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Full Length Research Paper

Impact of *Euphorbia milii* latex on infectivity of *Schistosoma mansoni* larval stages to their hosts

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The effect of sublethal concentrations of *Euphorbia milii* latex on the infectivity of the larval stages of *Schistosoma mansoni* to *Biomphalaria alexandrina* and to albino mice as well on these larval stages of *S. mansoni* (miracidia and cercariae) were studied. The results showed that *B. alexandrina* infection with *S. mansoni* miracidia was greatly reduced by exposure to LC_0, LC_{10}, and LC_{25} of the latex and also the infectivity of the *S. mansoni* cercariae shed from infected *B. alexandrina* post exposure to these concentrations to albino mice was suppressed. Exposure to LC_0, LC_{10} and LC_{25} of latex of *E. milii* caused a reduction in number of the produced cercariae per snail during the patent period and in the period of cercarial shedding. The mortality rates of miracidia and cercariae were elevated gradually by increasing the exposure period to latex of *E. milii*. The present results showed that the mean number of worms per mouse in the experimental groups was less than that of control as well. The total number of ova per g tissue; it can be concluded that the application of sublethal concentrations of latex of *E. milii* may be helpful in control of schistosomasis.

Key words: *Biomphalaria alexandrina*, *Schistosoma mansoni* miracidia, latex of *Euphorbia milii* plant.

INTRODUCTION

Schistosomiasis is a snail-borne trematode infection of humans, domestic and wild animals in different parts of Asia, Africa, the Middle East, South America and the Carribbean. The causative schistosome parasite is acquired trans-cutaneously while swimming or wading in most of contaminated waters; other trematodes infect their hosts only via ingestion (Chitsulo et al., 2004; Gryseels et al., 2006). Schistosomiasis remains one of the most prevalent parasitic infections in the tropical and subtropical regions and is endemic in 76 countries World Health Organization, 2008). *Biomplaraia alexandrina* snails are the snail intermediate host of *Schistosoma monsoni* with widespread distribution allover Egypt (Barlow and Munech, 1951; Heynman, 1978; Bakry et al., 2011). Controlling of the snail intermediate hosts of this parasite by molluscicides (synthetic and/or of natural origin) is still one of the most promising means in the battle against this parasitic disease (WHO, 2009). Molluscicides of plant origin appear to be environmentally as they have several advantages when compared with the synthetic chemicals (Perrett et al., 1996). So far, more than 1500 plant species have been screened for molluscicidal properties (Whitfield, 1996). In Egypt, molluscicides of plant origin have received an increasing attention, so several plant species were screened in this concept (Gawish et al., 2006; Hussein, 2005; Bakry, 2009). Some of these species proved to have promising molluscicidal properties against different snail species for example, *Ammi majus* (Rizk, 1995), *Anagallis arvensis* (El-Emam et al., 1996), *Solanum dubium* (Tantawy et al., 2000), *Dyzygotheca elegantissima* and *Dyzygotheca kerchoveana* (Refahy, 1994).

The present work aimes to study the effect of *Euphorbia milii* latex. on the infectivity of the free living

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larval stages of *S. mansoni* to *B. alexandrina* and to albino mice (miracidia and cercariae), as well on the viability of these larval stages.

MATERIALS AND METHODS

Snails

B. alexandrina snails were from the Schistosoma Biological Supply Centre (SBSC) at Theodor Bilharz Research Institute (TBRI), Imbaba Giza, Egypt. *S. mansoni* cercariae were from laboratory infected *B. alexandrina* snails.

Miracidia

S. mansoni ova were from the Schistosoma Biological Supply Centre (SBSC) at Theodor Bilharz Research Institute (TBRI), Imbaba Giza, Egypt. They were left in clean dechlorinated water for hatching under a desk lamp then fresh hatch miracidia were used in bioassay and infection tests.

Cercariae

S. mansoni cercariae were from laboratory infected *B. alexandrina* snails.

Plants

The plant material, seeds, leaves and stems of *E. milii* plant were collected from the fields of Giza governorate. They were kindly identified via a specialist in the Botany Department, Faculty of Science, Ain shams University, Cairo, Egypt. The plant latex was released when either stem, seedpod or leaves were cut or injured then collected in clear dry dark bottle refrigerator till use. The concentrations used in the bioassays were prepared from raw latex with distilled water.

