Trop J Nat Prod Res, April 2021; 5(4):707-714

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Inhibitory Effects of Some Jordanian Medicinal Plants on *In Vitro* Viability of Protoscolices of Hydatid Cysts

Yaseen T. Al Qaisi[1], Khaled M. Khleifat[2]*, Sawsan A. Oran[1]

[1]*Dept. of Biological Sciences, Faculty of Sciences, University of Jordan, Amman, Jordan*
[2]*Department of Biological Sciences, Mutah University, Mutah, Karak, 61710, Jordan*

ARTICLE INFO

Article history:
Received 21 february 2021
Revised 18 March 2021
Accepted 14 April 2021
Published online 03 May 2021

ABSTRACT

The common scolicidal agents used for the immobilization of protoscolices of hydatid cyst are mostly associated with side effects. The purpose of the current study was to investigate the methanol extracts of *Ruta graveolens*, *Peganum harmala* L. and seeds of *Citrullus colocynthis* L effects on hydatid cysts. The protoscolices were collected from hydatid cyst-infected sheep and goats and subjected to various concentrations of methanol extracts of the three plants (10, 20, 30 and 40 mg/mL) at different exposure times in vitro. Albendazole (ABZ) was used as a control. *R. graveolens* extract (40 mg/mL) killed 100% of the protoscolices in 75 minutes (1.25 hour) while the other concentrations (10, 20, 30 mg/mL) led to 100% mortality of the protoscolices at the maximum period of 12 hours, after treatment. Whereas, a 21% and maximum scolicidal effect (95%) were obtained using 20 mg/mL ABZ as control after 2 and 24-hour exposure period, respectively. The highest deadly effects obtained from *P. Harmala* and *C. colocynthis* extracts were 50 and 15%, respectively, using maximum concentrations (40 mg/mL), after 24 hours of treatment. Moreover, various concentrations of the *R. graveolens* extracts were very superior ($P < 0.05$) to the two plants and ABZ as control. The anti-fibroblast study showed that the use of 100 mg/mL of *R. graveolens* extract resulted in extremely low cytotoxicity with IC_{50} values of 65.78 mg/mL. Thus, the significant effects of this extract on the protoscolices reveal a new antihydatic activity using *R. graveolens* as a source of scolicidal agents.

***Keywords*:** Protoscolices, *Peganum harmala*, *Citrullus colocynthis*, *R. graveolens*, Scolicidal agents.

Introduction

Cystic Echinococcosis (CE) is an important zoonotic disease in many parts of the world, the disease is endemic in the Levant countries including Jordan.[1] This disease is caused by infection in animals or humans with the larval stage of the genus Echinococcus tapeworm.[2] The life cycle of the parasite involves canines as definitive hosts and herbivores as intermediate hosts where the hydatid cyst is found. Human acquires hydatidosis by ingesting the eggs of the parasite as the herbivores do.[3] It was found that, dogs are the only canids being infected by the adults of *Echinococcus granuolosus.*[4] The occurrence of cross infection in some occupations such as shepherds and farmers is as a result of the close proximity between dogs, sheep and humans.[5] Stray dogs congregate around slaughterhouses, farms, and among the local people living in this area. These residents use dogs to protect their sheep and livestock from predators such as Canis spp. and Hyena spp. The gathering of these carriers in one place enables the parasite to achieve its full life cycle and thus causing of echinococcosis. The treatment of human echinococcosis faces some complications and in such circumstances, intensive surgery is considered the best treatment option.[6] Since the spillage of the cyst within the body is the first reason of secondary parasitic infection.

*Corresponding author. E mail: alkh_kha@hotmail.com
Tel: 00962-7990-10339

Citation: Al Qaisi YT, Khleifat KM, Oran SA. Inhibitory Effects of Some Jordanian Medicinal Plants on *In Vitro* Viability of Protoscolices of Hydatid Cysts. Trop J Nat Prod Res. 2021; 5(4):707-714. doi.org/10.26538/tjnpr/v5i4.19

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

In purely cystic infectious liver disease, the risk of diffusion of the cyst contents can be averted by injection of an effective scolicidal agent.[7] Various scolicidal agents such as albendazole and mebendazole are widely used to neutralize cyst content during and after surgery. The use of chemically synthesized scolicidal agents such as hypertonic saline solution, Ag-nitrate, cetrimide, Albendazole and mebendazole may produce adverse side effects such as leukopenia, liver necrosis, elevated liver transaminase and sclerosing cholangitis.[2,8] Therefore, many researchers have attempted to find naturally sourced chemical components such as plants with anti-parasite activities. The majority of the world population (60-80%) still depends on traditional medicines to treat many of the current diseases.[9,10] Medicinal plants are an important source of various compounds containing effective biological substances for treating serious diseases all over the world.[11] Therefore, natural compounds of medicinal plants such as phenolic compounds and essential oils may have potent application as anti-parasitic, antimicrobial, anti-inflammatory and anti-pyretic. In Jordan, 363 species out of 2552 flowering plant species were reported of being used in traditional medicine.[12-16]

The plant *Ruta graveolens* L (*R. graveolens*) is a member of Rutaceae family and is commonly known as Rue.[13] Jordanians use the *R. graveolens* plant as food spices.[12] In traditional medicine, it is utilized as aphrodisiac, stimulant for fertility and is used to treat many ailments such as inflammations, wounds, parasitic infections, ulcers, low blood pressure, hypotension, reproductive defects and menstrual issues.[17-19] A schistosomicidal activity and anticancer effect of the *R. graveolens* extracts were reported by Carvalho *et al*.,[10] whereas its essential oil showed crucial antibacterial and antifungal activities.[18] The plant *P. harmala* L is commonly known as Syrian Rue belongs to family Zygophyllaceae, and is widely used in folk medicine. The plant *Citrullus colocynthis* L (*C. colocynthis*) belongs to the family Cucurbitaceae.[12] These plants are mostly endemic to Mediterranean

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

region particularly, Jordan [12]. *P. harmala* and its active alkaloids possess a wide range of pharmacological activities like anti-parasidal, antifungal, antibacterial, insecticidal and anti-leishmanial activities.[20,21] *P. harmala* seeds have a potential for treating several diseases such as anxiety, depression, fever, malaria and leukemia.[22,23] *C. colocynthis* is characterized by the presence of different chemical compounds such as alkaloids, carbohydrates, flavonoids, tannins, gums and mucilage's. Besides its usage as source of antioxidant, antimicrobial, insecticidal activity, anti-parasitic agent against Haemonchus contortus, Leishmania and Plasmodium and anti-diabetic activity, it is used in traditional medicine for treating cancer, carcinoma, endothelioma, leukemia, tumors of the liver, diabeties and spleen.[24-26]

The current study aims to investigate the activity of some wild plant extracts *(Ruta graveolens* L. (*Rutaceae*), *Peganum harmala* L. (*Zygophyllaceae*) and seeds of *Citrullus colocynthis* L. (*Cucurbitaceae*) collected from different regions of Jordan against *Echinococcus granulosus* protoscolices.

