, proteins and nucleic acids (DNA and RNA) were significantly higher at $P \leq 0.05$ in control group (62.6µg/ml, 31.0 mg/dl and (23.4 and 82.9 µg/ml, respectively) than that found in LC_{50} treated protoscolices (58.3 µg/ml, 15.3 mg/dl and (18.19 and 64.48 µg/ml, respectively). The study showed that oil extract of *N. sativa* seeds has a significant ($P \leq 0.05$) clear impact in reducing viability of *E. granulosus* protoscolices, as well as, mean concentrations of its biomolecules which may open away for further experiments about scolicidal validity of *N. sativa* seeds oil *in vivo*.

Keyword: *Nigella sativa*, *E. granulosus*, seeds oil, protoscolices, biomolecules.
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التغيرات في حيوية وتركيز الجزيئيات الكبيرة للرؤيسات الاولية في المشوكة الحبيبية المعزولة من المجترات إثر المعاملة بزيت بذور حبة البركة (دراسة في الزجاج)

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الخلاصة

إن الهدف من البحث الحالي لمعرفة التأثير القاتل لزيت حبة البركة (الحبة السوداء) على حيوية وتركيز الجزيئات الكبيرة (الكربوهيدرات، البروتينات والأحماض النووية) في الرؤيسات الأولية للمشوكة الحبيبية للكيس العدري (دراسة في الزجاج). استخرجت الرؤيسات الأولية من الأكياس الموجودة في أكباد ورئات الماعز والأغنام المصابة والتي تم ذبحها في المسلخ المحلي لمدينة الموصل/ نينوى /العراق في الفترة ما بين شهر تشرين الأول ٢٠١٧ والى شهر أيار ٢٠١٨. عرضت الرؤيسات التي تم جمعها اللي تراكيز متدرجة

من زيت بذور حبة البركة (٢٠-٦٠مايكرولتر/ ملليتر) وضمن جدول زمني (٥- ٤٠ دقيقة)، فحصت حيوية الرؤيسات الأولية باستعمال صبغة الأيوسين المائية (٠.١%). قدر تركيز الكربوهيدرات، البروتينات والأحماض النووية (DNA و RNA) بعد معاملة الرؤيسات بالتركيز القاتل لـ ٥٠% لزيت بذور حبة البركة بالمقارنة مع المجموعة السالبة (الغير معاملة). تم الكشف عن أن معدل الوفيات من الرؤيسات الأولية في المجموعة التي عولجت لمدة ١٠ دقائق مع ٤٠ مايكروليتر/ ملليتر و ٦٠ مايكروليتر /ملليتر من زيت بذور حبة البركة كانت ٥٠ و ١٠٠% على التوالي، وكانت نسبة حيوية الرؤيسات الأولية المعالجة بالمحلول الملحي مفرط التوتر بتركيز٢٠% لمدة ٥ دقائق بنسبة ٤٣%، بينما كانت ١٦% فقط من الرؤيسات حية عندما تم معاملتها بتركيز٦٠ مايكروليتر/ ملليتر من زيت بذور حبة البركة لنفس وقت المعاملة. أكدت الدراسة الحالية أن التأثير القاتل لزيت بذور الحبة السوداء يعتمد على تركيز الزيت قيد الدراسة وزمن تعريض الرؤيسات الأولية له، حيث وجد أن متوسط تراكيز الكربوهيدرات والبروتين والأحماض النووية للرؤيسات الأولية عند مستوى الاحتمالية ($P\leq0.05$) في مجموعة السيطرة ٦٢,٦ ميكروغرام/ملليتر، ٣١,٠ ملغرام/ديسيلتر و (٢٣,٤ و ٨٢,٩) ميكروغرام/ملليتر على التوالي. بينما تلك المعاملة بالتركيز القاتل لـ ٥٠% كان متوسط التركيز ٥٨,٣ ميكروغرام/ملليتر، ١٥,٣ ملغرام/ديسيلتر و (١٨,١٩ و ٦٤,٤٨) ميكروغرام/ملليتر على التوالي. أظهرت الدراسة الحالية أن زيت بذور حبة البركة لها أثر كبير في انخفاض حيوية ومتوسط تركيز الجزيئات الكبيرة في رؤيسات المشوكة الحبيبية المعالجة وبفرق معنوي عند مستوى الاحتمالية $P\leq 0.05$ والتي يمكن أن تكون فرصة جيدة لإجراء مزيد من التجارب حول صلاحية زيت بذور حبة البركة واستعمالها لقتل الرؤيسات الأولية للمشوكة الحبيبية داخل جسم الإنسان.