Bioassay tests

Molluscicidal screening

A stock solution of 1000 ppm was prepared (1 ml of the latex up to 1000 ml distilled water). After that a series of concentrations that would permit the computation of LC_{50} and LC_{90} values was prepared (WHO, 1965). Sublethal concentrations were calculated from the lethal-dose probability lines (Litchfieldand and Wilcoxon, 1949).

Effect on infection of *Biomphalaria alexandrina* snails with *Schistosoma mansoni* miracidia

The effects of sublethal concentrations of *E. milii* latex on infection rate of *B. alexandrina* with *S. mansoni* miracidia and cercarial production were examined. Three groups each of 50 snails were exposed individually to 10 miracidia/snail and maintained in each concentration of *E. milii* latex (LC_0, LC_{10} and LC_{25}) for 24 h under room temperature (24 ± 1°C) and ceiling illumination. After that, snails were continuously maintained in their corresponding sublethal concentrations. Another group of 50 snails was exposed to miracidia in the absence of the plant latex and maintained under the same conditions (control group). Examination of snails for cercarial shedding was carried out twice weekly, 25 days post miracidia exposure, and the cercarial suspension was poured in a graduated Petri dish, then few drops of Bown's fluid were added and all cercariae were counted using a dissecting microscope. Shedding snails were then isolated and kept in special aquaria in complete darkness.

Determining miracicidal activity of latex of *Euphorbia milii*

For determining miracidicidal activity of *E. milii* latex, 25 ml of water containing about 100 fresh hatched miracidia were mixed with 25 ml of double concentration of plant latex. 50 ml of dechlorinated water containing about 100 fresh hatched miracidia were used as control. 5 snails sublethal concentrations of latex LC_0 (1.9 ppm), LC_{10} (8 ppm), LC_{25} (12 ppm), LC_{50} (19 ppm) and LC_{90} (38 ppm) were used. During the treatment period, microscopical observations on the movement and mortality of miracidia were recorded at intervals of 1/2, 1, 2, 3, 4, 5, 6, 7, 8 and 24 h. LC_{50} and LC_{90} values of latex on *S. mansoni* miracidia for 8 h of exposure were determined.

Determining cercaricidal activity of *Euphorbia milii* latex

For determining cercaricidal activity of *E. milii* latex, cercariae in this experiment were obtained from laboratory infected *B. alexandrina* snails. A series of 5 ml samples of dechlorinated water containing 100 freshly shed cercariae was mixed with 5 ml of double concentration of latex of *E. milii*. 10 ml of dechlorinated water containing 100 freshly shed cercariae used as control. The aforementioned five sublethal concentrations of latex of *E. milii* were used at intervals of ¾, 1, 2, 3, 4, 5, 6, 7, 8 and 24 h. The cercariae were observed under a dissecting microscope to detect alterations happening in mobility and survival. LC_{50} and LC_{90} values of *E. milii* latex on *S. mansoni* cercariae for 8 h of exposure were determined.

Effect on infection of female albino mice with *Schistosoma mansoni* cercariae

Three groups each of ten laboratory-bred male and female albino mice (18 to 20 g) were exposed to 120 *S. mansoni* cercariae/mouse from *Biomphalaria* snails exposed to LC_0, LC_{10} and LC_{25} of latex of *E. milii*, respectively. A fourth mice group as control group was infected with *S. mansoni* cercariae from infected *Biomphalaria* snails which do not expose to sublethal concentrations of latex of *E. milii* by tail immersion method according to Oliver and Stirewalt (1952). 45 days after exposure to cercariae, mice in each group of the experiments were sacrificed individually and dissected. The worm load in each mouse was carried out by perfusion (hepatic and intestinal) according to the method of Kloetzel (1967). The different developmental stages of *S. mansoni* ova (the Oogram) was determined by the method of Pellegrino et al. (1962). The ova count/g tissue (digestion of liver and intestine) was calculated according to Cheever (1968) and Kamel et al. (1977).