Materials and Methods

Collection of the plant specimens

Aerial parts including stems and leaves of *R. graveolens* and *P. harmala* were collected from different regions of Jordan in June 2020 while seeds of *C. colocynthis* were collected in July 2020. The plant was authentically identified by Prof. Sawsan Oran[12, 13], Department of Biological Sciences, University of Jordan, Amman, Jordan. Voucher specimens numbers of *R.graveolens, P. harmala, and seeds of C. colocynthis* (MU2021-19, MU2021-20 and MU2021-21, respectively) were deposited in the Department of Biology, Faculty of Science, Mutah University, Jordan.

Preparing the extracts of R. graveolens

The fresh aerial parts of *R.graveolens, P. harmala*, and seeds of *C. colocynthis* were washed, dried under shade and grinded with a blender to powder. One hundred gram of each dried plant parts were soaked in 1000 ml methanol (10:1 v/w ratio) for 3 days with continuous shaking at room temperature. After filtration, the extract was dried using rotary evaporator at 45°C under reduced pressure. The obtained extracts were kept at -20°C in airtight containers. The extracts were weighed to calculate the yield percentage using the following equation: Yield% = (wt. of dry extract/ wt. of dry parts before extraction) x 100%.[26] Initially, each dried crude extract was dissolved in 30% dimethyl sulfoxide (DMSO). Stock solution containing 500 mg/mL of the extracts were prepared in sterile normal saline, and series of dilutions were made to obtain concentrations of 10, 20, 30 and 40 mg/mL.

Phytochemical analysis of R. graveolens, P. harmala, and seeds of C. colocynthis Determination of Total Phenolic Compounds

The whole phenol quantification process was carried out for R. graveolens, *P. harmala*, and seeds of *C .colocynthis* extracts using the Folin-Ciocalteu method.[27] 15 mL of Dimethyl sulfoxide (DMSO) was used in the dissolution of the crude extracts. 0.5 mL extract of *R. graveolens* was added to 2.5 ml of 0.2 N Folin-Ciocalteu reagent and left for 5 minutes at room temperature, then 2 mL aqueous solution of sodium carbonate (7.5 % w/v) was added. After 2 hours of incubation at room temperature in darkness, the measurements of absorbance were taken at 760 nm employing UV/Visible spectrophotometer (Elico, SL 150, India). Gallic acid concentrations (Sigma-Aldrich, USA) ranged between 0.01-0.05 mg/mL were applied for the calibration of standard curve. The unit (mg gallic acid equivalent/gram) was considered as an equivalent for measuring the total phenol content in these experiments.

Determination of total flavonoids

The amount of flavonoid content in each extract of *R. graveolens, P. harmala*, and seeds of *C. colocynthis* was estimated according the procedure of Zhishen *et al.*[28] The dissolution of the crude extracts was carried out in 15 mL of DMSO (dimethyl sulfoxide) (Hayman, England), in which 0.5 mL of each extract was mixed with 0.3 mL of 5 g/L sodium nitrite (Labchem, USA), 5 minutes later 0.3 mL aluminum chloride solution (1g/l) was added. After six minutes, 2 ml of 1M NaOH solution was added to the mixture and the total volume was adjusted to 10 mL with distilled water, and then sonication was carried out immediately. The absorbance was measured at 510 nm against water as control using a visible/UV spectrophotometer. The Calibration curve was made with employing a rutin solution preparation (0-200 µg/mL). The unit mg rutin/g extract of each plant was used to express the concentrations.

Determination of total tannins

The Folin and Ciocalteu method[29] was used for the estimation of the total tannins. Each extract of *R. graveolens, P. harmala*, and seeds of *C. colocynthis* was analysed by adding 0.1 ml of previously prepared 15 mL DMSO-crude extract solution dissolved in 7.5 mL of distilled water. To this mixture, 0.5 ml of Folin Ciocalteau phenol reagent and 1 ml of 35% (w/v) sodium carbonate solution were added and this was made up to 10 mL with distilled water. Samples were well mixed by shaking, left at room temperature for 30 minutes, and the optical absorbance read at 725 nm. Water was used for preparation of blank rather than of the sample. Pre-treatment was done for a set of standard solutions of gallic acid using the same method mentioned previously with making assessments against blank. The results of tannins were interpreted as the gallic acid equivalent/gram of the gallic acid extract.

The source of the hydatid cyst

The collection of hydatid cysts from infected organs was made from naturally infected sheep/goats that were slaughtered in Karak abattoir. The process of slaughtering animals in the slaughterhouse is subject to the supervision of the Veterinary Department for Meat Inspection under the administration of Karak Municipality, Jordan. The presence of cystic echinococcosis was examined by making regular visits (periodically) to the slaughterhouse to examine the various organs that could be affected, such as the liver, lung, and other organs in slaughtered animals.

The infected samples from each animal were taken separately, in clean plastic bags, 'in an icebox' and transferred within one hour to the research lab of the Department of Biological Sciences. Organs affected by hydatid cysts were recognized in slaughtered animals with the help of a veterinarian. After determining the site of infection in the examined samples, especially the liver and lungs, the incidence of infection was calculated as follows: Rate of infection = Infected animals/ Slaughtered animal's × 100%.

