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RESEARCH ARTICLE

Molluscidal activity of Mirazid on *Biomphalaria alexandrina* snails: biological and molecular studies

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Abstract

The growing awareness of the possible dangers from the use of molluscicides gives a great attention to the plant molluscicides for snail control. Mirazid was tested as a molluscicide against adult *Biomphalaria alexandrina* snails and a larvicide against S. mansoni larvae (miracidia and cercariae). LC_{50} and LC_{90} values were detected for both tests (19.19 and 41.26 mg/l, respectively). *B. alexandrina*, non-infected and infected with Schistosoma mansoni were exposed to 3 mg/l of Mirazid drug for 4 weeks compared to non-treated with Merazid and/or infected controls. Then the meracidea, cercariae, egg production, protein electrophoresis and DNA fragmentation analysis of B. alexandrina and organ histology were assessed. Mirazid treatment suppressed the growth rate by 11%, decreased the eggs/snail/week by 98 %, and delayed eggs hatchability to 13 days and complete cessation of cercarial shedding (100 %). Mirazid exhibited sever histological alterations at 4^{th} week of exposure on hermaphrodite and digestive glands of infected and non-infected *B. alexandrina* snails. It also led to decrease in total protein intensity of the hermaphrodite-digestive glands in both treated and treated-infected snails as absence of the protein band 54.77 kDa. The previous two groups of snails also showed DNA damage in hermaphrodite-digestive glands. It is concluded that, Mirazid can be used as a promising molluscicide for *B. alexandrina* snails' control.

1. Introduction

Schistosomiasis is an important public health problem of the century (Ross et al., 2001). The need for molluscicides from plant origin has received big attention because of the high cost and hazards accompanied with synthetic compounds for snail control in the endemic areas of the world (Adewunmi, 1991).

Myrrh (Family: Bruseraceae, *Commiphora molmol*) is a plant growing in northeast Africa and Saudi Arabia. The name (Myrrh) may derived from the Arabic and Hebrew word (Mur) which means bitter. Most of the secretion was obtained by spontaneous exudation from the cracks. The three main components of Myrrh are resin 7-17%, gum 57-61%, the volatile oil 2-10%, and 3-4% impurities, all are important in its activity (Michie and Cooper, 1991; Lemenih and Teketay, 2003 and Massoud et al., 2007). These authors added that Myrrh was widely used from past to today as herbal medicine and in several industries such as food, beverages, perfumes, pharmacology, pesticide (as insecticide and molluscicides) and others. Abdul-Ghani et al., (2009) recommended usage of Myrrh or Mirazid as a molluscicide for snail population control of schistosomiasis and *Biomphalaria* sp. and snails of *Fasciola* sp. (Massoud et al., 2000; and Massoud and Habib, 2003, Massoud et al., 2012). Al-Mathal and Fouad (2006) found that *C. molmol* from Saudi Arabia had a molluscicidal effect on *B. arabica* snails at low concentration, 40 mg/l after 48 h of exposure. In Egypt, Massoud et al. (2000) reported that *B. alexandrina* snails exposed to oleo-resin and oil extracts of *C. molmol* were killed at all experimental times. Many studies on the effect of oil extract of *C. molmol* on many intermediate host snails like *B. alexandrina*, *Bulinus truncates*, *Physa acuta*, *Melania*

tuberculata and *Cleopatra bulimoides* and *Lymnaea cailliaudi* and their eggs were proved its molluscicidal and ovicidal activity (Massoud and Habib, 2003; Massoud et al., 2010). Marked degenerative changes on the hermaphrodite gland and digestive gland tissues were observed after exposure of *Biomophalaria* sp. snails to different plant extractions (Al-Sharkawi and Rizk 1996; Rizk, 1995; Bakry et al., 2007, *Ammi majus*, *Sesbania sesban* and neem, respectively). *S. mansoni* infection caused great damage to the gametogenic stages, the epithelial, secretory cells, connective tissue and cysts formation by cercaria within the snail (Bakry et al., 2007; Bakry, 2009). El-Rigal and Hetta (2006) proved that administration of both Mirazid and C. reticulate extracts to S. mansoni infected mice increased level of protein fraction concentrations. In other plants, ethanol extract of Artemisia judaica L. (Family: Asteraceae) and Solanum siniacum have a molluscicidal effect against *B. alexandrina* snails, as they decrease snails fecundity and cercarial production of *S. mansoni* treated-infected snails as well as glycogen, total protein, pyruvate and lipids levels (Bakry et al., 2011).