Statistical analysis

Student's t-test and chi-square test (Petrie and Sabin, 2000) were used in comparing the means and rates of experimental and control

Table 1. Molluscicidal activity of latex solution of *Euphorbia milii* plant on *Biomphalaria alexandrina* snails under laboratory conditions for 24 h.

LC_{50} ppm	Confidence limit of LC_{50} ppm	LC_{90} ppm	Slope function	LC_0 ppm	LC_{10} ppm	LC_{25} Ppm
19	17.3 – 21	38	1.42	1.9	8	12

Table 2. Effect of sublethal concentrations of latex solution of *Euphorbia milii* plant on infectivity of *Schistosoma mansoni* miracidia to *Biomphalaria alexandrina* snails.

Treatment	Number of exposed snails	Survived snails at first shedding		Infected snails		% reduction
		Number	%	Number	%	
Control	50	45	90	32	71.1	
LC_0	50	32	64	15	46.9	34*
LC_{10}	50	30	60**	9	30	57.8**
LC_{25}	50	22	44***	3	13.64	80.82***

p<0.05, ** p<0.01 and *** p<0.001.

Table 3. Effect of sublethal concentrations of latex solution of *Euphorbia milii* plant on cercarial production of *Schistosoma mansoni* from infected *Biomphalaria alexandrina* snails (means ±S.D).

Concentration (ppm)	Number of cercariae/snail	Prepatent period (days)	Duration of shedding (days)
LC_0	685± 64.4**	28.2 ± 1.5	20.2 ± 1.2*
LC_{10}	366± 12.4***	29.6 ± 1.1	15.4 ± 1.6**
LC_{25}	114±16***	30.2± 1.2*	9.5 ± 0.82***
Control	1014 ± 167	31 ± 1.2	27.4 ±1.2

*p<0.05, ** p<0.01 and *** p<0.001.

groups statistically.

RESULTS

The molluscicidal activity of latex of *E. milii* on *B. alexandrina* snails after 24 h of exposure is presented in Table 1. The data obtained indicated that LC_{50} and LC_{90} values for *E. milii* latex were 19 and 38 pmm respectively. While the sublethal concentrations (LC_0, LC_{10} and LC_{25}) were found to be 1.9, 8 and 12 ppm respectively. The infection rate (Table 2) was significantly lower ($p< 0.001$) than that of the control snails (71.1%) being 46.9, 30 and 13.64% for snails exposed to LC_0, LC_{10} and LC_{25} of latex of tested plant, respectively. There was no significant difference between prepatent period of the snails exposed to sublethal concentrations of latex of *E. milii* and the control group. Table 3 showed that the duration of cercarial shedding among snails treated with this latex was shortened to 20.2 + 1.2, 15.4 + 1.6 and 9.5 + 0.82 days for LC_0, LC_{10} and LC_{25}, respectively, compared to 27.4 + 1.2 days for the control snails ($p< 0.001$). Also, there are highly significant reductions of total cercarial production per treated snail in comparison with control group were also detected. The effect of sublethal concentrations of latex on the infectivity of *S. mansoni* cercariae to albino mice is shown in Table 4. The lowest number of worms was obtained from mice infected with schistosome cercariae shed from *Biomphalaria* snails post exposure to LC_0, LC_{10} and LC_{25} of latex of *E. milii* with a reduction of -45.83, -72.12 and -89.94%, respectively than that of control. The mean number of the immature stages of Schistosome ova was higher in the experimental groups than control ones. Meanwhile, the rate of mature ova was lower, with highly significant difference than that detected in the control ($p<0.001$) being 34.4, 18.8 and 7.6% ova in the case of *Biomphalaria* snails post exposure to LC_0, LC_{10} and LC_{25} of Latex, respectively when compared with the value to 78.8% ova for control group.

It is clear from Figure 1 that the percentage of dead ova in mice infected with cercariae shed from *Biomphalaria* snails post exposure to LC_0, LC_{10} and LC_{25} of latex was higher being 10.8, 11.2 and 12.2%, respectively than that of control (3.8%) (Figure 1). The total number of ova per gram tissue decreased significantly in all experimental groups than that of control. Regarding the effect of latex of *E. milii* on miracidia of *S. mansoni*, Table 5 indicates

Table 4. The total number of worms in mice infected with *Schistosoma mansoni* cercariae from *Biomphalaria alexandrina* snails exposed to latex solution of *Euphorbia milii* plants.