Introduction

Echinococcosis is a well-known anthropozoonosis that is endemic in many regions of the world. It's one of the most important neglected parasitic diseases and has been identified as universal popular health problem especially in developing regions including Iraq (1). Hydatidosis may cause serious morbidity and death (2). Man, and grassing animals as intermediate host which have encysted larval stage (the hydatid cyst), dogs and other canines may act as final host in which adult cystoda inhabits small intestine. Man may get infection when ingesting ova with contaminated food or fluids (3). Number and localized of the cyst, general condition of the patient, the surgeon's experience and the presence of special services like intensive care unit was the main factors that affect the choice of therapy (4). Surgical removal of hydatid cyst remains the preferred choice (5,6). Furthermore, chemotherapy with benzimidazoles groups and PAIR (puncture, aspiration, injection, and respiration) are recommended as alternative treatments to surgery, especially for the patients who cannot tolerate surgery and to decrease the risk of intraoperative spillage of the cyst contents (protoscolices) (7,8). Subsequently recurrence of cystic echinococcosis and secondary infection, which is observed in about 10% of the postoperative cases, the use of effective scolicidal agents are essential (9,10).

Continuous trial to find agents those have high scolicidal effects in a shortest time to be used in surgery to prevent recurrence and secondary hydatidosis infections (8). Antihydatidosis agents like hypertonic saline, ethanol, silver nitrate, cetrimide and others used but have many serious adverse effects like liver necrosis, methemoglobinemia and sclerosing cholangitis (11). Therefore, developing new scolicidal agents with higher efficacy and less or no local or systemic adverse effects is necessary for treating hydatid cysts infections. Natural plant derivative has been widely used as antimicrobial agents either in folk medicine or as additive. As for hydatidosis infection, discovery of a novel scolicidal agent that has high efficacy against the parasites with low side effects is still the most important concern to eradicate the infection as reported by the World Health Organization (12). Specification of reliable scolicidal agents include the potency at lesser concentration in shortest time, the ability to permanents in the cyst fluid, minor toxicity, more complain to use and less side effects (13).

The most common chemotherapy used as anti-ecchinococcus agents is albendazole which is proved to have some adverse effects like poor solubility in gastrointestinal (GI) tract that is resulted in low drug concentration in liver, furthermore, encephalitis syndrome, influenza-like syndrome, allergic purpura, and drug rash (14), other researchers reported echinoccocal resistance to albendazole (14,15). Thus, there is an urgent need for new therapeutic strategies toward creative scolicidal agents that have rapid and complete inhibitory effects and few side effects. Many researchers focused their efforts to use natural products specially plant extracts as antimicrobial agents which is found to have considerable effective efficacy, validity and have low cost (16).

Nigella sativa Linn. (Ranunculaceae) or black seed commonly grows in many places of the world (Western Asia, Middle East, North Africa and Southern Europe). It has been recognized as one of the most popular herbs in many parts of the ancient world, which was used in folk medicine to help in cure different diseases like cough, dizziness, headache, hypertension, eczema, bronchitis, fever and diabetes in worldwide (17), in addition, to their nutritional and healthcare benefit for humans (18). Many

