Pak. J. Biotechnol. Vol 15 (3) 607-614 (2018) *ISSN Print: 1812-1837*
www.pjbt.org *ISSN Online: 2312-7791*

TOXICITY OF *Salvia officinalis* EXTRACTS AGAINST *Lymnaea auricularia* THE INTERMEDIATE HOST OF FASCIOLIASIS CYCLE DISEASE IN IRAQ

Mohammed J. Al-Obaidi, Ali H. Abbas, Mohammed Y. Abd Al-Hussain

Trop. Biol. Res. Unit, College of Science, University of Baghdad, Iraq.
E-mail: lftah@uo.scbaghdad.edu.iq

Article received 30.7.2018, Revised 31.8.2018, Accepted 8.9.2018

ABSTRACT

The objective of the present study is to determine the acute toxicity of aqueous extracts of *Salvia officinalis* against the snail *Lymnaea auricularia*, which is considered as the intermediate snail host of Fascioliasis. About 720 snails were used for the experiments. The LD50, LD100 and Dose-Response relationships of *S. officinalis* against the snail *L. auricularia* was calculated. The *S. officinalis* extracts concentrations were found to be potent against *L. auricularia*. The results of 96 hr-LD50 and LD100 of *S. officinalis* extract against the target snail were 6.6±1.1 and 18.4±3.4 g/L respectively. It was observed that the toxicity of different preparation for snails was both time and dose-dependent. The study suggested that the aquatic extract of *S. officinalis* could be used as a control substance for the target snail.

Keywords: *Lymnaea auricularia*, toxicity, Fascioliasis

INTRODUCTION

Fascioliasis is a disease caused by two known species of parasitic flatworms (Trematodes) that affect the liver of humans and animals. It is a zoonosis, which means that the infection may be transmitted to humans from an animal. The liver fluke or sheep liver fluke *Fasciola hepatica* and *Fasciola gigantica* cause Fascioliasis. Fascioliasis is an important veterinary disease in animals particularly sheep and cattle. However, in human, it considers as a secondary disease (CDC 2013, Jacob 2015).

Fascioliasis is distributed in South America, Northern Africa, Iran, and Western Europe (Johnston 2015). WHO was made an estimation, which 56 million humans were infected with one or more foodborne trematodes in the world (WHO 2016). In addition, there are 7071 Fascioliasis human cases reviewed from 51 countries in America, Europe, Africa, and Asia (Adnan Alatoom 2008, Madsena 2015). A prevalence of fascioliasis was reported in Peru and Bolivian Altiplano, (15.64% and 34.2%, respectively). In addition, the prevalence of fascioliasis was reported in Portugal, Egypt, Peru and Puerto Rico, (3.2%, 7.3%, 8.7%, and 10.9%, respectively) (Mas-Coma 1999, Soliman 2008, Jahangir Abdi 2013). However, low prevalence of fascioliasis was reported in France and Corsica, (0.34±3.1 and 0.83±1.16, respectively) (Periago 2008). Other previous reports demonstrated the increasing of Fascioliasis from 1970 to 1990 as 2594 cases, which were recorded in 42 countries (Mas-Coma 1999). High prevalence of fascioliasis was recorded in children under than 15 years old of age (Vicente Maco 2015, Helena Gretera 2016).

The reservoir host of fascioliasis parasites includes domestic and wild animals as sheep, cattle, pigs, donkeys and black rats (Mas-Coma 2005). *Lymnaea* (Mollusca: Gastropoda) was recorded such intermediate host of *Fasciola hepatica* and *Fasciola gigentica* in Iraq (Osama 2009). Lymnaeidae is used in control studies; because of its role in Fascioliasis endemics (Mas-Coma 2004). The *L. auricularia* snail is spreading in the central and southern provinces of Iraq and considered as an intermediate host for *F. gigentica* (Wajdi and Nassir 1983). The prevalence of this snail is depends on many physicochemical factors such nitrates (NO3), chloride (Cl), magnesium (Mg), bicarbonate (HCO3), calcium (Ca) and sulphate (SO4) (Raut 1996). The *L. truncate* snail is the intermediate host of fascioliasis in Europe, South America and Bolivia (Bargues 1997, Bargues 1998). *Biomphalaria alexandrina* is another intermediate snail host that belongs to the Planorbidae family was recorded In Egypt (Osman, Rashwan et al., 1995).

