Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 11708 - 11716
Received 05 March 2021; Accepted 01 April 2021.

Preparation and Study of a Novel Thiazole Derivative and Its Biological Efficacy on Male Rats Infected with *Echinococcus Granulosus* Using Electron Microscope

[1]Abdul–Jaleel Aziz Karim Alqaraghli and [*1]Mansoor Jadaan Ali AlKhaled
[1] Department of Biology, College of Education, University of Al-Qadisiyah, Iraq
[*1]Department of Microbiology, College of Vet. Medicine, University of Al-Qadisiyah, Iraq
abduljalil.aziz@qu.edu.iq ; mansoor.ali@qu.edu.iq

Abstract:

This study included the preparation of a novel thiazole derivative and its spectroscopic diagnosis, as it was prepared from crushing solid iodine with Thiourea well, then adding it with Pregnenolone. The compound was diagnosed using spectroscopic methods that included an IR Spectra, ^{1}H-NMR spectra and a mass spectra, and the results were virtually identical to expectations.

The lethal dose was determined to half 50 (LD_{50}) for the compound after it was dissolved with DMSO, and then the safe therapeutic dose was calculated, which was 0.18 g/ kg.

This compound was dosed orally as a therapeutic dose for a group of male rats, after they were infected with hydatid cysts. To find out the therapeutic efficacy of it on the hydatid cyst, and to compare it with a group that was infected with the same disease and treated with Albendazole, the dosing period lasted 30 days for each of the above groups.

The results of the Field Emission Scanning Electron Microscope (FE-SEM)study of the hydatid cystsof the positive control group showed that the hydatid cyst contains aGerminal Layer (GL) and contains large numbers of Protoscolices. In the infected group treated with Albendazole, the number ofProtoscolices in the GLwas very small, and the general cyst had a wrinkled appearance. As for the infected group and treated with the prepared compound, the hydatid cyst showed the presence of many erosion-like cracks in the walls of the cyst, and the absence of Protoscolices in GL.

Keywords: *Echinococcusgranulosus*, Thiazole derivative, Electron microscope

Introduction:

E.granulosus is one of the parasites that are transmitted from dogs to humans, and causes hydatid cysts, as it is one of the main diseases that affect humans as well as field animals [**1**].

Signs and symptoms of liver echinococcosis include enlarged liver (with or without a palpable mass in the right upper quadrant), epigastric pain, nausea and vomiting. If the cyst ruptures, the sudden release of its contents can lead to allergic reactions ranging from mild to fatal anaphylaxis[2].

Until recent decades, surgery was the only treatment option for hydatid cyst, and it was chemically treated with benzimidazole. More recently, Cyst puncture, Percutaneous aspiration, Injection of chemicals, and Reaspiration. It is seen as a better or alternative option to surgery [1].

Benzimidazoles (Albendazole and Mebendazole) are currently used either alone or in combination with Praziquantel for non-operative treatment of cysticercosis, and as a complementary treatment before and after surgery. This treatment has succeeded in many cases, especially in humans, as the health conditions of many individuals have improved when they use it. Because of stunted growth. However, failure of these drug treatments has been recorded, and there are severe side effects such as liver toxicity, which usually leads to discontinuation of its use [3].

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 11708 - 11716
Received 05 March 2021; Accepted 01 April 2021.

Azoles are classes of pentagonal heterocyclic nitrogenous compounds. They contain at least a non-carbon atom in their structure of nitrogen, sulfur or oxygen [4]. These compounds exhibit aromatic properties, two double bonds, and only one pair of electrons in each heterogeneous ion in the ring [4].
Mebendazole (an azole derivative) was first used to treat Hydatid cyst, however, its absorption was poor from the gastrointestinal tract [5], so it was soon replaced by Albendazole which is more easily absorbed.This drug had better efficacy due to its metabolite, albendazolesulfoxide, which expands easily from the cyst's membrane and concentrates in the cyst fluid [6]. Albendazole inhibits the polymerization of tubulin in the microtubules and has a higher affinity for the parasite tubulin compared to the host [7], by inhibiting glucose uptake in the parasite and depletingglycogen stores [8].