reports referred to the antioxidant, antitumor, immune-potentiation, anti-asthmatic and neuroprotective activities of *N. sative* seeds (17,19), other studies revealed the anti-inflammatory, general antimicrobial activities of *N. sativa* and its derivatives (20-22). Such pharmacological effects have been related its anti-inflammatory effects due to the presence of main active constituents that include thymoquinone (TQ), dithymquinone (DTQ), thymohydroquinone, dithymquinone (DTQ) and thymol (23). The cyst fluids and protoscolices contain biochemicals such as carbohydrates, proteins, lipids, vitamins, electrolytes and trace elements that may have role in the metabolism and growth of unilocular hydatid cyst (24). The carbohydrates of the protoscolices are glycogen, trehalose, glucose and alkali stable carbohydrates of particular interest was the detection of sucrose in both protoscolices and fluid (25). Proteins are the most important organic constituents of living things including cestode and play an important role in energy production. In the development process, various agents such as protein is required for the synthesis of ATP (26). The proteins of cyst fluid were mainly albumin and globulin, the latter having always double the concentration of the former (25).

Therefore, the aim of the present study is to estimate the scolicidal efficacy of *N. sativa* seed's oil against *E. granulosus* protoscolices and to evaluate its effect on the biomolecule contents of protoscolices compared with non-treated (negative control).

Materials and methods

Collection of plant materials

The seeds of *N. sativa* were purchased from local market in Mosul city. The identity was confirmed by Dr. May TH. Al-Wattar, Botanist, College of Science, Mosul University, Mosul, Iraq.

Parasite source

Fresh hydatid cysts were eradicated from livers and lungs of infected goats and sheep which had been slaughtered at Mosul local abattoir, Nineveh - Iraq. They were wrapped carefully in clean plastic bags, and carried to the microbiology laboratory at the Department of Clinical Laboratory Science, College of Pharmacy, University of Mosul where protoscolices were extracted.

Preparation of Protoscolices extract

Protoscolices (PSC) were aspirated according to Smyth (27). The outer surfaces of the cysts were sterilized with 70% ethanol before being dissected and withdrawn fluid sac by a 50ml syringe (suitable capacity), the aspirated fluid aseptically transferred into a flask. The cysts then washed from inside with Phosphate buffer solution (PBS) pH of 7.2, transferred to siliconized test tubes, underwent washing process and centrifuge two times per 3000 rpm, take the precipitation which contain the protoscolices with concentration of 7500 psc/ml (27). Viability of the protoscolices was accomplished by investigating peristaltic movement of the organism, flame cell and impermeability to the vital stain (0.1% aqueous eosin) under the light microscope. Take 2 ml of protoscolices suspension (15000 PSC) for the treatment study.

Evaluation of lethal concentration 50 (LC_{50}) and lethal concentration 90 (LC_{90}) of *Nigella sativa* oil extract to *E. granulosus* protoscolices

20 g of *N. sativa* seeds were grinded, poured into the flask mixed with 200ml of ethanol (95%) in a concentration of 1:10 (W:V) in a cooled site, then stirred for 1 hour using a blender, and kept in refrigerator overnight then filtered using gauze and whatman filter paper No.1. The oil was isolated according to Ghourchian *et al.* (28) with some modifications by hexane using Soxhlet apparatus. The resulting mixture was collected in a separator funnel and kept in cold place overnight, collect the oil layer and stored in a sealed vial dark colored at 4°C. In order to evaluate the scolicidal effects of *N. sativa* seed's oil, gradient concentrations of oil extract were prepared as following 20, 25, 30, 35, 40, 45, 50, 55 and 60 µl of oil / ml PBS (vol/vol) using DMF (Dimethyl formamide) as a solvent, in addition to negative (protoscolices in PBS at pH 7.4) and positive (protoscolices in 20% hypertonic saline) control group. Each concentration was put 2ml in Siliconized test tube, then 0.2ml of protoscolices suspension (about 15000 PSC) were added to each tube, mix gently and incubated at 37˚C for times interval (5, 10, 15, 20, 25, 30, 35, and 40 min.). the viability of protoscolices were determined depending on reaction with vital stain (0.1% aqueous eosin) and flame cells activity (29). Three replicates for each treatment were applied, LC_{50} and LC_{90} for extract was determined according to protoscolices viability. Furthermore, negative control group was protoscolices treated with buffer phosphate solution only while positive control group was protoscolices treated with 20% hypertonic saline solution.