Fascioliasis controlling, which was by done by using molluscicides plant origin, has wide applications; but the safety of the environment must be taken into its considerations (Barnes 2002). Another study suggested that *Thymus capitatus* has molluscicidal activity on adult and eggs of *Biomphalaria alexandrina*. The potential substance thymol of the thyme has an effective role on a terrestrial snail *Subulina octona*. While, others evaluated the molluscicidal activity of *Salvia officinalis* plant extracts against *Lymnaea auricularia* and *Nerium oleander* plant extracts against *Bulinus truncatus* snails (Khdier 2012, Al-Obaidi 2016). The aim of this study is to evaluate the molluscicidal activity of *Salvia officinalis* plant

extracts against the snail *L. auricularia* and explore the possibility of its effect in fascioliasis control.

MATERIAL AND METHODS

Collection of samples and preparing of solutions: This study was carried out during 2017 at the Tropical Biological Research Unit laboratories, College of Science, University of Baghdad, Iraq. The collection of *Lymnaea auricularia* snails was during the period from June to August 2016. The collection site was in Al-Rasheed district (30km) south Baghdad. The coordinates of the site are (33º32'83) longitude and (44º25'37) latitude. The snails were collected from a small irrigation canal branched from the main canal called (Muhyii Canal). Zooplankton net and laboratory steel spoon were used for collecting the snails, then the snails were kept in 5 Liters plastic containers with a perforated lid filled with a quantity of river water. The snails were fed with *alfa alfa* leaves extracts (10 milliliters per 50 Liters daily). The collected snails were isolated and identified according to standard keys of snails, then they are acclimatized to laboratory conditions (T: 25°C± 3) before testing. *S. officinalis* leaves extracts were prepared, concentrated, dried by a shade, shredded by a hand mill (Estrella®, model 41B) and electric mill (Moulinex®), sifted through a mesh (number 30), and kept in a cool, dry place (Guo-qing 2012).

Five and Ten milligrams of *S. officinalis* leafs powder were macerated in one liter of distilled water (DW) for 24 hours and placed in glass flasks. The macerate was filtered through cotton gauzes in a plastic funnel to get crude extracts. Stock solution (SS) was prepared by adding 5 milligrams to 1000 milliliters of distilled water to get 5% (0.05) concentration and a serial of dilutions were made from this SS.

Bioassays and Treatments: A serial of 1-5% concentrations was prepared from each SS (5 mg and 10 mg/L). Ten individuals of *Lymnaea auricularia* snail were tested in each replicate and the average was calculated. All tests were repeated three times at different times. The WHO method (II) for testing of molluscicides was used in this study. Exposure and recovery periods for 24 hours was depended on the all tests (Sukumaran 2004). Bioassays were conducted in the laboratory. The effects of aquatic extracts were applying different methods on snails of *Lymnaea auricularia*. Bioassays evaluated the parameters that included ED10, ED16, ED50, ED84, ED90, and ED100. Same parameters were performing to LD for each period exposure (24, 48, 72 and 96 hours) (Heinrichs 1981). The results were recorded at the end of each 24-hour of exposure.Dead snails removed and recorded at 24, 48, 72, 96 hr. after each application. No movement, no response to stimulation by the glass rod, no recovery after 24 hr. of putting in clean water and lack of the ability to adhere depending as the end point of experiments (Rand 1995).

Statistical analysis: Regression analysis depending on the probit units used to calculate different levels of LD and ED by using the provider of SPSS (V. 24) and Biostat (V. 5) programs (Li, Xing *et al*., 2012, Soni and Singh 2015, Wang, Qin *et al*., 2016). The results corrected by Abbott equation, calculating with two analysis methods included Log of Dose and Dose, and relationships between Logarithm of concentrations and probit units plotted (WHO **2003**).