This study was aimed to investigate the effect of Mirazid on some biological, histological and molecular parameters of *S. mansoni* infected *B. alexandrina* snails as a safe molluscicide of plant origin.

2. Materials and methods

2.1. Experimental animals

The experimental snails used in the present study were adult *Biomphalaria alexandrina* and albino CD1 mice. The snails and mice were obtained from Schistosome Biological Supply Centre (SBSC), Theodor Bilharz Research Institute, Giza, Egypt. Snails reared in Invertebrate Research Lab in Zoology Department, Faculty of Science, Menufiya University. *B. alexandrina* snails were maintained under standard laboratory conditions according to (Oliver et al., 1962). Snails were fed daily with fresh lettuce leaves and provided with foam pieces for oviposition. Snails were allowed to lay egg masses on small foam pieces placed on the water surface of the aquaria as described by El-Emam and Ebeid (1989).

2.2. Experimental material

Mirazid drug was used in the form of capsules (each contain 300 mg of purified *Commiphora* extract) obtained from Pharco Pharmaceuticals Company, Egypt.

2.3. Biological study

2.3.1. Mice infection

Male CD1 mice were infected individually by paddling method in dechlorinated tap water contains 80-100 freshly emerged *S. mansoni* cercariae for 2 h (Liang et al., 1987).

2.3.2. Snail Infection

After 6 - 8 weeks post mice infection liver and intestine were homogenized and eggs were extracted and washed in saline. The miracidia were gathered and snails were exposed individually to 2 ml dechlorinated tap water contains 6 - 8 miracidia for 3h (Anderson et al., 1982).

2.3.3. Molluscicidal activity of Mirazid

Snails were exposed to a series of concentrations of Mirazid for 48h at room temperature. Triplicates of tanks (10 snails/treatment) were kept under the same laboratory conditions in dechlorinated tap water (WHO, 1965). Dead snails were removed and recorded from the container.

2.3.4. Larvicidal and cercaricidal activity

For miracidicidal activity, 40 - 50 freshly hatched miracidia were used. Tested concentrations of Mirazid (50, 100, 200, 300, 400 and 500 mg/l) were prepared in dechlorinated tap water (Tchounwou et al., 1991). The activity of the miracidia at time intervals of 5, 10, 15, 20, 30, 45, and 60 min and dead miracidia were recorded. For cercaricidal activity, 20 - 30 freshly shed cercariae were exposed to 50, 100, 200, 300 and 400 mg/l of Mirazid in triplicates. Control group was represented as clean dechlorinated tap water containing the same number of cercariae (Ritchie et al., 1974). Dead (motionless) cercariae were recorded at the time intervals of 5, 10, 15, 20, 30, 45 and 60 min. The LC_{50}, LC_{90} and slope function of molluscicidal, lavicidal and cercaricidal activity of Mirazid was computed using Probit Proban analysis (Ver.1.1).

2.3.5. Survival rate, growth rate and egg laying capacity of B. alexandrina snails

90 adult *B. alexandrina* snails were continuously exposed to 3 mg/l (LC_4) of Mirazid for 4 weeks as a sublethal concentration in plastic containers. The treatment was changed weekly with freshly prepared one. Triplicates of

control, Mirazid and/or *S. mansoni* infected groups (10 snails each) were used. Mortality, egg laying and growth were recorded daily. Survival rate was calculated according to Frank (1963).

The growth rate was calculated by calculating the mean values of the shell diameter of the snail form each experimental group weekly using a caliper according to Chernin and Michelson (1957).