Biochemical studies

20 µl of suspended protoscolices in BPS (pH 7.2) were put on glass slide, stained with aqueous eosin (0.1%), spread, covered with cover glass, scanned for the number of vital protoscolices, 1500 vital protoscolices / 20 µl, the volume then was adopted for the subsequent study. Stock solution of 1000 µl *N. sativa* seed's oil /ml of the solvent (DMF) were prepared, then graduated concentration of the oil were prepared (20, 25, 30, 35, 40, 45, 50, 55 and 60 µl/ml PBS). Prepared *E. granulosus* protoscolices were added to each treatment group. 0.2 ml of the prepared suspension were treated with LC_{50} (40µl/ml) of *N. sativa* oil extracts for 10 min, the mixtures then were precipitated

using MSE (super speed ultracentrifuge) at 10000 rpm. for 10 minutes. Sediments were washed with distilled water several times at 8°C, centrifuged using the previous method, the protoscolices sediment were collected, crushed by 1 gram parasites : 5ml of 50 millimolar trichloro acetic acid (TCA) solution at pH 7.2 using ultrasonic disintegration (Andreas Hettich, Germany) being crude homogenate (crude extract), the protoscolices were further centrifuged in ice bath (30), deep freeze for subsequent biochemical studies.

Carbohydrates

Herbert *et al.* (31) method was applied to estimate carbohydrates concentration in the extract. Absorbance was measured at 488nm depending on carbohydrate standard curve that has been prepared different concentrations of glucose.

Total proteins

Lowry *et al*. (32) colorimetric method used to evaluate protein concentration. Folin Ciocalteus Reagent react with protein to produce blue complex in alkaline medium, absorbance measured at 750nm. Concentration of protein was estimated using protein standard curve applied by bovine albumin.

Separation of nucleic acids from *E. granulosus* protoscolices

Quantitative determination of nucleic acids (DNA and RNA) was determined according to Schneider's (33) method as available materials in our laboratories. Protoscolices extract was washed two times with ethyl alcohol (95%) to discard lipids, 1.3 ml of distilled water and 1.3 ml of 10% TCA were added to the sediment, the mixture then heated at 90 C° for 15 minutes with shaking from time to time. In this step protoscolices proteins were disposed. The mixture then precipitated using cooled centrifuge (10000 rpm) for 10 minutes. Filtrate kept away as filtrate (1), the sediment was then suspended again in 2.5 ml of 5 % TCA, and then separation was performed using the formerly used method to gain filtrate (2). concentration of nucleic acids in the filtrates was assessed.

Deoxyribonucleic acid (DNA)

DNA concentration was estimated utilizing DNA calibration curve, that already prepared using calf thymus DNA. Absorbance of blue color sample was measured spectroscopically at 600 nm (33).

Ribonucleic acid (RNA)

Concentration of RNA was estimated in each sample quantitatively by measured the absorbance of green color sample at 660 nm, then rely on RNA calibration curve that had been prepared using yeast RNA (33).

Statistical Analysis

In the present research, all experiments were accomplished in triplicate. Data analysis was carried out using SPSS statistical package version 19), which was sought to analyze the data by computer. Paired-Samples T Test, Two-Way ANOVA and Duncan test were used to analyze the differences between treatment and control groups. All differences were considered as significant statistically at $P \leq 0.05$ (34).