RESULTS AND DISCUSSION

Mortality rates (SS- 5 &10mg/l of *S. officenalis*): The results of the study were shown that the lowest value of mortality was 9 (probit percent 0.264) and the highest value was 15 (probit percent 0.4398) for SS (5 mg/L) experiments. No significant differences of concentrations effect on mortality rates ($p = 0.8$). The lowest value of mortality was 6 (probit percent 0.2213) and the highest value was 9 (probit percent 0.3207) for SS (10 mg/L) experiments. No significant differences of concentrations effect on mortality rates p=0.8 (Table-1). A serial of doses and their percentile must use to get the percentile of mortality which recorded in the study was summarized below. These doses limited in range 0.0034-20, 481.6 mg /L of the SS-5 mg/L and 0.0176-23, 952.4 mg/L for SS-10 mg/L. The Dose, which needs to achieve 50% mortality, is 8.3 mg/L for the SS-5 mg/L and 20.5mg/L for SS-10 mg/L. According to regression analysis, correction must make doses used in the experiments as it appears below with complete the series of doses (Table2).

Table 1: Probit Analysis - Finney Method (Lognormal Distribution) for a mortality rate of *L. auricularia* snail exposed 10mg/l of *S. officials* extracts for 96 hours.

Log10[Dose (Stimulus)]	Actual (%)	Probit (%)	N	R	E(R)	Difference	Chi-square
			5 mg/L				
0.	0.3	0.264	30	**9.**	7.921	1.079	0.147
0.301	0.3	0.3356	30	**9.**	10.0686	-1.0686	0.1134

0.4771	0.3	0.3807	30	**10.**	11.4211	-1.4211	0.1768
0.6021	0.4	0.4137	30	**12.**	12.4123	-0.4123	0.0137
0.699	0.5	0.4398	30	**15.**	13.1944	1.8056	0.2471
				10 mg/L			
0.301	0.2	0.2213	30	**6.**	6.6401	-0.6401	0.0617
0.4771	0.3	0.2630	30	**9.**	7.8905	1.1095	0.156
0.6021	0.3	0.2948	30	**9.**	8.8455	0.1545	0.0027
0.699	0.3	0.3207	30	**9.**	9.621	-0.621	0.0401

	5 mg/L	**10 mg/L**
Chi-square	0.698	0.2605
Degrees Of Freedom	3	2
P-level	0.8737	0.8779
Alpha value (for confidence interval)	0.001	

Table 2: Dose percentile of mortality rates of *L. auricularia* exposed to 5mg/l S. *officinalis* for 96hr.

%	**Probit (Y)**	**Log10 Dose**	**SE**	**Dose**	**SE**	**LCL**	**UCL**
				5 mg/L			
1	2.6732	-2.4719	1.8397	0.0034	0.1166	0.	13.6136
5	3.3548	-1.4784	1.2149	0.0332	0.2715	0.0001	7.9939
10	3.7183	-0.9486	0.8836	0.1126	0.4231	0.0021	6.0703
16	4.0056	-0.5298	0.6245	0.2953	0.5868	0.0176	4.9452
20	4.1585	-0.3068	0.489	0.4934	0.6806	0.0543	4.4841
25	4.3258	-0.063	0.346	0.8649	0.7644	0.1815	4.1227
30	4.476	0.1559	0.2302	1.4319	0.795	0.5066	4.0468
40	4.7471	0.551	0.1719	3.5566	1.4446	1.6374	7.7252
50	**5.**	**0.9197**	**0.3467**	**8.3123**	**7.3646**	**1.7382**	**39.751**
60	5.2529	1.2884	0.5653	19.4271	33.0616	1.5147	249.1595
70	5.524	1.6835	0.8087	48.2539	151.5486	1.2548	1,855.6963
75	5.6742	1.9025	0.9449	79.8828	347.3174	1.123	5,682.3475
80	5.8415	2.1463	1.0973	140.0463	870.5616	0.9897	19,817.9889
84	5.9944	2.3692	1.2371	234.0139	2,013.0123	0.8801	62,222.4803
90	6.2817	2.788	1.5002	613.8119	9,700.143	0.7041	535,107.2924
95	6.6452	3.3179	1.8338	2,079.0591	70,878.7595	0.5293	8,166,975.1676
99	7.3268	4.3114	2.4603	20,481.6472	2,955,474.6759	0.3084	1,360,028,849.79
				10 mg/L			
1	2.6732	-1.7538	2.507	0.0176	2.8327	0.	1,445.276
5	3.3548	-0.8555	1.5252	0.1395	2.3351	0.0001	136.1279
10	3.7183	-0.3765	1.0043	0.4203	2.1018	0.0045	39.0901
16	4.0056	0.0022	0.5982	1.0051	1.8657	0.0676	14.9518
20	4.1585	0.2038	0.3899	1.5988	1.6362	0.2752	9.2902
25	4.3258	0.4242	0.1971	2.6561	1.2475	1.0911	6.466
30	4.476	0.6222	0.192	4.1897	1.913	1.7616	9.9648
40	4.7471	0.9794	0.5213	9.5376	14.4039	0.907	100.2909
50	**5.**	**1.3128**	**0.8763**	**20.5489**	**75.9124**	**0.3938**	**1,072.3517**
60	5.2529	1.6461	1.2375	44.2729	381.1672	0.1662	11,791.4983
70	5.524	2.0034	1.6267	100.7837	2,132.2432	0.0653	155,510.2747
75	5.6742	2.2013	1.8429	158.9747	5,534.2701	0.0388	650,605.4446
80	5.8415	2.4218	2.0838	264.105	16,014.5053	0.0218	3,206,399.7399
84	5.9944	2.6234	2.3043	420.1219	42,327.6378	0.0128	13,796,933.5953
90	6.2817	3.002	2.7187	1,004.6815	262,839.8943	0.0047	214,126,062.6977
95	6.6452	3.4811	3.2433	3,027.4471	2,650,555.8835	0.0013	6,885,350,072.4592
99	7.3268	4.3793	4.2275	23,952.4319	202,214,047.6866	0.0001	4,626,295,359,968.58