Treatment	Mean No of worms/mouse			Total mean no of worms/mouse	Percent worm reduction %
	Male	Female	Pairs		
Control	15.4±0.44	6.6±0.51	9.2±0.42	31.2 ± 1.6	
LC_0	6.2±0.48**	4.1±0.5**	6.6±0.7**	16.9±1.4**	-45.83
LC_{10}	4.8±o.22***	2.1±0.5***	1.8±0..6***	8.7±1.4***	-72.12
LC_{25}	1.8±0.3***	0.86±0.4***	0.48±.23***	3.14±1.2***	-89.94

*p<0.05, **p< 0.01 and ***p< 0.001.

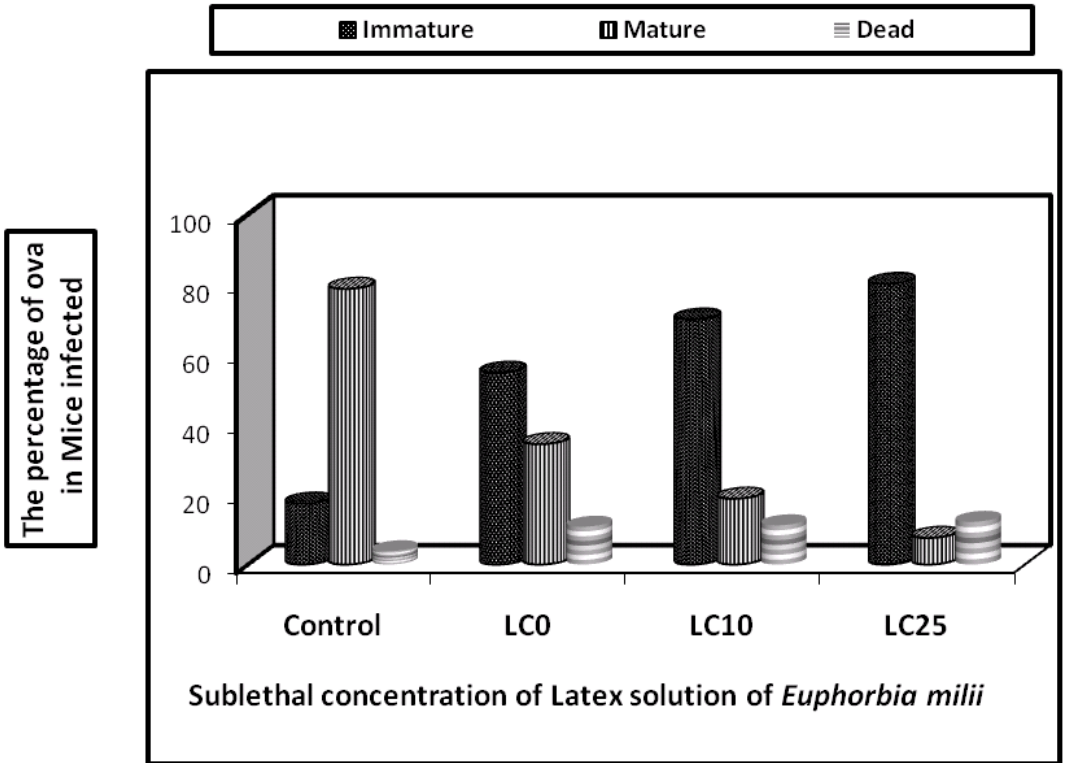

Figure 1. The oogram pattern in mice infected with *Schistosoma mansoni* cercariae from *Biomphalaria alexandrina* snails exposed to sublethal concentrations of latex solution of *Euphorbia milii* plant.

that sublethal concentrations of *E. milii* latex did not exert any detectable harm effect to miracida after ½ and 1 h of exposure to 1.9, 8 and 12 ppm. However, after 2 and 8 h, these concentrations exhibited considerable harmful effect, where 88 and 96% moralities were observed among miracidia exposed to LC_{50} and LC_{90} for 8 h respectively. The results also indicated that LC_{50} and LC_{90} values of *E. milii* latex on the miracidia after 8 h were 11 and 24 ppm respectively (Table 6). Elongation of the exposure period of cercariae to *E. milii* latex (Table 6) showed a gradually increase of mortality rate. It became 100% for group exposed to 19 and 38 ppm after 8 h of exposure when compared to 18% for control group. The recorded LC_{50} and LC_{90} values for 8 h for latex of *E. milii* on *S. mansoni* cercariae were 8.2 and 16 ppm, respectively, (Table 7 and 8).