The egg masses laid on foam pieces or on the walls of the aquaria of all experimental groups were daily removed and counted by a hand lens (x 10). The egg-lying capacity was calculated according to El Gindy and Radhawy, (1965). The effect of sublethal concentrations of Mirazid on hatchability of *B. alexandrina* eggs was investigated. 5 control egg masses (each contains 10 ± 2 eggs) aged 24 h were exposed to 20 ml of the tested solutions in triplicates, alongside with a non-exposed control group. All groups were maintained at 25 ± 2 ºC till hatching (Oteifa et al., 1975). Eggs were examined daily under a stereomicroscope and the number of normal viable eggs and hatched embryos were recorded (Oliver et al., 1962). The survived infected and treated-infected snails were individually examined for cercarial shedding to detect the infection rate starting from the 3rd week post miracidial exposure till the end of the experiment (4^{th} week, Coles, 1973). For each positive shedding snail, cercariae were counted in 100 µl (3 replicates) as the mean number of cercariae/snail.

2.4. Histological study

For histological investigation**,** 5 snails were selected randomly from each experimental group. Shells were crushed and fragments were removed. Hermaphrodite and digestive glands were separated and immediately fixed in aqueous Bouin's fluid for 24 h. Then specimens were processed for histological examination according to the method described by Romeis (1989).

2.5. Molecular study

2.5.1. Sodium Dodecyle Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS- PAGE was performed under reducing conditions according to the protocol of Laemmli (1970) to separate *B. alexandrina* tissue proteins. Total tissue proteins of hermaphrodite-digestive gland complex were separated on 8% resolving gel with 3.75% stacking gel using electrophoresis apparatus (Bio-Rad USA vertical minigel, double side). Hermaphrodite-digestive glands were dissected out from 3-5 snails and pooled in 1 ml Ependorf tube with tissue-extracting buffer was added in a ratio of 1:10 w/v (Bradford, 1976). The protein marker (molecular weight of 205 - 29 kDa) from Sigma Chemical Company was used. Protein bands were visualized by staining the gel with Coomassie Brilliant Blue (CBB) stain (De-Moreno et al., 1985). The resultant gel was analyzed using Gel pro analyzer software (Ver. 3.0) cypermedica USA and gel densitometer Bio-rad G- 70, USA.

2.5.2. DNA analysis

DNA extraction from hermaphrodite-digestive glands was done according to "salting out extraction method" of Aljanabi and Martinez (1997) and modification introduced by Hassab El-Nabi (2004a). From each experimental group 20 mg of tissue were taken at time intervals of 24h, 48 h and 1st, 4^{th} weeks post exposure. Tissue samples were lysed, and then samples loaded in wells. The gels were photographed using a digital camera on 312 UV light transeliminator. The optical density of bands was measured by software Gel program as maximum optical density values.

2.6. Statistical analysis

Data were analyzed using Statgraphics 5.1 Plus software. All data are expressed as means ± S.D. for the biological parameters in different groups. One-way ANOVA were conducted for treatment/infection effects. Where ANOVA could not be applied, a non-parametric ranking test was used (Kruskal Wallis test). The level of significance was accepted when $P < 0.05$.

3. Results

3.1. The molluscicidal and larvicidal activity of Mirazid

Molluscicidal activity of Mirazid against adult B. alexandrina snails after 48h of exposure was measured. The obtained results revealed that Mirazid had a molluscicidal activity against adult *B. alexandrina* snails. The results showed that LC_{50} and LC_{90} values after 48h of exposure for Mirazid were 19.19 and 41.26 mg/l, respectively with slope function values of 3.83. Mirazid had a larvicidal activity against *S. mansoni* miracidia and cercariae. For miracidia and cecariae, the LC_{50} and LC_{90} values were 39.62, 198.53, 22.74 and 122.31 mg/l, with slope function values were 2.45 and 3.21, respectively.

3.2. Effect of Mirazid on biological activities of B. alexandrina snails

3.2.1. Survival rate

The results indicated that the survival rate of B. alexandrina snails treated with Mirazid exhibited non-significant reduction throughout the experimental period when compared to control group (Table 1, $P \geq 0.3$, ANOVA). The mean number of survived snails were 3 ± 0.6, 6.6 ± 1.6 and 2 ± 0.5 for Mirazid treated, infected and treated-infected snails, respectively compared to 9 ± 2 for control group by the end of the 4th week of exposure.