LD 50 (LCL-UCL) of *S. officinalis* extracts for SS 5 mg/L to the snail *L. auricularia* was 21.3 (13.3-9.8) mg/L with standard errors for Dose and log Dose (7.3 and 0.3) respectively. According to the analysis of log of Dose used in the study, the Beta value was 0.6 with standard error 0.4 and intercept 4.3. LD 50 (LCL-UCL) of *S. officinalis* extracts for SS 10 mg/L to was 20.5 (0.3-1072.3) mg/L with standard errors for Dose and log Dose (75.9 and 0.8) respectively. According to the analysis of log of Dose used in the study, the Beta value was 0.7 with standard error 0.8 and intercept 4 (Table 3). The study was recorded the clear significant relationship between *S. officinalis* extracts and *L. auricularia* response represented by mortality rates. Increasing of Dose extracts was followed by increasing of mortality and increasing of the log of Dose was followed by increasing of the mortality for 5 and 10 mg/L experiments (Figure 1).

Table 3: The median lethal dose of *L. auricularia* snail exposed to 5 mg/L of *S. officials* extracts for 96 hours.

5 mg/L			
LD50	**21.3146**	**LD50 Standard Error**	**7.3646**
LD50 LCL	13.3134	*LD50 UCL*	39.751
Log10[LD50]	1.3287	*Standard Error*	0.3467
Beta	3.9401	*Intercept*	4.369
Beta Standard Error	1.7356		
10 mg/L			
LD50	**20.5489**	**LD50 Standard Error**	**75.9124**
LD50 LCL	0.3938	*LD50 UCL*	1,072.3517
Log10[LD50]	1.3128	*Standard Error*	0.8763
Beta	0.7588	*Intercept*	4.0039
Beta Standard Error	0.8321		