DISCUSSION

The data obtained in the present work showed that LC_{50} and LC_{90} values for latex of *E. milii* were 19 and 38. Closely related results were previously reported by Bakry et al. (2004) where they found that LC_{90} of plant extracts of *Oreopanax reticulum* and *Furcraea selloea* were 68 and 28 ppm for *B. truncatus* snails, respectively. Also,

Table 5. Effect of latex solution of *Euphorbia milii* plant on *Schistosoma mansoni* miracidia.

Conc. (ppm)	% mortality of miracidia after the following intervals (hour)									
	½	1	2	3	4	5	6	7	8	24
1.9	0	0	0	2	4	8	12	15	25	100
8	0	0	2	4	8	24	33	34	42	100
12	0	0	8	12	22	30	42	52	64	100
19	4	10	16	28	36	48	60	72	88	100
38	6	14	26	32	40	58	70	84	96	100
Control	0	0	0	0	0	1	4	6	10	100

Table 6. Activity of latex solution of *Euphorbia milii* plant against miracidia of *Schistosoma mansoni* after 8 h of exposure under laboratory conditions.

LC_{50} ppm	Confidence limit of LC_{25} ppm	LC_{90} ppm	LC_0 ppm
11	9.16-13.2	24	1.1

Table 7. Effect of latex solution of *Euphorbia milii* plant on *Schistosoma mansoni* cercariae.

Conc. (ppm)	% mortality of miracidia after the following intervals (hour)									
	1/2	1	2	3	4	5	6	7	8	24
1.9	0	2	8	10	12	20	30	34	44	100
8	2	8	10	18	24	32	40	56	60	100
12	4	16	22	30	34	58	66	70	88	100
19	8	18	36	42	52	64	76	92	100	100
38	20	26	40	52	64	72	88	94	100	100
Control	0	0	0	2	4	8	12	14	18	100

Table 8. Activity of latex solution of *Euphorbia milii* plant against cercariae of *Schistosoma mansoni* after 8 h of exposure under laboratory conditions.

LC_{50} ppm	Confidence limit of LC_{25} ppm	LC_{90} ppm	LC_0 ppm
8.2	6.83-9.84	16	0.82

Mantawy et al. (2004) recorded the LC_{50} of the plants *Capparis spinosa* and *Acacia arabica* to be 500 and 66 ppm respectively. Thus, the latex of the plant discussion may show a similarity in its molluscicidal activity to the previously mentioned plants. This may be attributed to their molluscicidal potency which causes disturbance in metabolic pathways and protein contents of the snails. In the present work, the infectivity of *S. mansoni* miracidia to *B. alexandrina* was greatly reduced by LC_{25} of *E. milii* latex. The present result agrees with that recorded by Bakry et al. (2011) using *Solanum siniacum* and *Artemisia judaica L* plants. As these plants interrupt the biochemical parameters, as well, the activities of enzymes of treated snails that render their physiological processes unsuitable for the parasite development and reduce cercarial production. However, comparable results were obtained in literature (Bakry and Hamdi, 2006) using *Agave celsii*, *Ammi visnaga* and *Chenopodium ambrosioides* and Bakry (2006) using *Furcraea gigantean* and *Lampranthus spectabilis* plants. This was also recorded by Ibrahim et al. (2004) on significant reduction of *B. alexandrina* infection rates with *S. mansoni* miracidia post their exposure to LC_{25} of *P. repens* dry powder.