3.2.2. Egg laying capacity

The egg laying capacity of *B. alexandrina* snails markedly affected as a result of Mirazid treatment and/or *S. mansoni* infection (Table 1). The exposure of mature snails to sublethal concentration of Mirazid led to significant decrease in No. of egg masses/snail/week when compared to the control ones ($P \leq 0.002$, Kruskall wallis). At the 4th week post exposure, Mirazid-treated group mean number of egg masses/snail/week was 0.5 ± 0.1 and eggs laid/snail/ week was 2.8 ± 0.6 ($P \leq 0.001$, Kruskall wallis). Mirazid treated-infected group mean numbers were 0.3 ± 0.1 and 2.7 ± 0.5 when compared to 1.8 ± 0.2 and 33.6 ± 3.0 of control snails, respectively.

The net reproductive rate (R0) of *B. alexandrina* snails under continuous exposure of Mirazid and/or *S. mansoni* infection was assessed. The results indicated that R0 was greatly affected in all experimental groups when compared to the control group. The values of net reproductive rate for infected, Mirazid treated and Mirazid treated-infected groups were 56.14, 12.7 and 5.14, respectively when compared to the control 118, with reduction percentages of 52.4, 89.2 and 95.6 %, respectively when compared to the control group.

3.3. Effect of Mirazid on B. alexandrina eggs hatchability

Data showed that Mirazid treatment greatly inhibited the hatchability of snail's eggs. The treated and control eggs hatched after 13 and 7 days of exposure, respectively. The mean number of hatched eggs of treated and control eggs were 26 ± 7.3 and 123 ± 11.5 with hatchability percentage of 20.5 and 91.1 %, respectively.

3.3.1. Growth rate

The results in Table (1) revealed that there is a gradual decrease in growth rate but recorded non-significant decrease in shell diameter in all experimental groups ($P \geq 0.07$, ANOVA). At the 4th week of exposure, infected, Mirazid-treated and treated-infected snails recorded decrease in the shell diameter mean number 9.3 ± 0.4, 9 ± 0.4 and 9 ± 0.2 when compared to the control group 10.5 ± 0.4 mm.

3.3.2. Cercarial production

The cercarial production of *B. alexandrina* snails infected with *S. mansoni* was completely stopped by exposure to sublethal concentration of Mirazid after 4 weeks of exposure. Data indicated that infection rate was reduced by 100 % in treated-infected group when compared to the infected one.

3.4. Effect of Mirazid on histological structure of hermaphrodite and digestive gland's

3.4.1. Hermaphrodite gland

Hermaphrodite gland of control *B. alexandrina* snails consists of acini connected together by connective tissue. Each acinus lined with germinal epithelial layer that differentiate into successive developmental stages of spermatogenesis and oogenesis of male/female gametocytes (Fig. 1A).

The examination of *B. alexandrina* snails infected with *S. mansoni* sections revealed obvious histological alterations in the whole architecture of the hermaphrodite gland. After 4 weeks post infection, the acini have a deformed shape separated by loose connective tissue. Degeneration in epithelial cells and ova were observed besides the presence of sporocysts (Fig. 1B). Exposure of *B. alexandrina* snails to Mirazid for 4 weeks caused a series of deleterious effects on hermaphrodite gland structure (Fig. 1C and D). Deformed acini with severe destruction of germinal epithelium layer and degenerated spermatocytes and ova were detected. Widespread atrophy was also observed in completely deformed acini with scattered and irregular sperms. Hermaphrodite gland of B. alexandrina snails infected/treated with Mirazid exhibited degenerated epithelial layer of completely deformed acini with degenerated and scattered gametocytes and almost replaced by immature and malformed sporocysts (Fig. 1E).