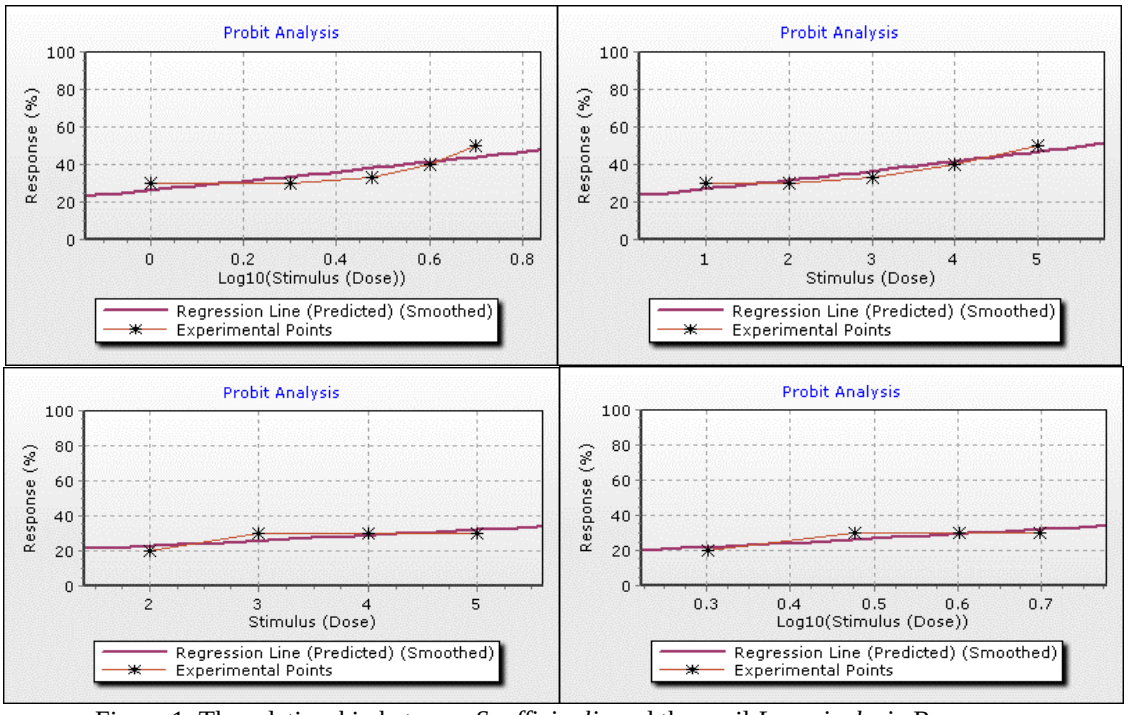

Figure 1: The relationship between *S. officinalis* and the snail *L. auricularia* Response

Table 4: The least squares of escaping activity numbers of *L. auricularia* exposed to S. *officinalis* Dose for 96hr (Normal Distribution).

Dose	Actual (%)	N	Probit (Y)	Weight (Z)
		5mg/l		
1.	0.3	30.	4.476	4.452
2.	0.3	30.	4.476	4.452
3.	0.3333	30.	4.5697	4.5697
4.	0.4	30.	4.7471	4.7471
5.	0.5	30.	5.	5.
		10 mg/L		

2.	0.2	30.	4.1585	3.8171
3.	0.3	30.	4.476	4.452
4.	0.3	30.	4.476	4.452
5.	0.3	30.	4.476	4.452

The results of the study found that the Dose SS-5 and SS-10 mg/L, which used in the experiments, was suitable to record the LD50. According to the least squares of mortality rates, the actual percent, probit, and weight of Dose used in the experiments were suitable for determining the LD50 of extracts to target snail (Table 4). LD50, 84, 90, and 100 of *S. officinalis* extracts to snail *L. auricularia* were 5.5, 13, 15.1, and 16.8 mg/L respectively. The lower confident level of LD50 was 2.6 while upper one was 8.4 mg/L. According to probit analysis for Dose used in the study, the Beta value of regression line was 0.1 with SE 0.1 and intercept 4.2. While for the SS-10 mg/L, the LD50, 84, 90 and 100 were 10.1, 21.1, 24.2 and 26.6 mg/L respectively. The lower confident level of LD50 was 5.3 while upper one was 14.6 mg/L. According to probit analysis for Dose used in the study, the Beta value of regression line was 0.09 with SE 0.2 and intercept 4 (Table 5).