Collecting the protoscolices

Protoscolices were obtained after cleaning the surfaces of the cysts twice before dissecting using 70% ethyl alcohol.[30,31] Hydrophilic fluid was extracted from every cyst using a sterile syringe, transported it to a sterile box and left for 30 minutes to allow precipitations of protoscolices. The obtained protoscolices after subtracting the supernatant were rinsed thrice in Phosphate Physiological Stock Saline (PBS) (pH 7.2) [1,9] .The extracted protoscolices were kept in a sterile mixture composed of Kreb's Ringer Solution (KRS) and hydatid cyst fluid (4:1) supplied with 100 µg/mL penicillin and streptomycin sulphate to make it bacteria free media.[32]

Evaluating the viability of the protoscolices

Protoscolices vitality was evaluated through monitoring the motility of flame cells and staining with 0.1% aqueous eosin solutioin. Living protoscolices do not absorb the eosin and remain uncoloured, while dead protoscolices absorb the eosin and appear distinctively blue under a microscope.[30,31]

The percentage of alive protoscolices in the sample was calculated by dividing the number of alive protoscolices in the sample to the total number of the calculated headings x 100. When the percentage of viable protoscolices exceeded 90%, it was utilized in the next phase of experiments.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

In vitro effect of R. graveolens, P. harmala, and seeds of C. colocynthis methanol extracts on the viability of E. granulosus protoscolices

Hydatid cyst protoscolices were treated independently with 10, 20, 30 and 40 mg/ml of each plant extract for 1, 3, 6, 12, 18 and 24 h. In more details, one milliliter (approximately 2 x 10^3 protoscolices/mL) from protoscolices suspension (Kerb's ringer's and Hydatid cyst fluid 4:1) was placed in test tubes. Then various concentrations of plants' extracts (10, 20, 30 and 40 mg/mL) were added and the effect of each concentration was evaluated as a function of different specified time periods (1, 3, 6, 12, 18 and 24 h) at room temperature.[2,6] The scolicidal activity of extract was determined by staining the protoscolices with 0.1% eosin for 15 min and the rate of mortality was monitored using the light microscope. Protoscolices viability was tested by the motility of flame cells and by their impermeability to a 0.1% eosin solution. Protoscolices were rated as viable when kept unstained and 100 protoscolices were included for each estimate of protoscolices viability.[9] To monitor the efficacy of the methanol plant extract on the viability of protoscolices, 20 mg / mL of ABZ was used for comparison. Mixture of 30% DMSO and protoscolices maintained in a sterile medium (1:1000) were used as positive control. Normal saline and the maintenance medium was used as negative control [33]. In all cases, experiments were performed in three replicates. All images were captured by Pro-MicroScan, Microscope Digital Camera, 8M Pixels High-Speed and light microscope OLYMPUS Model CX21FS1 (100x)

In vitro cytotoxicity test:

Cytotoxicity of *Ruta graveolens* extract was assessed against fibroblast cell lines. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 100 µg/ml of penicillin and streptomycin antibiotics. The cells were grown in humidified 5% CO_2 incubator at 37°C. The cells were splitted each 3-4 days by the removal of the culture media followed by cell detaching using 1-2 ml of trypsin and adding fresh warmed DMEM medium.[34-36] Concentration ranges tested were between 10-100 mg/ml for plant extracts. All cultures were performed in triplicates. IC_{50} was calculated from dose-response curve.

Culturing of cell lines

The cytotoxicity of *Ruta graveolens* extract was determined microscopically. Different concentration of the samples (10, 25, 50 and 100 mg/ml) were applied into 24-well microtiter plates containing 5×10^4 cells/ml of the tested cell line. The cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2. The test was done in duplicate and the cytotoxic effect was observed daily up to 72 hr of cultivation.[37,38] Changes in cell shape morphology including loss of monolayer, rounding, and shrinking was considered signs of cytotoxic effect of the tested samples under microscopy. Cultures of cell line without tested extract was implemented as negative controls.

Antiproliferative assay

Following cultivation of cell lines with tested compounds, the inhibition in cell proliferation was monitored using Giemsa staining method.[39] The media from wells will be aspirated followed by washing with 0.5 ml PBS and fixation with 0.3 ml methanol for 10 min at 37°C. Methanol was aspirated and plates were left to 2 min for drying. 0.5 ml of Giemsa stain (1:10 in PBS) was applied to each well and left for 10 min, after which, the stain was aspirated and the wells were washed with 0.5 ml deionized water. The bounded stain will be extracted using 0.3 ml of 0.1N HCl and antiproliferative activity will be estimated using enzyme-linked immunosorbent assay (ELISA) microplate reader at 630 nm. The cell viability will be expressed as percentage of living cells relative to control. The percentage cell mortality was calculated using the formula:

%Mortality rate = Absorbance of control− Absorbance of treated cells ×100/ Absorbance of control.

Statistical analysis

All the experiments were carried out in triplicate. Data analysis was made by using version 17.0 of SPSS statistical package (SPSS Inc., Chicago, Illinois, USA). The results were analysed by finding differences between the test and control groups by means of t-test. Statistical significance was considered at $P < 0.05$.

Results and Discussion

The plants endemic to Jordan are part of the Mediterranean flora, a number of them are medicinal and they show different ways of acclimatization through the creation of bioactive ingredients. *R. graveolens*, *P. harmala*, and *C. colocynthis* are among the most important plants used in Jordanian traditional medicine.[12]

Rate of infection

In this study, 3725 slaughtered animals were examined (1675 indigenous and 2050 imported) during the period of 1/8/2020 to 1/10/2020. The rate of infection among imported and indigenous animals were 0% and 1.49%, respectively. Among all the slaughtered animals that was examined, about 0.91% were infected (Table 1).

Yield and Phytochemicals analysis of methanol extracts

The yielded extracts obtained by methanol extraction of aerial parts of *R. graveolens*, and *P. harmala* and seeds of *C. colocynthis* (Table 2) were about 23.51, 20.50 and 12.0% w/w on dry weight basis, respectively. The results of this study showed that methanol extracts of studied plants in the order as mentioned above, contained phenolic compounds (25.53 ± 0.49, 18.25 ± 0.04 and 17.60 ± 0.39), as well as flavonoids (6.66±0.31, 8.385 ± 0.71 and 9.81 ± 0.53) and tannins (8.035 ± 0.049, 6.41 ± 0.80 and 5.70 ± 0.07). These results reflect their considerable potential as antioxidants, antimicrobial and antiparasitic due to the presence of hydroxyl groups in phenolic compound which serves as a key to their scavenging capacity.[26,37-40]

In vitro treatment of protoscolices

The viability of the protoscolices was significantly minimized when treated with the methanol extract of *R. graveolens* for different times in comparison with the ABZ as control. The methods of treating hydatid cysts are faced with some restrictions; the azole compounds are effective in conditions where the cysts are scarce and tiny, however they are not efficacious in all circumstances and may lead to some harmful and unwanted consequences.[8,41] Consequently, more attention must be paid to the field of ethnobotanical exploration.