The present article showed no significant difference between the prepatent period of the snails exposed to latex of *E. milii* and the control. Despite that, a highly significant reduction in the duration of cercarial shedding and total cercarial production per infected snails were detected. These phenomena were stated by many authors using different plant species as molluscicidal agents. Thus, Badawy (2007), Gawish (2008) and Bakry

(2009) found that the plants *Viburnum tinus, Syzygium jambos, Euphorbia splendens* and *Atriplex stylosa* have a remarkable decrease in the duration of cercarial shedding and cercarial production/snail from *B. alexandrina* infected with *S. mansoni* miracidia. This reduction in cercarial shedding period and total cercarial production per snail is probably due to rupture of snails' tissues through miracidial penetration in the presence of these molluscicides which increased the harmful effects of these plants on the subsequent development of the parasite within snail's tissues (Bakry, 2006). These observations are in accordance with many authors using different plant species as molluscicides. Thus, El-Ansary et al. (2000) reported that *Ambrosia maritima* caused a remarkable decrease in cercarial shedding and cercarial production in *B. alexandrina* snails treated with this plant powder. Sharaf et al. (2001) obtained similar reduction in cercarial shedding and cercarial production from *B. alexandrina* treated with sublethal concentrations of aqueous suspension of *Z. simplex*. The present results showed that the mean number of worms per mouse in the experimental groups was less than that of control. The lowest number of worms was obtained from the mice infected by schistosome cercariae shed from *Biomphalaria* snails exposed to LC_{25} of *E. milii* latex with a reduction of 89.94%. This result approaches the observation of Ritchie et al. (1974) who showed that infectivity of *S. mansoni* cercariae was inhibited after treatment with 100 ppm of bis tri-nbutyltin oxide for 5 min. The same finding were previously observed by Viyanant et al. (1982) who used sublethal concentrations of copper sulphate and tributyltin fluoride and Gawish (1997) who used sublethal concentrations of Niclosamide.

In the present work, the total number of ova per gram tissue decreased significantly in mice infected with the schistosome cercariae shed from *Biomphalaria* snails exposed to latex of *E. milii* plant. This agrees with the observations of El-Ghayeb (1970) who declared that exposure of *S. mansoni* cercariae to 0.25 ppm of copper sulphate for 15 to 60 min markedly decreased the number of recovered worms per infected mouse and the number of ova/g tissue of liver. The reduction in the number of ova was explained by WHO (1963) that the few specimens of the survived cercariae that survived the treatment with molluscicides could infect the exposed mice and developed to adult worms laid low number of ova. This may be due to disturbance in their physiological activities as Bayluscide affects the respiratory enzymes which are essential factors in physiological processes of cercariae and adult worms. Also, in the present work, the number of mature ova in the experimental groups was lower significantly in groups of mice infected with the 'schistosome cercariae' shed from *Biomphalaria* snails exposed to latex of *E. milii* plant. The present authors noticed an increase in the mortality rates of *S. mansoni* miracidia and cercariae by increasing sublethal concentrations of latex of *E. milii* and the exposure period. Similar observations were previously recorded against *B. alexandrina* obtained by Mahmoud (1993) using pesticides, Mohamed et al. (2000) using Abamectin, and Bakry et al. (2002) using plant molluscicides.

From the aforementioned data, it can be concluded that the application of sublethal concentrations of *E. milii* latex may be helpful in control of schistosomasis.

REFERENCES

Badawy AMS (2007). Studies on Dracaena draco (Agavaceae) and Viburnum tinus (Caprifoliaceae) as Plant Molluscicides Against the Snail Vectors of Schistosomiasis and the Larval Stages of this Parasite, Ph.D. Thesis, Zoology Dept. Girls College for Arts, Science and Education, Ain Shams University, Cairo, Egypt.

Bakry FA (2006). Effect of methanol extracts of Furcraea gigantean and Lampranthus spectabilis plants on Biomphalaria alexandrina infection by Schistosoma mansoni and on energy metabolism indicator, Egypt. J. Schist. Infect. Endemic Dis., 28: 1–14.

Bakry FA (2009). Use of some plant extracts to control *Biomphalaria alexandrina* snails with emphasis on some biological effects. World. Appl. Sci., 1(6):1335-45.

Bakry FA (2009). Use of some plant extracts to control *Biomphalaria alexandrina* snails with emphasis on some biological effects, Pestic. Biochem. Physiol., 95: 159–165.