3.4.2. Digestive gland

The control B. alexandrina digestive gland composed of tubules. Each tubule consists of a single layer of columnar epithelial cells differentiated into digestive and secretory cells which surround a central lumen. The later cells are settled on their basal portion. The tubules connected together by connective tissue (Fig. 2A).

After 4 weeks of exposure to *S. mansoni* infection, the gland structure was deformed. Great damage occurred in the epithelial region, some cells become necrotic and other disappeared. The acini had deformed shape and the connective tissue appeared necrotic. Large number of sporocysts was recorded (Fig. 2B). Exposure of B. alexandrina snails to Mirazid resulted in completely necrotic connective tissue led to appearance of scattered acini, which lost their normal shape, and notable vacuoles in secretory and digestive cells were found (Fig. 2C). Exposure

to Mirazid combined with S. mansoni infection led to degeneration of germinal epithelial layer, disappearance of central lumen, appearance of immature sporocysts and vacuoles (Fig. 2D).

3.5. Effect of Mirazid and/or S. mansoni infection on hermaphrodite-digestive glands total proteins

SDS-PAGE profile of tissue proteins extracted from *B. alexandrina* snails treated with Mirazid during 4 weeks of exposure is illustrated in Fig. (3A). The results indicated a decrease of total proteins intensity during 4 weeks of exposure when compared to control group. Protein electrophoretic analysis of hermaphrodite-digestive glands complex yielded complex patterns of polypeptide with different molecular weights in both control and treated groups ranging between 4.99 - 258.54 kDa. The dominant protein bands were 104.85; 86.07 and 66 kDa in both treated and control groups. There was an occasional appearance and absence of some proteins in different experimental time intervals. Mirazid treatment resulted in appearance of protein bands at 41.38 kDa. The protein band 54.77 kDa disappeared at 3rd and 4th week but appeared at other time intervals. 123.09 and 16.57 kDa protein bands disappeared only at 3rd and 1st weeks respectively. On the other hand, the protein band 129.19 kDa completely disappeared during the period from 48 h till the 4th week.

Protein patterns of hermaphrodite-digestive glands extracted from infected/treated *B. alexandrina* during 4 weeks of exposure were illustrated in Fig. (3B). The data recorded decrease as an occasional absence of some protein fractions and new protein bands appeared as a result of infection combined with treatment at 80.8 and 42.39 kDa. The dominant protein bands in both control and treated-infected groups were 258.54, 104.85, 73.01 and 66 kDa. 20.74 kDa bands disappeared in control group and appeared after 24 h and 4th week in treated-infected groups because of treatment.

3.6. Electrophoretic pattern of hermaphrodite-digestive glands DNA

There are two types of DNA fragments induced. The first one is apoptotic fragments and the second one is necrotic fragments. The apoptotic bands appeared at 200 bp and its multiples while the necrotic ones appeared as a smear. As shown in Table (2) and Fig. (4 A), the amount of intact DNA decreased from 102.9 in control snails to 7.71 and 30 in hermaphrodite-digestive glands of infected and Mirazid treated snails after 24 h of exposure, respectively. However, DNA bands at 200 bp and its muultiples showed an increase in maximal optical density after infection and Mirazid exposure when compared to control snails in all time intervals. After 48 h of exposure to Mirazid, DNA fragmentation appeared as necrotic form in infected and treated-infected groups and the amount of fragmented DNA were increased with values 70 and 160, respectively when compared to control value 61.31. After one week of exposure, in Mirazid treated-infected group, the optical density of intact DNA decreased (170) when compared to control (200.7), however an increase in the amount of DNA at 800, 600 and 400 bp recorded in Table (2) and Fig. (4C). After four weeks of exposure to Mirazid treatment, DNA fragmentation appeared in necrotic form in hermaphrodite-digestive glands. The amount of intact DNA in hermaphrodite-digestive glands of S. mansoni infected and Mirazid treated snails were increased values 130.6 and 50.13 maximal optical density when compared to control snails value 32.71 (Fig. 4D and Table 2).