The current study showed that the different concentrations of the methanol extracts of *R. graveolens* and *P. Harmala* displayed a significant effect ($P<0.05$) on the viability of the protoscolices at different exposure times, but the *R. graveolens* extract (Figures 1, 2 and 3) was very high compared to ABZ. All concentrations used (10, 20, 30 and 40 mg/mL) for *R. graveolens* extract (Table 3) showed potent protoscolicidal activity in a short period against protoscolices obtained from the organs of infected sheep and goats. The use of 40 mg / mL of *R. graveolens* extract resulted in the 100% death of protoscolices in 75 minutes (1.25 h) whereas when using 20 mg/mL ABZ as the control, a lethal effect of 21% was obtained after 2 h of treatment. In fact, the scolicidal impact of 20 mg/mL ABZ lessened the viability of the protoscolices up to 52.5% after exposure time of 12h and thus showed an efficacy of approximately 50% of what was obtained through the *R. graveolens* extract. In this study, different concentrations of *C. colocynthis* extract (Figure 4) that were tested against protoscolices did not show any significant changes ($P < 0.05$) except a 15% reduction in viability that was obtained after 24 h incubation at the highest concentration (40 mg/mL).

The *R. graveolens* L extracts are considered very effective protoscolicidal agents at lower concentrations compared to different plants tested for the same objective (Figure 5). The use of different plant extracts against protoscolices showed a large extent of variability in the strength of its activity. For example, *Oleaeuropaea* leaf, Nectar scordumkoelzi and *N. tripedale* extracts were effective through achieving complete killing at varying lengths of time, using different extent of concentrations and with varying degrees of toxicity.[41-43] In addition, when assessing the best factor for scolicidal efficacy, many

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

factors must be taken into consideration including the use of lower concentrations, stronger efficacy in the shortest possible period of exposure, cystic fluid stability, scolicidal capacity inside a cyst, less toxicity, more accessibility and capability for quick preparation.[8] The results of using *R. graveolens* in this study were comparable to the scolicidal action of 20% silver nitrate (20 minutes), 95% ethyl alcohol (15 min), 20% hypertonic saline (15 minutes), H_2O_2 3% (15 minutes) and 0.5-1% cetrimide (10 minutes).[44,45] Moreover, inclusion of these scolicidal drugs may lead to manifestation of harmful side effects such as sclerosing colitis, methemoglobinemia and liver necrosis.[46] However, the results of toxicity and inhibition by plant extracts are subject to several factors such as optimal conditions, concentration and purity of the bioactive compounds.[45, 47-50]

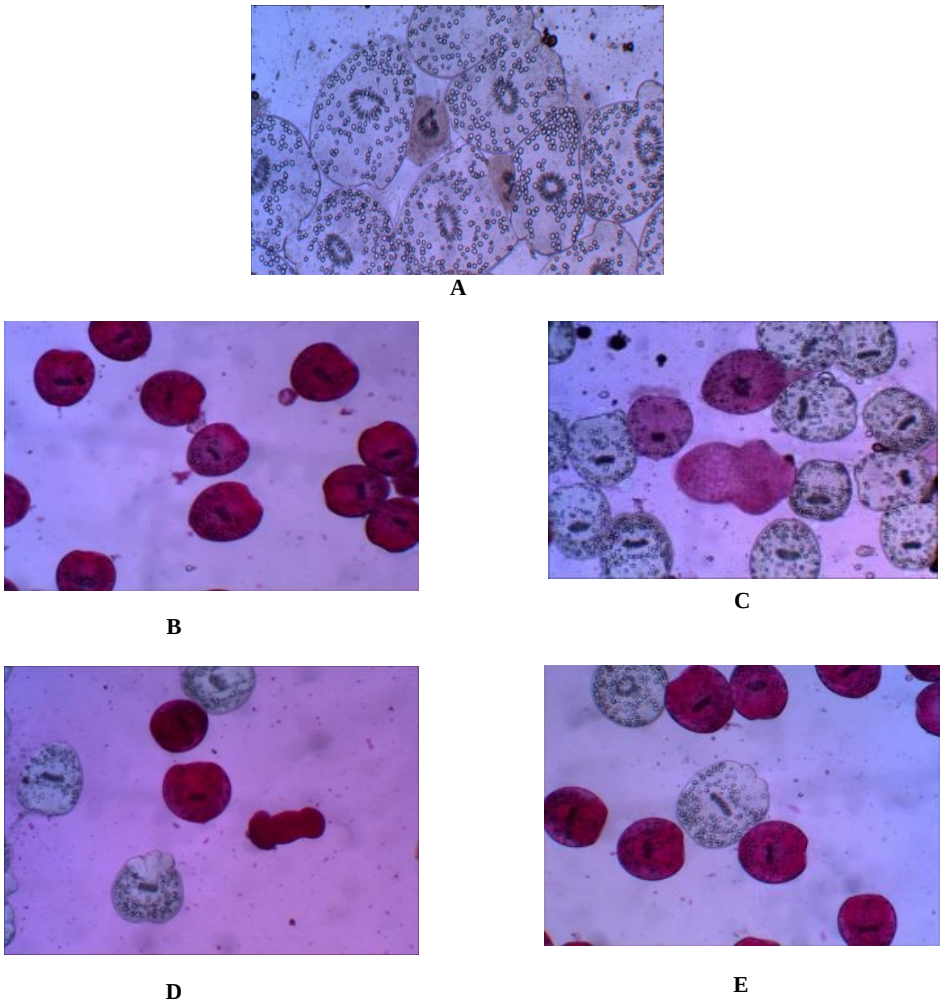

Figure 1: Images of living, dead and partialy dead protoscolices of *E. granulosus* after being stained with 0.1% eosin;
(a), untreated protoscolices with any of extracts. (b), complete mortality of protoscolices after exposure time of 1.25 h (75 minutes) to 40mg/mL of *R. graveolens* extract. (c), partially dead protoscolices (15%) of *E. granulosus* obtained after exposure to 40mg/mL of *C. colocynthis* extract for 24h (d), partially dead protoscolices of *E. granulosus* obtained (50%) after exposure to 40mg/mL of *P. harmala* extract for 24h (d), 95% deadly effect of 20mg/ml ABZ on protoscolices after exposure time for 24h. (Total magnification 100X).