Material and methods

All chemicals were purchased from BDH, FT-IR spectra were measured in KBr, a Shimadzu spectrophotometer within the range of 4000-200 cm^{-1} was used in the KBr disk of the synthesized compound. Melting points were measured with a thermoelectric device, the Bruker DRX System AL500 (500 MHz) spectrometer in DMSO, the chemical transformation in ppm relative to the internal TMS. The partial (C, H, N) analysis of the ligands is performed with the CHNS-O PerkinElmer model 2400-11-Mass spectra recorded using Agilent 5975 mass spectrometry techniques.

Synthesis of chemical compound

The component mixedby crushed 0. 075 mol (19.035 g) of iodine was steel with 0. 15 mol (11.418 g) of thiourea well, then added to 0. 075 mol (23.7367 g) of pregnenolonewas placed in a three-hole reaction flask, and then the reaction mixture was heated on a water bath at a temperature between 80 – 90 ℃ with stirring for 8 hours. Then it was cooled and washed with diethyl ether and washed twice and saturated aqueous solution of sodium thiosulphate, then pour in ice water, then nominated and sediment collected and dry, and then recrystallized from hot ethanol/ the acetone cold [9]. Physical properties and analytical data were recorded in Table 1.

Table 1: Physical properties and analytical data for the synthesized compound

Molecular formula	M. Wt	m.p C°	Color	Yield %	P H
$C_{22}H_{32}N_2OS$	372.57	243 – 244	Light brown	77	5.2

Determination of median lethal dose

Male rats were divided into random groups, each group of 10 rats, and dosed with the compound prepared orally using a stomach tube syringe, after dissolving it with DMSO to determine LD_{50}. Rats were followed for 72 hours after each dose; To note weakness, unsteady gait, loss of balance, and death for these animals, the dosing was in progressive proportions. After the trial ended, the therapeutic dose of the compound was determined based on the Wilcoxon (1949) equation [10] below.

$$LD_{50} = \text{highest dosage} - \frac{\sum ab}{n}$$

Where, LD_{50} is the lethal dose 50, highest dosage is the dose with 100% mortality of rats , a is the value of difference between the previous and next dose, b is the summation of dead

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 11708 - 11716
Received 05 March 2021; Accepted 01 April 2021.

animal for each dose (previous dose + next dose/ 2) and n is the number of animals used for each dose.

Parasite study

Hydatid cyst samples were obtained from infected sheep from the Najaf massacre in Najaf province. Those samples were transferred directly to the Department of Biology/ College of Education/ University of Al-Qadisiyah after placing them in plastic containers and placing ice in those containers. To preserve Protoscolices inside the cyst [11].

Isolate of Protoscolices

Protoscolices were obtained from hydatid cysts in the laboratory by placing the livers of infected sheep containing these cysts in a sterile dish, and sterilizing its surface with a piece of cotton moistened with ethyl alcohol at a concentration of 70%, then puncturing the cyst with a medical syringe of 10 ml. to withdraw as much liquid of the cyst as possible to reduce the pressure inside.

The cysts were then opened with a scalpel, and the secondary cysts and GL were extracted; It contains the largest number of Protoscolices. After that, the capsules were kept in 4 ml of Kerb's Ringer Solution per 1 ml of suspension; to estimate the vitality of Protoscolices and calculate their number [12].

Injection of rats with viable protoscolice

Male laboratory rats were injected after knowing the appropriate number of live protoscolices in a volume of 0.7 mL of protoscolices suspension and Phosphate Buffered Saline (PBS). After dividing the laboratory animals into random groups, with 10 rats per group, the injection site was sterilized with 70% ethyl alcohol then rats were injected with 2×10^3 live protoscolices.In the intraperitoneal (IP) cavity[13].