Results

In the present work, essential oil of *N. sativa* seed was extracted, its scolicidal activity against *Echinococcus granulosus* protoscolices was tested. Different concentrations of *Nigella sativa* seed's oil following different incubation times interval were applied to estimate the lethal concentration of *N. sativa* oil for 50% of protoscolices (LC_{50}). Table 1 showed that the viability of treated protoscolices was decreased with the concentration increase, for example, treating protoscolices with 55 µl/ml (vol/vol) of oil was reducing protoscolices survival to 34.66±1.2%, 12.0±0.57%, 2.66±0.33% and 0% after 5, 10, 15 and 20min of treatment respectively; while 82.67±1.45%, 74.33±0.33%, 67.67±1.2%, 53.67±1.2%, 37.33±0.33%, 19.67±1.2%, 7.67±1.2% and 0% of protoscolices were survive for treating with 20 µl/ml after 5, 10, 15, 20, 25, 30, 35 and 40 min of exposure respectively. On the other hand, treating protoscolices with 60 µl/ml seed's oil for 10 min were approximately eliminate all protoscolices. It seems that LC_{50} of seed's oil for *E.g.* protoscolices was 40 µl/ml after 10 min of brood, while LC_{90} was 55 µl/ml of the oil at the same incubation period (Figure 1).

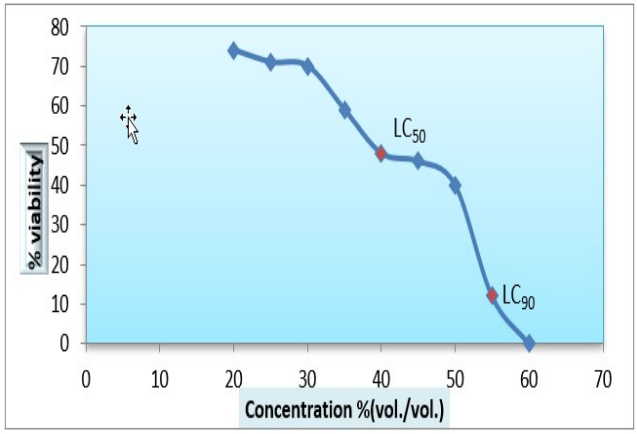

Figure 1: Effect of LC_{50} and LC_{90} of *Nigella sativa* seed's oil on the *E.granulosus* protoscolices.

Table 1: The scolicidal effect of *Nigella sativa* seed's oil on the viability% of *E. granulosus* protoscolices at various concentrations following different exposure times *in vitro*, at 37°C ±2 compared with negative and positive control groups (Duncan test at P≤0.05)

Treatment	Concentration µl/ml	Mean viability% of Protoscolices ±SE after:							
		5min.	10min.	15min.	20min.	25min.	30min.	35min.	40min.
negative control (PBS fluid pH7.4)	0	99.9±0.13 I	99.9± 0.1 A	99.8±0.16 A	99.8±0.13 a	99.7±0.33 A	99.7±0.33 A	99.7±0.23 a	99.6±0.33 B
positive control (hypertonic saline)	20	42.6±0.88 C	0.0±0.00 A	0.0±0.0 A	0.0±0.0 A	0.0±0.0 A	0.0±0.0 A	0.0±0.0 A	0.0±0.0 A
Nigella sativa seed's oil	20	82.67±1.45 H	74.33±0.33 I	67.67±1.2 H	53.67±1.2 F	37.33±0.33 E	19.67±1.2 D	7.67±0.88 D	0.0±0.00 A
	25	81.66±0.33 H	69.33±0.88 H	60.0±1.0 G	52.0±0.0 F	34.66±0.66 E	18.33±0.88 D	4.0±0.57 C	0.0±0.0 A
	30	77.66±0.88 G	69.0±0.88 G	55.0±0.57 F	47.33±1.33 E	21.33±0.88 D	10.33±1.32 C	0.66±0.53 B	0.0±0.0 A
	35	75.66±0.33 G	59.33±0.33 F	50.66±0.33 E	38.0±0.57 D	13.33±0.88 C	3.33±0.88 B	0.0±0.0 A	0.0±0.0 A
	40	68.66±0.66 F	48. 0±0.57# E	36.33±1.33 D	20.0±0.57 C	11.33±0.88 C	0.0±0.02 A	0.0±0.0 A	0.0±0.0 A
	45	55.33±1.2 E	45.66±0.88 D	14.0±0.0 C	5.66±0.33 B	0.3±0.3 B	0.0±0.0 A	0.0±0.0 A	0.0±0.0 A
	50	47.66±0.88 D	40.33±0.88 C	10.33±0.33 C	2.66±0.66 B	0.0±0.0 A	0.0±0.0 A	0.0±0.0 A	0.0±0.0 A
	55	34.66±1.2 B	12.0±0.57## B	2.66±0.33 B	0.0±0.0 A	0.0±0.0 A	0.0±0.0 A	0.0±0.0 A	0.0±0.0 A
	60	16.33±0.88 A	0.0±0.3 A	0.0±0.0 A	0.0±0.0 A	0.0±0.0 A	0.0±0.0 A	0.0±0.0 A	0.0±0.0 A
F		759.893	2217.512	2958.967	2285.507	2925.127	1251.373	5696.653	28383