Table 1: Infection rates of hydatidosis among all slaughtered nimals

Infected organs examined			Infection	No. of	No. of Slaughtered	Slaughtered animals
both liver and lung	lung	liver	rates (%)	Infected animals	animals	
0	0	0	0	0	2050	Imported sheep /goats
19	2	4	1.49%	25	1675	Indigenous sheep /goats
19	2	4	0.91%	25	3725	Total slaughteredanimals

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: Percentage yield (%) and Phytochemicals of *R. graveolens*, *P. harmala*, and seeds of *C. colocynthis* methanol extract

Plants	Weight of MeOH plant extract [g]	(%) Yield of MeOH plant extract	Total Phenol's (GAE mg/g)	Total Flavonoids (Rutin equ. mg/g)	Tannin (GAE mg/g)
R. graveolens	16.46	23.51	25.53 ± 0.49	6.66 ± 0.31	8.035 ± 0.049
P. harmala,	14.36	20.50	18.25 ± 0.04	8.385 ± 0.71	6.41 ± 0.80
C. colocynthis	8.40	12.0	17.60 ± 0.39	9.81 ± 0.53	5.70 ± 0.07

Table 3: Effect of treatment with methanol extracts of *R. graveolens*, *P. harmala* and *C. colocynthis* on the relative viability of protoscolices as a function of time (h). Data represent mean values ± SD; n = 3. The significance of the differences was calculated using Tukey's test, with $P < 0.05$ indicating a significant difference.

Source of extract	Concentration mg/mL	Relative viability of protoscolices Treatment duration (h)					
		1	3	6	12	18	24
R. graveolens∗	10	0.360 ± 0.05	0.125 ± 0.03	0.040 ± 0.03	0.00	0.00	0.00
	20	0.150 ± 0.02	0.025 ± 0.0015	0.015 ± 0.002	0.00	0.00	0.00
	30	0.155 ± 0.03	0.020 ± 0.001	0.00	0.00	0.0	0.00
	40	0.020* ± 0.001	0.00	0.00	0.00	0.00	0.00
P. harmala	10	0.971 ± 0.015	0.960 ± 0.004	0.923 ± 0.010	0.903 ± 0.012	0.884 ± 0.014	0.853 ± 0.020
	20	0.952 ± 0.009	0.909 ± 0.022	0.816 ± 0.013	0.717 ± 0.017	0.685 ± 0.008	0.663 ± 0.010
	30	0.916 ± 0.006	0.834 ± 0.015	0.778 ± 0.023	0.721 ± 0.027	0.597 ± 0.047	0.543 ± 0.014
	40	0.855 ± 0.023	0.808 ± 0.024	0.737 ± 0.035	0.663 ± 0.021	0.573 ± 0.056	0.500 ± 0.017
C. colocynthis	10	0.97 ± 0.01	0.95 ± 0.02	0.94 ± 0.03	0.92 ± 0.02	0.90 ± 0.02	0.90 ± 0.02
	20	0.98 ± 0.01	0.96 ± 0.01	0.96 ± 0.01	0.94 ± 0.02	0.92 ± 0.02	0.90 ± 0.02
	30	0.96 ± 0.01	0.95 ± 0.01	0.93 ± 0.02	0.91 ± 0.02	0.90 ± 0.01	0.87 ± 0.02
	40	0.95 ± 0.01	0.93 ± 0.01	0.92 ± 0.01	0.90 ± 0.01	0.88 ± 0.02	0.85 ± 0.02
ABZ	20	0.97 ± 0.02	0.90 ± 0.02	0.77 ± 0.05	0.50 ± 0.05	0.28 ± 0.03	0.04 ± 0.03
Control		0.99 ± 0.01	0.99 ± 0.01	0.98 ± 0.02	0.98 ± 0.01	0.98 ± 0.01	0.97 ± 0.00

∗ Taking into account that methanol extract of *R. graveolens* showed complete killing (0.0 viability) of protoscolices (40 mg/ml) after 1.25 h (75 minutes), the two hours' time point was considered as interval instead of three hours' time point.

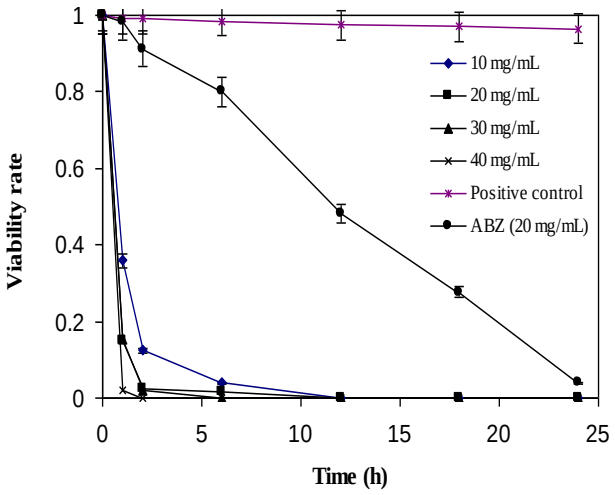

Figure 2: Effect of various concentration of *R. graveolens* methanol extract on the protoscolices viability of hydatid cysts at different exposure periods compared with 20 mg/ml (ABZ).

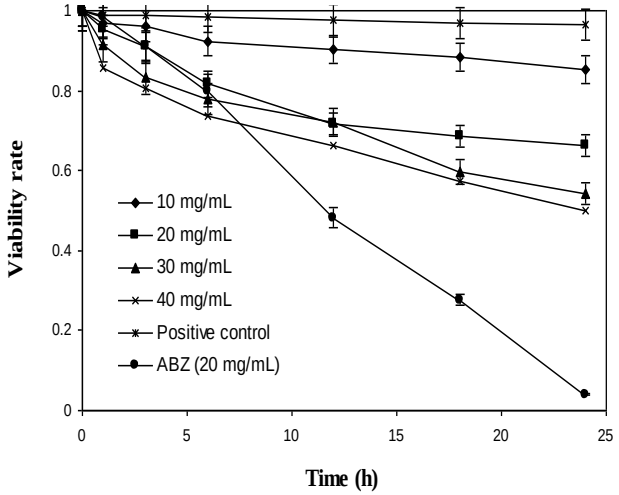

Figure 3: Effect of various concentration of *P. harmala* extract methanol extract on the protoscolices viability of hydatid cysts at different exposure periods compared with 20 mg/ml (ABZ).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

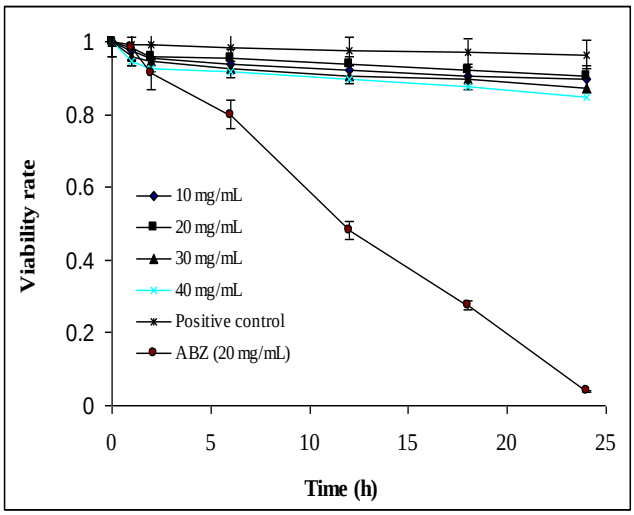

Figure 4: Effect of various concentration of *C. colocynthis* methanol extract on the protoscolices viability of hydatid cysts at different exposure periods compared with 20 mg/mL (ABZ).