Treatment of the infected rats

Rats infected with hydatid cysts were treated 6 months after injection, as a group of oral administration was dosed with the prepared compound at a dose of 0.18 g/ kg (after dissolving it with 0.1 ml of DMSO), which is the therapeutic dose of the compound, and the dose was at a rate of once daily for a month, the second group was dosed with the drug Albendazole at a dose of 0.5 mg/ ml/ rat at a rate of once daily for a period of a month. The third group was infected and was not treated as a positive control group. The fourth group was not infected nor treated, and it was used as a negative control group.

Field Emission Scanning Electron Microscope (FE-SEM)

FE-SEM was used; this is for the purpose of revealing the external shape of the protoscolices and layers of the hydatid cyst. The slides were prepared according to the method used befor[14].

Results

The novel thiazole derivative synthesized in a good yield as follows Scheme 1.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 11708 - 11716
Received 05 March 2021; Accepted 01 April 2021.

Scheme 1: Synthesis of thiazole derivative

^{1}H-NMR spectra

The ^{1}H-NMR spectraof the first component was marked by the appearance of a single signal at a chemical displacement of 6.21 ppm. They belong to the protonated group of amines in the thiazole ring. The proton signal of the thiazole ring also showed a single signal at 5.94 ppm near the signal of the double junction protons (C = CH) at 5.84 ppm. In addition, the alcoholic OH group proton signal appeared at 5.01 ppm. The appearance of the signal of the protons of the aliphatic rings of the cholesterol nucleus also showed in the form of multiple signals at 0.43 - 2.02 ppm belonging to 26 protons . Fig. 1.

IR Spectra

IR Spectra showed the prepared compound a group of beams, using the KBr disk, as the compound showed six distinct beams due to the stretchy vibrations of the groups :
[OH and NH2, Ar – H and C=CH, C–H aliphatic, C=N, C=C, C–O] which occurred within ranges:
[1195, 1442, 1643, 2800 - 2919, 3116, 2700 - 3500] cm $^{-1}$ respectively.

The disappearance of the amplitude oscillation beam of the C=O ketone group of the pregnenolone complex and the appearance of the amplitude oscillation beam of the C = N group of the thiazole ring formed at 1643 cm -1 confirms the validity of the proposed composition of the prepared compound. The spectrum also showed a broad band due to the beams of amplitude oscillation of the two groups OH and NH2 from 2700 to 3500 cm -1 due to the hydrogen interaction. The spectrum also showed infrared absorption beams for the aurmic and olefinic group C – H at 3116 cm -1. Table (2) and Fig. (2) illustrate this.

Mass Spectra

The mass spectra of the compound was characterized by the appearance of the molecular ion ($M^{+\cdot}$) signal at m/ z It is equal to 372, as fig. (3) shows the pattern of fragmentation of the prepared compound, which accurately shows the formation of the compound according to the method of fragmentation, which indicates the validity of the proposed structures for the compound [15].

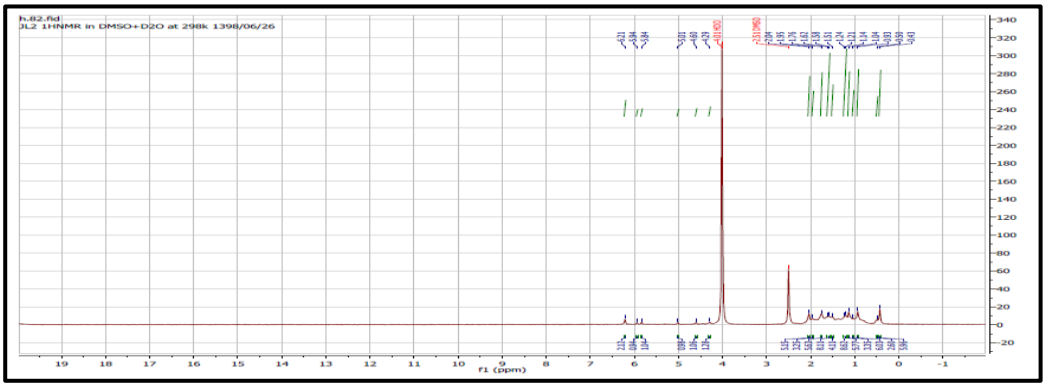

Fig. 1: ^{1}H-NMR spectra of thiazole derivative

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 11708 - 11716
Received 05 March 2021; Accepted 01 April 2021.