Significant at P≤0.05, different letters vertically means significant difference, Each test tube was contained ≈ 15000 protoscolices at zero time, Three replicates were applied for each treatment, Viability % at zero time was considered as 100%, Initial number of protoscolices used in each treatment were≈ 1500 protoscolices /20µl, # lethal concentration 50 (LC_{50}), ## lethal concentration 90 (LC_{90}).

Statistical analysis using Two-Way ANOVA and Duncan test was showed significant different between protoscolices viability% before and after treatment at P≤0.05. It is seemed that reduction percentage in viability% was inversely proportional to the seed's oil concentration and exposure time. Ultimately, mean reduction percentage in viability was 100% as shown in table 1.

Viability signs those adopted to evaluate the alive protoscolices were showed obvious different between protoscolices groups those treated with *N. sativa* oil and those in negative control group, especially after staining with the vital stain (red pigmentation in aqueous eosin 0.1%), breakdown of flame cells movement and so that the peristaltic movement of the whole organisms those treated with the scolicidal oil (Figure 2).

Evaluation of protoscolices biomolecules after treating with Lethal concentration for 50% (LC_{50}) of *Nigella sativa* seed's oil were made according to Herbert *et al.* (31), Lowry *et al.* (32) colorimetric and Schneider (33) method colorimetric method and compared with that of negative non-treated control group as in table 2, there were a significant P≤0.05 decreased in the biomolecules contents of *E. granulosus* protoscolices with LC_{50} treated group compared with untreated group using Paired-Samples T Test.

Discussion

Antimicrobial properties of remedial herb medicines had been logically revealed in experiments with their oils, aqueous or alcoholic extracts, and other constituents (35). Clinically, albendazole was naturally used to treat echinococcosis with or without surgery, however, its severe side effects and poor solubility limit its application (36). The results revealed viability % of protoscolices treated with 20% hypertonic saline solution (actually used in our hospital) for 5 minutes was 43%, whereas, 16% of protoscolices were viable when treated with 60 µl/ml of

seed's oil at the same time. The result of the present study was coming agree with that of Keyhani *et al.* (37), those concluded that scolicidal concentration of 20% hypertonic saline (the positive control in the present study) was terminate protoscolices viability 100% after 10 min of application. The same result is reviled in our work when *E. granulosus* protoscolices were treated with 60% *N. sativa* seed's oil. Therefore, the scolicidal activity of seed's oil at the concentrations 60 % after 10 min of incubation was approximately similar to that of positive control, whereas, the viability percentage was higher than 60% seed's oil at 5 min which indicate it is more active against protoscolices than our surgically used. It was proved to have inhibitory effects against *E.granulosus* that agree with Ali *et al.* (17).

Recently, *N.sativa* seeds oil has an effective antimicrobial activities considering their low coasty and good safety products (37). Agrawal *et al*. (38) manifested significant *N. sativa* seed's oil antihelmintic activity against some pathogenic nematode and cestode. Thymoquinone has hepatoprotective properties due to its antioxidant activity (28). Either whole seeds or fixed and essential oils have therapeutic effects on respiratory symptoms (28) The oil extract of *Nigella sativa* seeds have a clear impact in inhibiting protoscolices viability that agree with Mahmoudvand *et al.* (10). However, further studies will be required to approve these results by inspecting other derivatives of *N. sativa* and comparing with its active constituents in the *in vitro* and *in vivo* model and to estimate its mode of actions.