Table 5: Different ED levels of escaping activity of *L. auricularia* exposed to *S. officinalis* (Dose-Response analysis)

5mg/l			
LD10	-4.0112	*Beta Standard Error*	0.146
LD16	-1.9018	*Beta*	0.1336
LD50	**5.5855**	*Intercept*	4.254
LD50 Standard Error	**0.8646**	*LD84*	13.0728
LD50 LCL	2.6831	*LD90*	15.1822
LD50 UCL	8.4878	*LD100*	16.8164
10mg/l			
LD10	-4.02	*Beta Standard Er*	0.2194
LD16	-0.9141	*Beta*	0.0907
LD50	**10.11**	*Intercept*	4.0829
Standard Error	**1.4232**	*LD84*	21.1341
LD50 **LD50** *LCL*	5.3079	*LD90*	24.24
LD50 UCL	14.9122	*LD100*	26.6462

Mortality rates do not appear in the first hours of exposure, which may indicate to the No Observed Effect Level (NOEL). Also, indicate killing all tested snails. Generally, the study reported that the increased concentration of stock solution followed by increasing of mortality rates. As well as, the increasing of exposure period followed by increasing of mortality rates. The results showed significant differences of mortality rates in experiments comparatively compared to control group. Also, the complete death of snails did not mark in stock solution (5mg/L) experiments but the complete death was marked in stock solution (10mg/L) experiments. This finding agreed with previous finding, which showed a marked reduction in the survival rate of snails treated with concentrations of different plant extracts compared to control (Finney 1952). In addition, the present findings showed that these extracts were caused effect and death to snail of *L. auricularia* with dose and time dependent. These results agreed with other studies of using different concentration of *T. tetraptera* aquatic extract (15, 20, and 25mg/liter) in Nigeria (Ryan 2004, Benelli, Bedini *et al*., 2015, Nema 2015, Wang and Wu 2015). In the present study, noticed that not removing of dead snails out of exposure media to avoid contamination risk; might affect on the reading of results.

The estimation of mortality endpoints determined by the disability to attached, down bottom the beaker and no response to needle probe (Sutton 1996). The mechanism of snail-killing extract is not known exactly and requires further studies. Some studies were suggested that the mortality occurred in snails due to the presence of toxic chemicals as wedel lactone (Vijay 2010), steroidal saponin Diosgenin (Tang, Yang *et al*., 2007), deltonin and isodeltonin (Brimer, ElSheikh *et al*., 2007), cardenolides (anti-feedings against snails) (Al-Sarar, Hussein *et al*., 2012). But, the mode of action of cardenolides was recorded in mammals as an inhibition of Na^+, K^+, ATPase and may cause cholinesterase inhibition in some organisms (Adewunmi, Oguntimein *et al*., 1990). Another author suggested an action mechanism of *T. tetraptera* compounds, as aridanin and serotonin on the

rhythmicity of the intestinal smooth muscle and slowing the heart of *Biomphelaria glabrata* snail (Aladesanmi, 2006). In addition, the exposure of *Bulinus globosus, Biomphalaria glabrata* and *Physa waterlotti* snails to *T. tetraptera* extract caused histopathological changes on tissues, so the epithelium was the primary affected by the molluscicide (Brackenbury 1999).

CONCLUSIONS

The study concluded that the extracts are effective against freshwater snails and can be used to control snails. The mode of action, chemical structure and other derivatives of these extracts should be studied and investigated more detail.

ACKNOWLEDGMENTS

The authors are grateful to the Prof Ihsan Al-Saqure, the minister of Tropical Biological Research Unit for managers supporting and to the Prof Assistant Shihab Ahmed Salman to snail identification supporting.

REFERENCES

Adewunmi, C.O., B.O. Oguntimein and P. Furu, Molluscicidal and antischistosomal activities of Zingiber officinale. Planta medica 56(04): 374-376 (1990).

Adnan Alatoom, D.C., Paul Southern, Rita Gander, Fasciola hepatica Infection in the United States (2008).

Al-Obaidi, M.J., Applied study to evaluate the molluscicidal activity of Nerium oleander extracts to the snail of Bulinus truncatus.. Al-kufa University J for Biology Pp. 127-133 (2016).

Al-Sarar, A., H. Hussein, Y. Abobakr and A. Bayoumi, Molluscicidal activity of methomyl and cardenolide extracts from Calotropis procera and Adenium arabicum against the land snail Monacha cantiana. Molecules 17(5): 5310-5318 (2012).

Aladesanmi, A.J., Tetrapleura tetraptera: Molluscicidal activity and chemical constituents. African Journal of Traditional, Complementary and Alternative medicines (AJTCAM) 4 (1): 23-36 (2006).

Bargues MD, M.C.S., Phylogenetic analysis of lymnaeid snails based on 18S rDNA sequences. Molecular biology and evolution 14: 569-577 (1997).