Table 2 : Selected Bands of Diagnostic Importance from the IR Spectrafor the prepared compound

Compound	N–H and O–H Str.	Ar–H and C=CH Str.	C–H Aliphatic Str.	C=N Str.	C=C Str.	C–O Str.
$C_{22}H_{32}N_2OS$	3500 – 2700 s	3116 m	2919 – 2800 m	1643 s	1442 s	1195 m
Str. = Stretching , m= medium , s=strong , w=weak						

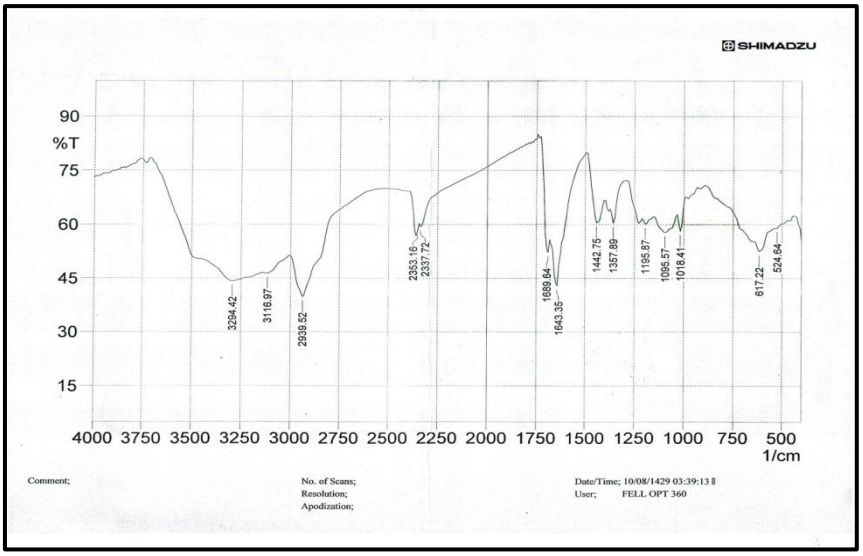

Fig. 2: IR Spectrafor the prepared compound

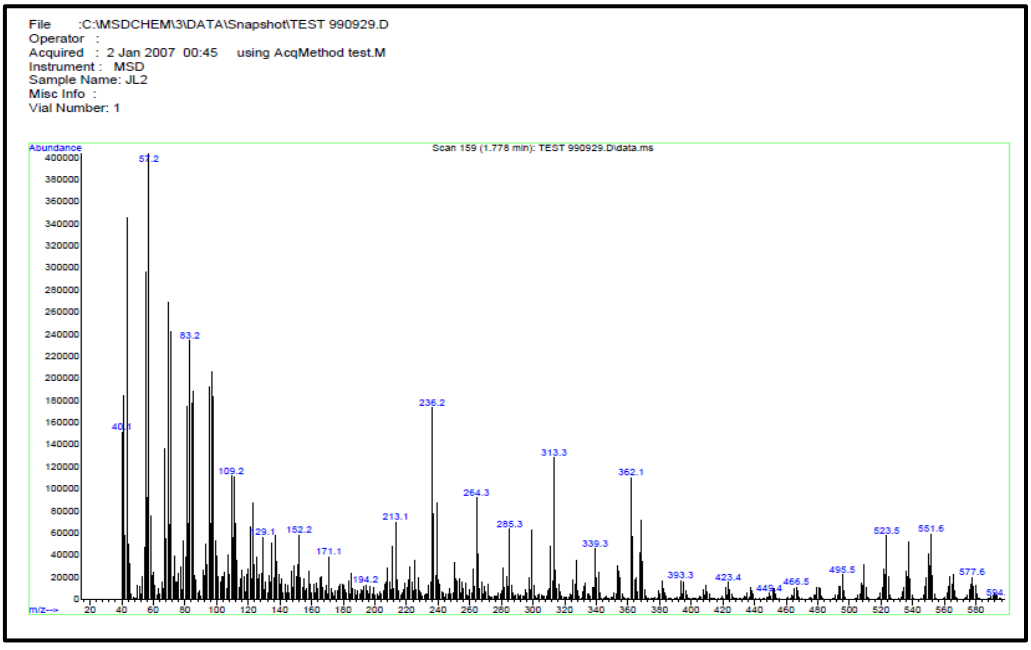

Fig. 3: mass spectra for the prepared compound .

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 11708 - 11716
Received 05 March 2021; Accepted 01 April 2021.

Determination of LD 50

The LD_{50} has been determined for the compound , by dosing elevated concentrations of this compound by rats oral administration, After dissolving it with DMSO. The death of rats was followed up with increasing dose concentrations, and the concentration of the dose that killed all rats was determined, and then the value of the safe therapeutic dose was calculated for it. In order to dose them for male rat as table 3.

Table 3: value LD_{50} for compound applied tomale rats

Dose G / kg	Number of animals	number of dead animals	a	b	a × b
1	10	0	–	–	–
2	10	0	1	0	0
3	10	2	1	1	1
3.5	10	3	0.5	2.5	1.25
4	10	6	0.5	4.5	2.25