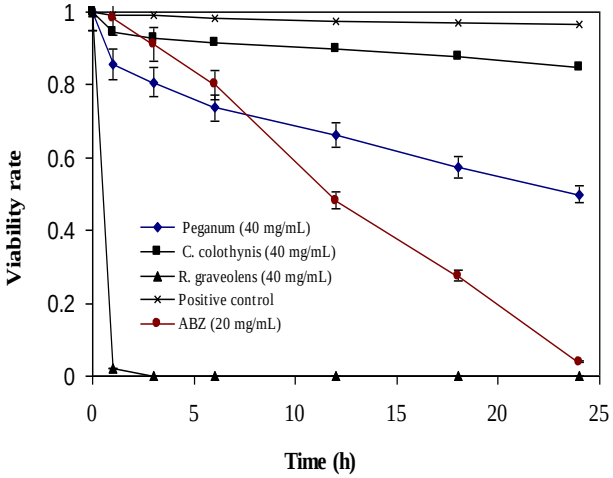

Figure 5: The effect of using 40 mg/mL extract for each of the three plants (R. graveolens, C. colocynthis and P. harmala) on the viability of *E. granulosus* protoscolices as a function of time as compared with using 20 mg/ml of ABZ**.**

The cytotoxic activity of R. graveolens extract

Previous studies on the alcoholic extract of *R. graveolens* proved its non-toxicity to rat liver by studying pathological changes.[51] Since only *R. graveolens* L extract gave promising results, unlike the other two plants (*P. harmala and C. colocynthis*), it was necessary to examine its methanol extract toxicity against fibroblast cells. It was found that the use of *R. graveolens* extract at a concentration of 100 mg/mL exhibited very low cytotoxicity against the fibroblast producing 72.8%, mortality of fibroblast cells with IC_{50} values of 65.78 mg/ml. The treatment of fibroblast cells with 100 mg/mL of *R. graveolens* L. extract for 72 hours showed a significant decrease in their proliferation compared with control. Carvalho et al. [10] showed that *R. graveolens* extract and its rich alkaline fractions (Rg-FAE) were effective against the adults of *Schistosoma mansoni* worms, while no significant cytotoxicity to macrophages was demonstrated.

On the other hand, the result of Schelz *et al.*[52] who demonstrated that the alkaloid constituents (isogravacridone chlorine (IGC), rutacridone) isolated from *R. graveolens*, had a pronounced effect on cell proliferation of MDA-MB-231 with an IC_{50} value of 2.27 µM. However, the same extract had a very cytotoxic action against Mehr-80 (IC50 = 46.2 µg/mL).[53] In addition, chalepin compound isolated from *Ruta angustifolia* L. showed excellent cytotoxicity against A549 cell line with an IC_{50} value of 8.69 ± 2.43 µg/mL.[54] Arora and Tandon, [55] reported that *R. graveolens* 30CH had high ability to decrease cell viability and cell migration by increasing apoptosis of the human colon cancer. These results open the track potential use of *R. graveolens* extract as ideal agent that is both effective and safe for the host at the same time.

Conclusion

The methanol extract of *R. graveolens* (40 mg/ml) caused strong protoscolicidal effect at a shorter exposure time (1.25 hr) compared to 96% lethal effect obtained by using 20 mg/ml ABZ after 24 h treatment. The scolicidal effect of various concentrations of the *R. graveolens* methanol extract was significantly ($P < 0.05$) higher than that of the standard drug (ABZ) at all exposure times, thus, it can be used instead of other protoscolicidal agents that usually have side effects. Therefore, it is necessary to emphasize the urgent need for surgeons to overcome the obstacles of hydatid cyst surgery to conduct in vivo studies to monitor any side effects on the human body through using R. graveolens extract as natural scolicidal agents in surgery.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The researchers would like to thank the deanship of scientific research at the University of Jordan, for offering financial support of this project.

References

1. Al-Musawi SAA. Evaluate effectiveness of aqueous extract of the Eucalyptus glubules plant leaves on the growth and vitality of Hydatid Cysts Protoscolices of Echinococcus granulosus *In Vitro*. Paper presented at: Journal of Physics. 2019; Conf. Ser. 1234(1):1-6.
2. Malekifard F and Keramati F. Susceptibility of Protoscoleces of Hydatid Cyst to Various Concentrations of Oak Gall (Quercus infectoria Olivier) Extract at Different Exposure Times In Vitro. Zahedan J Res Med Sci. 2018; 20(5):1-6.
3. Hijjawi NS, Al-Radaideh AM, Rababah EM, Al-Qaoud KM, Bani-Hani KE. Cystic echinococcosis in Jordan: a review of causative species, previous studies, serological and radiological diagnosis. *Acta tropica*. 2018; 179:10-16.
4. Nasrieh MA and Abdel-Hafez SK. Echinococcus granulosus in Jordan: assessment of various antigenic preparations for use in the serodiagnosis of surgically confirmed cases using enzyme immuno assays and the indirect haemagglutination test. Diagn Microbiol Infect Dis.2004; 48(2) 117-123.
5. Ajlouni AQ, Saliba EK, Disi AM. Intestinal cestodes of stray dogs in Jordan. *Z Parasitenk.* 1984; 70(2):203-210.