Bargues MD, M.O.A.C., Mas-Coma S., A novel molecular marker in the 18S rRNA gene of lymnaeids and its use in studies of the transmission of fascioliasis]. Congreso de la Sociedad EspanÄ ola de Medicina Tropical y Salud Internacional, ChinchoÂ n, Spain: 22-24 (1998).

Barnes, J., Herbal Medicines, Barnes J, Anderson AL, Phillipson JD, Pharmaceutical Press: London, Chicago (2002).

Benelli, G., S. Bedini, G. Flamini, F. Cosci, P.L. Cioni, S. Amira, F. Benchikh, H. Laouer, G. Di Giuseppe and B. Conti, Mediterranean essential oils as effective weapons against the West Nile vector Culex pipiens and the Echinostoma intermediate host Physella acuta: what happens around? An acute toxicity survey on non-target mayflies. Parasitology research 114(3): 1011-1021 (2015).

Brackenbury, T., Gross histopathological effects of an extract of Agave attenuata on the epithelium of the digestive tract of Bulinus africanus. Annals of Tropical Medicine & Parasitology 93(5): 519-526 (1999).

Brimer, L., S.H. ElSheikh and P. Furu, Preliminary investigation of the disposition of the molluscicidal saponin deltonin from Balanites aegyptiaca in a snail species (Biomphalaria glabrata) and in mice. Journal of Pesticide Science 32(3): 213-221 (2007).

CDC. Parasites - Fascioliasis (Fasciola Infection) (2013).

Finney, D.J., Probit Analysis. Cambridge, England, Cambridge University Press. (1952).

Guo-qing, L. Acute Toxicity Tests of Four Heavy Metal Salts to Juvenile Snail of Babylonia lutosa. Fujian Journal of Agricultural Sciences (2012).

Heinrichs, E., Manual for testing insecticides on rice. Int. Rice Res. Inst. (1981).

Helena Gretera, B., Noemi Cowana, B., Bongo N. Ngandoloc, Hamit Kesselyd, Idriss O. Alfaroukhc, Jürg Utzingera, B., Jennifer Keisera, B., Jakob Zinsstaga. Treatment of human and livestock helminth infections in a mobile pastoralist setting at Lake Chad: Attitudes to health and analysis of active pharmaceutical ingredients of locally available anthelminthic drugs. Acta Tropica 141(B): 295-302 (2016).

Jacob, A., Singh, P. and A. Verma, Effect of feeding deoiled mahua (Bassia latifolia) seed cake on the growth performance, digestibility and balance of nutrients in cross-bred calves during pre-patent period of Fasciola gigantica infection. Journal of Animal Physiology and Animal Nutrition 99(2): 299-307 (2015).

Jahangir Abdi, R.N., Mohammad Rostami Nejad, Vahid Mansouri, New features of fascioliasis in human and animal infections in Ilam province, Western Iran. Gastroenterol Hepatol Bed Bench 6(3): 152-155 (2013).

Johnston, C., Toxicology of model naphthenic acids in the great pond Snail Lymnaea stagnalis, University of Calgary (2015).

Khdier, F.A., The Study of the effect of some medical plant extracts on snail Lymnaea auriculari. Journal of Kerbala University 10(1): (2012).

Li, Y.Z., Y.T. Xing, H.J. Li, G.L. Qu, W. Wang, J.Y. Wei, Y.S. Liang and J.R. Dai, Studies on standardization of methods for screening molluscicides in laboratory IV sensitivity of Oncomelania snails from different months to niclosamide. Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi 24(1): 35-39 (2012).

Periago, M.A.V., M. El Sayed, K. Ashrafi, A. El Wakeel, M.Y. Mohamed, M. Desquesnese, F. Curtalef, S. Mas-Coma, First phenotypic description of Fasciola hepatica/Fasciola gigantica intermediate forms from the human endemic area of the Nile Delta, Egypt. Infection, Genetics and Evolution 8(1): 51-58 (2008).

Madsena H., H.N.M. Reprint of An overview of freshwater snails in Asia with main focus on Vietnam. Acta Tropica 141: 372-384 (2015).