Bakry FA, Hamdi SAH (2006). Effect of different plant molluscicides on bioiogical and biochemical of Biomphalaria alexandrina snails, Egypt. J. Exp. Biol., 2: 99–106.

Bakry FA, Ismail SM, Abd El- Monem S (2004). Effect of two plant extracts on some Biological and enzymatic activities of *Bulinus truncatus* with *Schistosoma haematobium*. J. Aqual. Biol. Fish., 8(4): 313-446.

Bakry FA, Mohamed RT, El-Hommossany K (2011). Biological and biochemical responses of Biomphalaria alexandrina to some extracts of the plants Solanum siniacum and Artemisia judaica L.Pesticide. Biochem. Physiol., 99: 174–180.

Bakry FA, Ragab FMA, Sakran AMA (2002). Effect of some plant extracts with molluscicidal properties on some biological and physiological parameters of *Biomphalaria alexandrina* snails. J. Ger. Soc. Zool., pp: 30-234.

Barlow CH, Munech H (1951). Life span and monthly mortality rate of Bulinus truncates and Plannorbis boissyi the intermediate hosts of schistosomiasis in Egypt. J. Parasitol., 37: 165.

Cheever AW (1968). Condition affecting the accuracy of potassium hydroxide digestion technique for counting *Schistosoma manson* eggs in tissues. Bull. Wld.Hlth. Org., 39: 328.

Chitsulo L, Loverde P, Engels D (2004). Schistosomiasis. Nat Rev Microbiol., 2: 12-13.

El-Ansary A, El-Bardicy S, Solima SM, Zayed N (2000). Sublethal concentration of Ambrosia maritime (Damsissa) affecting compatibility of *Biomphalaria alexandrina* snails to infection with *Schistosoma mansoni* through disturbing the glycolytic pathway. J. Egyptian Soc. Parasitol., 30: 809–819.

El-Emam MA, El-Amin SM, El-Sayed MM, El-Nahas HA (1996). Field trials to control the snail vectors of schistosomiasis and fascioliasis by the plant *Anagallis arvensis* (Primulaceae). Egypt. J.Bilh. 18: 89-100.

El-Ghayeb FM (1970). Effect on of certain molluscicides on *Bulinus truncatus* ,*Biomphalaria alexandri*na and *Schistosoma manson* M.Sc. Thesis. Fac. Agric. Cairo Univ.

Gawish FM (1997). Evaluation of combination of certain molluscicides against *Biomphalaria alexandrina* and the free living stages of *Schistosoma mansoni.* Ph.D. Thesis Zoology Department Girls Coll for Arts and Science, Ain Shams University. Cairo,Egypt.

Gawish F, El-Bardicy S, Tadros MM, Mahmoud K, Yousief F (2006).

Biocidal activity of twenty eight plant species from two families, Myrtaceae and Agavaceae on *Biomphalaria alexandrina* snails and *Sichistosoma mansoni* free larval stages. J. Egypt. Ger. Soc. Zool. 50: 89.

Gawish FA (2008). Activity of the plant *Syzygium jambos* against *Biomphalaria alexandrina* snails' reproduction and infection with *Schistosoma mansoni.* New Egypt. J. Med., 39: 103-110.

Gryseels B, Polman K, Clerinx J, Kestens L (2006). Human schistosomiasis. Lancet, 368: 1106–1118.

Heynman D (1978). Manmade lakes and schistosomiasis, Proc.Inter Conference on schistosomiasis Oct. 18-25 (1975). The Minstry of Health, Egypt.

Hussein KT (2005). Suppressive effect of *Calendula micrantha* essential oil and gibberelic acid (PGR) on reproductive potential of the Mediterranean fruit fly *Ceratitis capitata.* Wied (Diptera:Tephritidae), J. Egyptian Soc. Parasitol., 35: 365-377.

Ibrahim AM, El-Emam MA, El-Dafrawy SM, Mossalem HS (2004). Impact of certain plant species on Schistosoma mansoni Biomphalaria alexandrina system. Proc. 3rd Int. Conf. Sci., 3: 390-413.