4.5	10	8	0.5	7	3.5
5	10	10	0.5	9	4.5
					$\sum$ ab = 12.5

$$LD_{50} = 3 - \frac{12.5}{10}$$

$$= 3 - 1.25 = 1.75 \approx 1.8\ \text{g/Kg}$$

The LD_{50} was then divided by 10, so that the safe therapeutic dose was 0.18 g/ kg.

Field Emission Scanning Electron Microscope (FE-SEM)

After dissecting the animals and sending hydatid cyst samples to the University of Tehran/ Iran. The results for the different groups showed depth details, whether it was phenotypic details of hydatid cyst composition of the positive control group in which the rats were infected with *E. granulosus* without treatment, or their combination in the albendazole treated groups, and the prepared compound.

In the positive control group, the hydatidcyst sections demonstrated the GL, which contained large numbers of Protoscolices. Fig. 4 illustrates this.

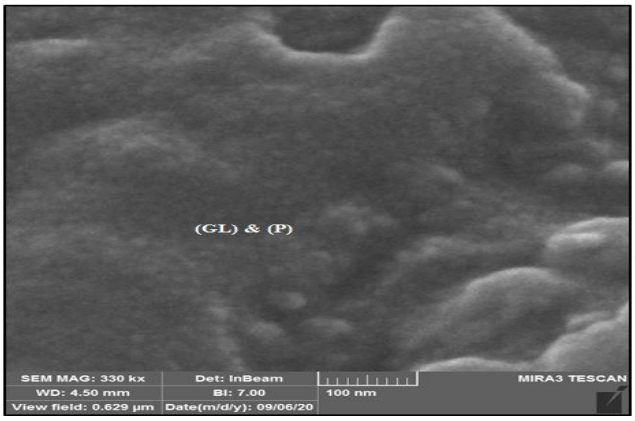

Fig. 4: Germinal layer (GL) containing large numbers of Protoscolices (P).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 11708 - 11716
Received 05 March 2021; Accepted 01 April 2021.

The second group infected and treated with Albendazole had very few protoscolicesin the germinal layer, and the general appearance of the cyst was wrinkled. Fig. 5 illustrates this.