6. Monteiro DU, Azevedo MI, Weiblen C, Botton SDA, Funk NL, Da Silva CDB, Zanette RA, Schwanz TG, DE LA RUE ML. *In vitro* and *ex vivo* activity of Melaleuca alternifolia against protoscoleces of Echinococcus ortleppi. Parasitol. 2017; 144(2):214.
7. Ohiolei JA, Yan H-B, Li L, Zhu G-Q, Muku RJ, Wu Y-T, Jia W-Z. Review of cystic echinococcosis in Nigeria: a story of neglect. Acta parasitol. 2020; 65(1):1-10.
8. Mahmoudvand H, Harandi MF, Shakibaie M, Aflatoonian MR, ZiaAli N, Makki MS, Jahanbakhsh S. Scolicidal effects of biogenic selenium nanoparticles against protoscolices of hydatid cysts. Int J Surg. 2014; 12(5):399-403.
9. Yones DA, Taher GA, Ibraheim ZZ. *In vitro* effects of some herbs used in Egyptian traditional medicine on viability of protoscolices of hydatid cysts. Korean J Parasitol. 2011; 49(3):255.
10. Carvalho LSAd, Queiroz LS, Alves Junior IJ, Almeida AdC, Coimbra ES, de Faria Pinto P, Silva MPNd, De Moraes J, Da Silva Filho AA. *In vitro* schistosomicidal activity of the alkaloid-rich fraction from Ruta graveolens L.(Rutaceae) and its characterization by UPLC-QTOF-MS. Evid-Based Compl Altern Med. 2019; 2019:1-8.
11. Benedito RB, Alves MF, Pereira WB, de Arruda Torres P, Costa JP, da Rocha Tomé A, de Cássia da Silveira e Sá R, de Sousa DP, Ferreira PMP, de Freitas RM. Perillyl Alcohol: Antinociceptive Effects and Histopathological Analysis in Rodent Brains. Nat Prod Commun. 2017; 12(9):1385-1389.
12. Oran S and Al-Eisawi D. Check-list of medicinal plants in Jordan. *Dirasat.* 1998; 25(2):84-112.
13. Oran SA. The status of medicinal plants in Jordan. J Agric Sci Technol. 2014; 4:46-467.
14. Althunibat OY, Qaralleh H, Al-Dalin SYA, Abboud M, Khleifat K, Majali IS, Aldal'in H, Rayyan WA, Jaafraa A. Effect of thymol and carvacrol, the major components of Thymus capitatus on the growth of Pseudomonas aeruginosa. J Pure Appl Microbiol. 2016; 10(1):367-374.
15. Alqaraleh M, Kasabri V, Aljaafreh A, Al-Majali I, Al-Othman N, Khleifat K, Khaleel S, Al-Tawarah NM, Qaralleh H, Al-majali M. Evaluation of the antiglysation effect of branched chain amino acids and phytochemical compounds on RAW 264.7 cell line and their synergistic effect on colorectal cell line panel. Trop J Nat Prod Res. 2021; 5(1):88-93.
16. Yousef I, Oran S, Alqaraleh M, Bustanji Y. Evaluation of cytotoxic, antioxident and antibacterial activities of *Origanum dayi, Salvia palaestina* and *Bongardia chrysogonum* plants growing wild in Jordan. Trop J Nat Prod Res. 2021; 5(1):66-70.
17. De Queiroz AC, Dias TdLMF, Da Matta CBB, Cavalcante Silva LHA, de Araújo-Júnior JX, Araújo GBd, Moura FdBP, Alexandre-Moreira MS. Antileishmanial activity of medicinal plants used in endemic areas in northeastern Brazil. Evid-Based Compl Altern Med. 2014; 2014:1-9.
18. Amabye TG and Shalkh TM. Phytochemical screening and evaluation of antibacterial activity of Ruta graveolens L.-A medicinal plant grown around Mekelle, Tigray, Ethiopia. Nat Prod Chem Res. 2015; 3(6):115-118.
19. Alqaraleh M and Kasabri V. The antiglycation effect of monomethyl branched chained fatty acid and phytochemical compounds and their synergistic effect on obesity related colorectal cancer cell panel. Romanian J Diabetes Nutr Metab Dis. 2019; 26(4):361-369.
20. Moloudizargari M, Mikaili P, Aghajanshakeri S, Asghari MH, Shayegh J. Pharmacological and therapeutic effects of Peganum harmala and its main alkaloids. Pharmacogn Rev. 2013; 7(14):199.
21. Moazeni M, Borji H, Darbandi MS, Saharkhiz MJ. *In vitro* and in vivo antihydatid activity of a nano emulsion of Zataria multiflora essential oil. Res Vet Sci. 2017; 114:308-312.
22. Mamedov NA, Pasdaran A, Mamadalieva NZM. Pharmacological studies of Syrian rue (*Peganum harmala* L., Zygophyllaceae). I J Second Metab. 2018; 5(1):1-6.
23. Rezaee M and Hajighasemi F. Sensitivity of hematopoietic malignant cells to *Peganum harmala* seed extract *in vitro*. J Basic Clin Pathophysiol. 2019; 7(1):21-26.
24. AL-Khlifeh, EM, Khleifat KM, Al-Tawarah N, Al-Limoun M, Abdel-Ghani A, Alsharafa K, Qaralleh H. Genetic diversity and chemical composition of *Juniperus phoenicea* L reflect on Its antimicrobial activity. Int J Pharm Res. 2021; 13(1):3409-3426
25. Ahmed CN, Hamad KK, Qadir FA. Haemonchus contortus as a model in assessing activity of *Citrullus colocynthis* fruit extract to control benzimidazole-resistant parasitic nematodes. Zanco J Pure Appl Sci. 2019; 31(5):61-70.
26. Al Qaisi YT, Khleifat KM, Oran SA. *In Vitro* Quantitative Assessment of Viability of Echinococcus granulosus Protoscoleces after Treatment with Ruta graveolens L. Methanol Extract. Int J Pharm Res. 2021; 13(1):3841-3850.
27. Singleton VL and Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965; 16(3):144-158.
28. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 1999; 64(4):555-559.
29. Tamilselvi N, Krishnamoorthy P, Dhamotharan R, Arumugam P, Sagadevan E. Analysis of total phenols, total tannins and screening of phytocomponents in Indigofera aspalathoides (Shivanar Vembu) Vahl EX DC. J Chem Pharm Res. 2012; 4(6):3259-3262.
30 Smyth J and Barrett N. Procedures for testing the viability of human hydatid cysts following surgical removal, especially after chemotherapy. Trans R Soc Trop Med Hyg. 1980; 74(5):649-652.
31. Al-Arabi F, Mehdi M, Farooqui M, Pradhan V. The effect of Aloe vera extracts on the viability of Echinococcus granulosus protoscolices. Int Res J Pharm. 2019; 10(4):184-189.
32. Al-Eryani M. Comparative study on the efficiency on Ivermectin drug and Peganum harmala seed extract on protoscoleces hydatid cyst, MSc Thesis, Al-Mustansiriya University, Baghdad; 2002.
33. Blanton RE, Wachira TM, Zeyhle EE, Njoroge EM, Magambo JK, Schantz PM. Oxfendazole treatment for cystic hydatid disease in naturally infected animals. Antimicrob Agents Chemother. 1998; 42(3):601-605.
34. Tarawneh K, Irshaid F, Jaran A, Ezealarab M, Khleifat K. Evaluation of antibacterial and antioxidant activities of methanol extracts of some medicinal plants in northern part of Jordan. J Biol Sci. 2010; 10(4):325-332.
35. Alqaraleh M, Kasabri V, Al-Othman N. Evaluation of Pancreatic and Extra Pancreatic Effects of Branched Amino Acids. Romanian J Diabetes Nutr Metab Dis. 2019; 26(2):199-209.
36. Alqaraleh M, Kasabri V, Mashallah S. Evaluation of Anticancer and Anti-Inflammatory Properties of Branched Chain Amino Acids. J Biochem Cell Biol. 2018; 1(2):108-118.
37. Khleifat KM, Matar SA, Jaafreh M, Qaralleh H, Al-limoun MO, Alsharafa KY. Essential Oil of Centaurea damascena Aerial Parts, Antibacterial and Synergistic Effect. J Essent Oil-Bear Plants. 2019; 22(2):356-367.
38. Al-Tawarah NM, Qaralleh H, Khlaifat AM, Nofal MN, Alqaraleh M, Khleifat KM, Al-limoun MO, Al Shhab MA. Anticancer and Antibacterial Properties of Verthemia Iphionides Essential Oil/Silver Nanoparticles. Biomed Pharmacol J. 2020; 13(3):1175-1185.