Kamel IA, Cheever AW, Elwi AM, Mosimann JE, Danner R (1977). *Schistosoma manson* and *Schistosoma haematobium* infections in Egypt.Technique for recovery of worms at necropsy. Am. J. Trop. Med Hyg., 26: 696.

Kloetzel K (1967). Egg and pigment production in *Schistosoma manson* infections of the white mouse. Am. J. Trop. Med. Hyg., 12: 293.

Litchfieldand JT, Wilcoxon F (1949). A simplified method of evaluating dose-effect experiment. J. Pharm. Exper. Therap., 96: 99-113.

Mahmoud MB (1993). Effect of certain pesticides *on Biomphalaria alexandrina* and the intramolluscan larval stages of *Schistosoma mansoni.* M.Sc. Thesis, Fac. Sci., Cairo Univ. Cairo,Egypt.

Mantawy MM, Hamed M, Sammour M, Sanad M (2004). Influence of Capparis spinosa and Acacia Arabica on certain biochemical haemolymph parameters of Biomphalaria alexandrina. J. Egypt. Soc. Parasitol., 34(2): 659-677.

Mohamed AM, Bakry FA, Heiba FN (2000). Molluscicidal effects of Abamectin on *Biomphalaria alexandrina* and its infection with *Schistosoma mansoni*. J. 1st Int. Conf. Biol. Sci., (ISBS). 1(2): 207-216.

Oliver L, Stirewalt MA (1952). An efficient method for exposure of mice to cercariae of *Schistosoma mansoni*. J. Parasit., 38: 19.

Pellegrino J, Oliveira CA, Faria J, Cunha A (1962). New approach to the screening of drugs in experimental *Schistosoma manson in* mice. Am. J. Trop. Med. Hyg., 11(1): 301.

Perrett S, Whitfild PJ (1996). Currently available molluscicides, Parasitol. Today, 12:156-159.

Petrie A, Sabin C (2000). Medical statistics at a Glance.Blackwell Science Ltd, Oxford.

Refahy LA (1994). Studies on the molluscicidal activity of some plants of Family Araliaceae. Ann. Agric. Sci. Moshtohor., 32(4): 2105-2116.

Ritchie LS, Lopez VA, Cora JM (1974). Prolonged applications of an organotin against Biomphalaria alexandrina and *Schistosoma manson in Molluscicides in schistosomiasis* control, TC. Chenge Ed. Academic press New York and London, pp: 77-88.

Rizk ET (1995). Studies on the effect of certain molluscicidal agents on the snail intermediate host of *Schistosoma mansoni*. Ph. D. Thesis, Fac. Science. Tanta Univ., Egypt.

Sharaf El-Din AT, Bakry FA, Tantawy A (2001). Molluscicidal activity of *Zygophyllum simplex* (Family; Zygophyllaceae) against *Biomphalaria alexandrina* and *Bulinus truncatus*, Egyptian J. Aqua. Biol. Fisheries. 4: 131–143.

Tantawy A, Sharaf El-Din AT, Bakry FA (2000). Laboratory evaluation of the mollusciciding activity of *Solanum dubium* (Solanaceue) against *Biomphalaria alexandrina* snails. J. 1st Int. Cong. Biol. Sci., 1(2): 307-318.

Viyanant V, Thirachantra S, Sornmani S (1982). The effect of controlled release copper sulphate and tributyltin fluoride on the mortality and infectivity of *Schistosoma manson miracidia.* Southeast Asian J. Trop. Med. Pub. Hilth., 13(2): 225.

Whitfield PJ (1996). Medicinal plants and control of parasites. Noval antihelmintic compounds and molluscicides from medicinal plants. Trans. Roy. Soc. Trop. Med. Hyg., 20: 596-600.

WHO (1963). Data sheet on pesticides. No.63,Niclosamide.WHONBC/DC/88.63.

WHO (1965). Molluscicide screening and evaluation. Bull. WHO., 33: 567-581.

WHO (2009).The control of schistosomiasis. Technical Report Series No. 922, Geneva.

World Health Organization (WHO) (2008). The Social Context of Schistosomiasis and its Control: An Introduction and Annotated Bibliography. Birgitte Bruun, Jens Aagaard-Hansen.