P

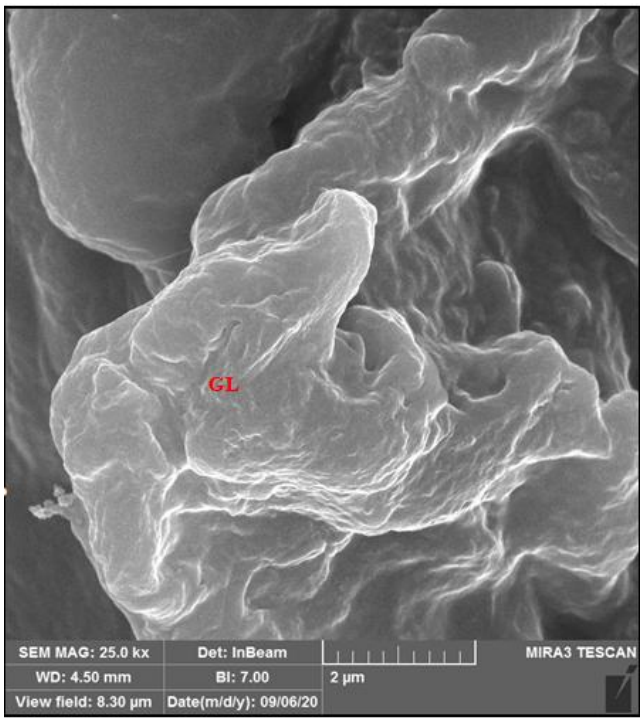

Fig. 5: Protoscolices in the germinal layer are few, and the general appearance of the cyst is wrinkled.

As for the group infected with cysts and treated with the prepared compound, the hydatid cyst showed the presence of many abrasion-like cracks in the walls of the cyst, and it was also observed in this group that Protoscolices were not present in the germinal layer. As shown in the fig. 6.

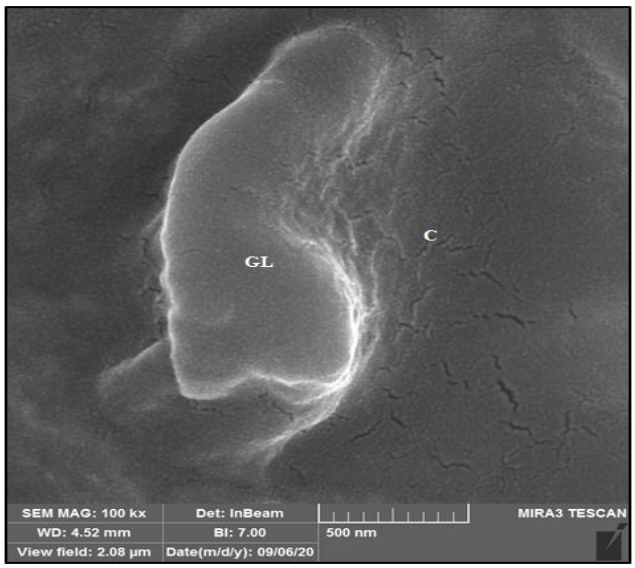

Fig. 6: There are many Cracks (C) in the hydatid cyst, and no Protoscolices in the (GL).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 11708 - 11716
Received 05 March 2021; Accepted 01 April 2021.

Discussion

Currently, the treatment of echinococcosis depends on surgery and/ or chemotherapy, depending on various factors such as the size and location of the cyst, the vitality, the interaction between the cyst and the adjacent tissues of the host, the bacterial and fungal infection accompanying the infection, and possible complications such as the rupture of the cyst and the spillage of its contents from the protoscolices[16].

For non-operative cases, chemotherapy with albendazole, a broad-spectrum anthihelminthic anthelmintic, mebendazole and praziquantel is the only option [17].

[18] evaluated more than 2,000 well-documented cases of hydatid cyst treated with albendazole. It has been noted that upon evaluation for up to 12 months after starting chemotherapy, the cyst disappeared in 30% of patients, and the cyst degeneration at 50-70%, which indicates improvement (which corresponds to our current study), while 20-30% of patients did not respond to chemotherapy.

In our current study, the results of electron microscopy of the prepared compound showed a greater effect than the effect that resulted from the use of Albendazole, which is represented by the cracks caused by the compound in the hydatid cyst.