39. Vijay Avin BR, Thirusangu P, Lakshmi Ranganatha V, Firdouse A, Prabhakar BT, Khanum SA. Synthesis and tumor inhibitory activity of novel coumarin analogs targeting angiogenesis and apoptosis. Eur J Med Chem. 2014; 75:211-221.
40. Tarawneh M, Al-Jaafreh AM, Al-Dal'in H, Qaralleh H, Alqaraleh M, Khataibeh M. Roasted date and barley beans as an alternative's coffee drink: micronutrient and caffeine composition, antibacterial and antioxidant activities. Syst Rev Pharm. 2021; 12(1):1079-1083.
41. Niazi M, Saki M, Sepahvand M, Jahanbakhsh S, Khatami M, Beyranvand M. *In vitro* and *ex vivo* scolicidal effects of Olea europaea L. to inactivate the protoscolecs during hydatid cyst surgery. Ann Med Surg. 2019; 42:7-10.
42. Mahmoudvand H, Fallahi S, Mahmoudvand H, Shakibaie M, Harandi MF, Dezaki ES. Efficacy of Myrtus communis L. to inactivate the hydatid cyst protoscoleces. J Invest Surg. 2016; 29(3):137-143.
43. Galehdar N, Niazi M, Jahanbakhsh S, Mahmoudvand H, Rouientan A. Evaluation of Protoscolicidal Effects of Nectaroscordum Koelzi Methanol Extract Against Hydatid Cyst Protoscoleces. Entomol Appl Sci Lett. 2018; 5(1):72-76.
44. McCutcheon A, Ellis S, Hancock R, Towers G. Antibiotic screening of medicinal plants of the British Columbian native peoples. J Ethnopharmacol. 1992; 37(3):213-223.
45. Qaralleh H, Khleifat KM, Al-Limoun MO, Alzedaneen FY, Al-Tawarah N. Antibacterial and synergistic effect of biosynthesized silver nanoparticles using the fungi Tritirachium oryzae W5H with essential oil of Centaurea damascena to enhance conventional antibiotics activity. Adv Nat Sci Nanosci Nanotechnol. 2019; 10(2):025016.
46. Mahmoudvand H, Dezaki ES, Kheirandish F, Ezatpour B, Jahanbakhsh S, Harandi MF. Scolicidal effects of black cumin seed (Nigella sativa) essential oil on hydatid cysts. Korean J Parasitol. 2014; 52(6):653-659.
47. Al-Asoufi A, Khlaifat A, Tarawneh A, Alsharafa K, Al-Limoun M, Khleifat K. Bacterial Quality of Urinary Tract Infections in Diabetic and Non Diabetics of the Population of Ma'an Province, Jordan. Pak J Biol Sci. 2017; 20(4):179-188.
48. Khleifat KM, Abboud M, Omar S, Al-Kurishy J. Urinary tract infection in South Jordanian population. *J Med Sci.* 2006; 6(1):5-11.
49. Khleifat KM, Abboud MM, Al-Mustafa AH, Al-Sharafa KY. Effects of carbon source and Vitreoscilla hemoglobin (VHb) on the production of β-galactosidase in Enterobacter aerogenes. Curr Microbiol. 2006; 53(4):277-281.
50. Abboud MM, Aljundi IH, Khleifat KM, Dmour S. Biodegradation kinetics and modeling of whey lactose by bacterial hemoglobin VHb-expressing Escherichia coli strain. Biochem Eng J. 2010; 48(2):166-172.
51. Nabaei M, Mesbah A, Ghavami H, Saeidinia A, Keihanian F. Effects of Ruta graveolens extract on Histopathologic changes in mice livers. Int J Pharm Res Scholars. 2014; 3(2):675-680.
.52 Schelz Z, Ocsovszki I, Bozsity N, Hohmann J, Zupko I. Antiproliferative effects of various furanoacridones isolated from Ruta graveolens on human breast cancer cell lines. Anticancer Res. 2016; 36(6):2751-2758.
53. Varamini P, Soltani M, Ghaderi A. Cell cycle analysis and cytotoxic potential of Ruta graveolens against human tumor cell lines. *Neoplasma.* 2009; 56(6):490-493.
54. Richardson JSM, Aminudin N, Abd Malek SN. Chalepin: A compound from Ruta angustifolia L. pers exhibits cell cycle arrest at S phase, suppresses nuclear factor-kappa B (NF-κB) pathway, signal transducer and activation of transcription 3 (STAT3) phosphorylation and extrinsic apoptotic pathway in non-small cell lung cancer carcinoma (A549). Pharmacogn Mag. 2017; 13(Suppl 3):489-498.
55. Arora S and Tandon S. DNA fragmentation and cell cycle arrest: a hallmark of apoptosis induced by Ruta graveolens in human colon cancer cells. *Homeopathy.* 2015; 104(1):36-47.