Mas-Coma, S., Human fascioliasis: epidemiological patterns in human endemic areas of South America, Africa and Asia. Southeast Asian Journal of Tropical Medicine and Public Health 35: 1-11 (2004).

Mas-Coma, S.E., J.G. Bargues, Epidemiology of human fascioliasis: a review and proposed new classification. Bulletin of the World Health Organization 77: 340-346 (1999).

Nema, P., Effect of some plant extracts on the development of Lymnaea spp. (2015).

Osama, M.S., Effect of salinity and drought on the survival of Biomphalaria arabica, the intermediate host of Schistosoma mansoni in Saudi Arabia. Egypt. Acad. J. biology Sci. 1(1): 1-6 (2009).

Osman, M., E. Rashwan and H. Farag, Phagccytic activity of neutrophils in human fasciolosis before and after. Journal of the Egyptian Society of Parasitology 25(2): (1995).

Rand, G.M., Fundamentals Of Aquatic Toxicology: Effects, Environmental Fate And Risk Assessment, Taylor & Francis (1995).

Raut, S.K., Thermal Effect on the life _cycle parameters of the medically important fresh water snail species Lymnaea (Radix) luteola (Lamarck). Mem. Inst. Oswaldo. Cruz, Riodejaneiro 91(1): 119-128 (1996).

Ryan, J.K., C. George Ray, An introduction to infection diseases. Sherris Medical Microbiology 4th edition, Mcgraw-hill medical publishing division (2004).

Mas-Coma, S., Bargues, M.A., Valero Fascioliasis and other plant-borne trematode zoonoses. International Journal for Parasitology 35(11-12): 1255-1278 (2005).

Soliman, M.F.M., Epidemiological review of human and animal fascioliasis in Egypt. J Infect Developing Countries 2(3):182-189 (2008)

Soni, N., V.K. Singh, Molluscicidal activity of Tamarindus indica and Terminalia Arjuna against Indoplanorbis exustus: a causative agent of trematodiasis. Scientia 12(3):163-170(2015)

Sukumaran, D., Parashar, B.D., Gupta, A.K., Jeevaratnam, K., S. Prakash, Molluscicidal Effect of Nicotinanilide and its Intermediate compounds against a freshwater snail Lymnaea Inteola, the vector of animal schistosomiasis. Mem. Inst. Oswaldo Cruz 99(2): 205-210 (2004).

Sutton, R.S. Generalization in reinforcement learning: Successful examples using sparse coarse coding. Advances in neural information processing systems 1038-1044 (1996).

Tang, S.R., R.T. Yang, F.S. Pan, A.M. Zhao, Z.J. Pang, Steroidal saponin and steroidal sapogenin in Chinese Dioscorea L. Journal of Plant Resources and Environment 16(2): 64 (2007).

Vicente Maco, L.M., Jaime Delgado, Julio Herrera, José Nestares, Angelica Terashima, Frine Samalvides, Eduardo Gotuzzo, Efficacy and tolerability of two single-day regimens of triclabendazole for fascioliasis in Peruvian children. Rev. Soc. Bras. Med. Trop. 48(4): (2015).

Vijay, P., Evaluation of mollucicidal activity of some Indian medicinal plants against lymnaea acuminata (2010).

Wajdi, N., J. Nassir, Studies on the parasitic helminths of slaughtered animals in Iraq: I. Parasitic helminths of the liver of herbivores. Annals of Tropical Medicine & Parasitology 77 (6): 583-585 (1983).

Wang, P., Y.J. Wu, Applications of metabonomics in pesticide toxicology. Current drug metabolism 16(3): 191-199 (2015).

Wang, W., Z. Qin, D. Zhu, Y. Wei, S. Li and L. Duan, Synthesis, Bioactivity Evaluation, and Toxicity Assessment of Novel Salicylanilide Ester Derivatives as Cercaricides against Schistosoma japonicum and Molluscicides against Oncomelania hupensis. Antimicrobial agents and chemotherapy 60(1): 323-331 (2016).

WHO. Communicable Disease Working Group on Emergencies, HQ Division of Communicable Disease Control, EMRO, WHO OFFICE, Baghdad. WHO Office, Baghdad. Communicable Disease Toolkit, IRAQ CRISIS 39-44 (2003).

WHO. Foodborne trematode infections, Fascioliasis. (2016).