The reason for the compound may be due to the use of Pregnenolone in its composition (prenanolone and progesterone form the precursors for all other steroid hormones [19]), which may facilitate the penetration of this compound into the layers of the hydatid cyst, and the presence of the thiazol-5-amine group in This compound may activate the albendazole compound in mechanistic treatment.

References:

1. Morar, R. and C. Feldman, *Pulmonary echinococcosis.* European Respiratory Journal, 2003. **21**(6): p. 1069-1077.
2. Moro, P. and P.M. Schantz, *Echinococcosis: a review.* International journal of Infectious diseases, 2009. **13**(2): p. 125-133.
3. Hemphill, A. and M. Walker, *Drugs against echinococcosis.* Drug Design Reviews-Online, 2004. **1**(4): p. 325-332.
4. Eicher, T., S. Hauptmann, and A. Speicher, *The chemistry of heterocycles: structures, reactions, synthesis, and applications*. 2013: John Wiley & Sons.
5. Teggi, A., M.G. Lastilla, and F. De Rosa, *Therapy of human hydatid disease with mebendazole and albendazole.* Antimicrobial agents and chemotherapy, 1993. **37**(8): p. 1679-1684.
6. Morris, D.L., et al., *Albendazole—objective evidence of response in human hydatid disease.* JAMA, 1985. **253**(14): p. 2053-2057.
7. Chu, S.W., et al., *Potent inhibition of tubulin polymerisation and proliferation of paclitaxel-resistant 1A9PTX22 human ovarian cancer cells by albendazole.* Anticancer research, 2009. **29**(10): p. 3791-3796.
8. Shams-Ul-Bari, S.H.A., et al., *Role of albendazole in the management of hydatid cyst liver.* Saudi journal of gastroenterology: official journal of the Saudi Gastroenterology Association, 2011. **17**(5): p. 343.
9. Ghali, T.S., *Synthesis, Characterization and Study The effect of The Chemical Constitution on Liquid –Crystalline Behaviour of New Heterocyclic Compounds Derived from Acetophenone Compounds.* Ph. D. Thesis, College of Education for pure science Ibn AL-Haitham. University of Baghdad, 2017.
10. Litchfield, J.T.a. and F. Wilcoxon, *A simplified method of evaluating dose-effect experiments.* Journal of pharmacology and experimental therapeutics, **1949**. **96**(2): p. 99-113.
11. Smyth, J.D. and D. Wakelin, *Introduction to animal parasitology*. 1994: Cambridge university press.

12. Smyth, J., *In vitro culture of Echinococcus spp.* Proceedings of the 13 th Int. Congr. Hydatidology. Madrid, 1985: p. 84-89.
13. Wangoo, A., N. Ganguly, and R. Mahajan, *Phagocytic function of monocytes in murine model of Echinococcus granulosus of human origin.* The Indian journal of medical research, 1989. **89**: p. 40-42.
14. MURAKAMI, T., *A revised tannin-osmium method for non-coated scanning electron microscope specimens.* Archivum histologicum japonicum, 1974. **36**(3): p. 189-193.
15. Silverstein, R.M., et al., *Spectrometric identification of organic compounds*. 2015, Hoboken, NJ: John Wiley and Sons, Inc.
16. Reuter, S., et al., *In vitro activities of itraconazole, methiazole, and nitazoxanide versus Echinococcus multilocularis larvae.* Antimicrobial agents and chemotherapy, 2006. **50**(9): p. 2966-2970.
17. Hemphill, A. and H.J. Müller, *Alveolar and cystic echinococcosis: towards novel chemotherapeutical treatment options.* Journal of helminthology, 2009. **83**(2): p. 99-111.
18. Pawłowski, Z., et al., *Echinococcosis in humans: clinical aspects, diagnosis and treatment.* WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern, 2001: p. 20-66.
19. Sanderson, J.T., *The steroid hormone biosynthesis pathway as a target for endocrine-disrupting chemicals.* Toxicological sciences, 2006. **94**(1): p. 3-21.