Generally, different concentrations of *N. sativa* oil tested in the present work were proved to have significant scolicidal effects at P≤0.05. However, an ideal scolicidal agent is defined by its potency at lower concentrations, high efficacy in a shortest time of exposure, stability in the presence of cystic fluid, scolicidal ability inside a cyst, lower toxicity, higher availability, and ability to be prepared rapidly. Therefore, evolution of new scolicidal agents with higher efficacy and low or no local or systemic side effects is insistent need for surgical hydatidosis success.

Biochemical substances (metabolites) within hydatid cysts play definitive role in the metabolism, physiology and immunology of cystic echinococcosis (39, 40). This study observed decreased in carbohydrate, protein and nucleic acids (DNA and RNA) concentrations when treated with LC_{50} of seeds oil compared with non-treated group in which carbohydrate, proteins, DNA and RNA concentration was agree with AL-Abady (41) and Barazesh *et al* (42) in untreated group. There is significant p≤0.05 relationship between metabolites quantities in *E.granulosus* protoscolices before and after treated with *N. sative* seed's oil.

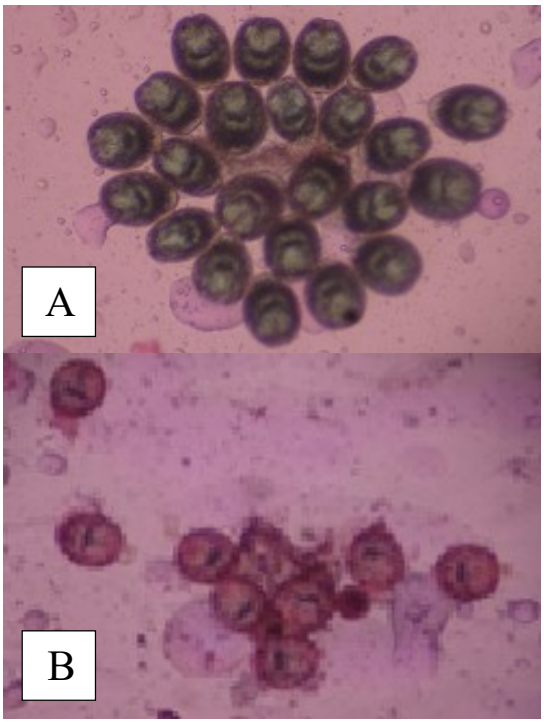

Figure 2: A; Living protoscolices of hydatid cyst in negative control group (note green color of vital protoscolices). B; note the red color of dead protoscolices those treated with 60% of *N. sativa* oil for 10 min. (40X).

Table 2: Concentration of biomolecules in *E. granulosus* protoscolices after treating with Lethal concentration for 50% (LC_{50}) of *Nigella sativa* seed's oil compared with negative control group

Biomolecule	Mean concentration in group treated with LC_{50} of *N. sativa* oil ±S.E	Mean concentration in negative control group± S.E	T value	DF	P value
Carbohydrates (µg/ml)	58.33±2.33	62.66±2.33	- 6.50	2	0.023
Proteins (mg/dl)	15.34±0.05	31.0±1.03	- 24.90	2	0.002
DNA (µg/ml)	18.19±0.1	23.43±0.27	- 36.10	2	0.001
RNA (µg/ml)	64.48±0.3	82.96±0.36	- 33.79	2	0.001

Negative control group= protoscolices in PBS (pH 7.4), Significant at P≤0.05.

Conclusion

The present study demonstrated oil extract of *N. sativa* seed have effective scolicidal activity against protoscolices of *Echinococcus granulosus* in vitro, as well as, decrease biomolecules (protein, carbohydrate and nucleic acids) concentration of treated protoscolices. Further studies could be applied to check the ability of *N.sativa* seeds oil to inhibit growth of the hydatid cysts *in vivo*.

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