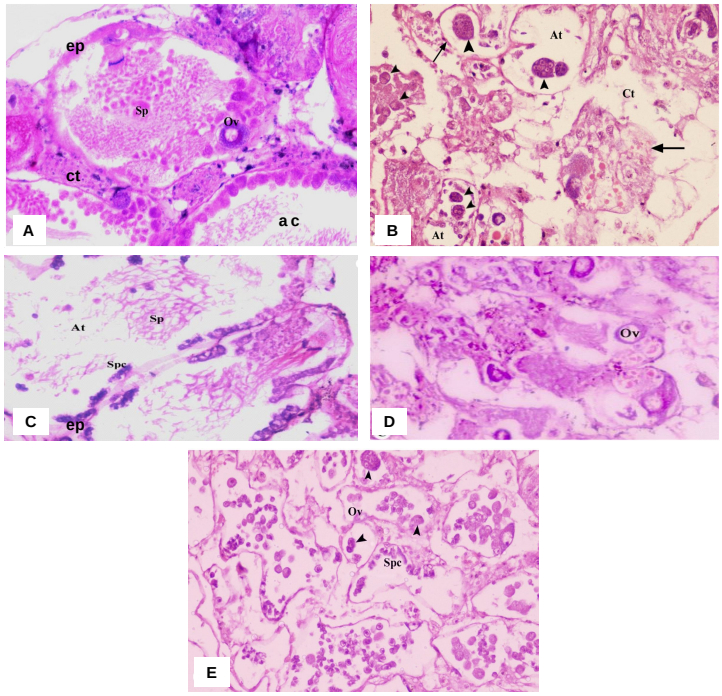

Figure (1): Light photomicrographs of hermaphrodite gland stained with E and H of control *B. alexandrina* snails, (A) control (B) infected snial, showing deformed acini (ac, arrows) and separated by loose connective tissue (Ct). Severe degeneration and atrophy (At) in all gland structure were noticed as well as the presence of sporocysts (arrow heads); (C and D) Mirazid treated snails showing atrophy in all gland structure, scattered and irregular sperms (Sp) and degenerated spermatocytes (Spc), degenerated ova (Ov) and destroyed epithelial cells (ep) were noticed. (E) treated/infected snails showing completely deformed acini (arrows) with degenerated and scattered spermatocytes, degenerated ova and undeveloped sporocysts (arrow heads), (x 400).

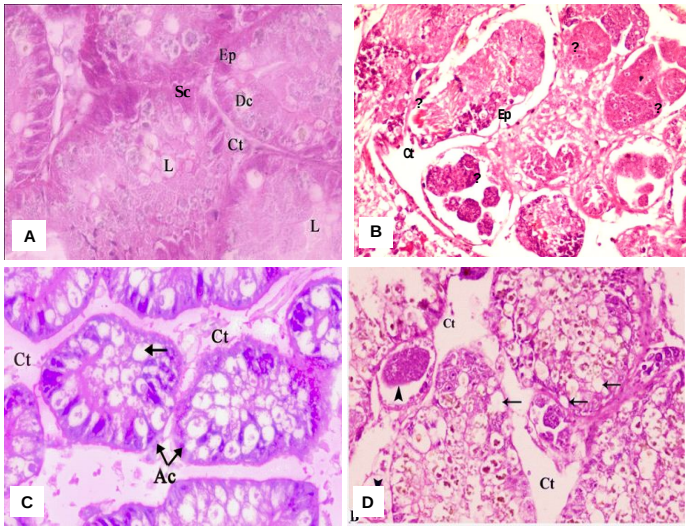

Figure (2): Light photomicrographs of digestive gland sections stained with E and H of *B. alexandrina* snails. (A) Control. (B) B. alexandrina snails infected with S. mansoni at 4th week post infection, the acini (ac) had deformed shape connected together with loose connective tissue (Ct) and the presence of sporocysts (arrow heads). (C) Mirazid treated snails showing scattered acini, completely necrotic connective tissue and cellular vacuoles (arrows) were noticed. (D) Infected treated snails showing destroyed connective tissue, cellular vacuoles besides the presence of sporocysts. epithelial cells (Ep); digestive cells (Dc); secretory cells (Sc); lumen (L); and acini (Ac), (x 400).

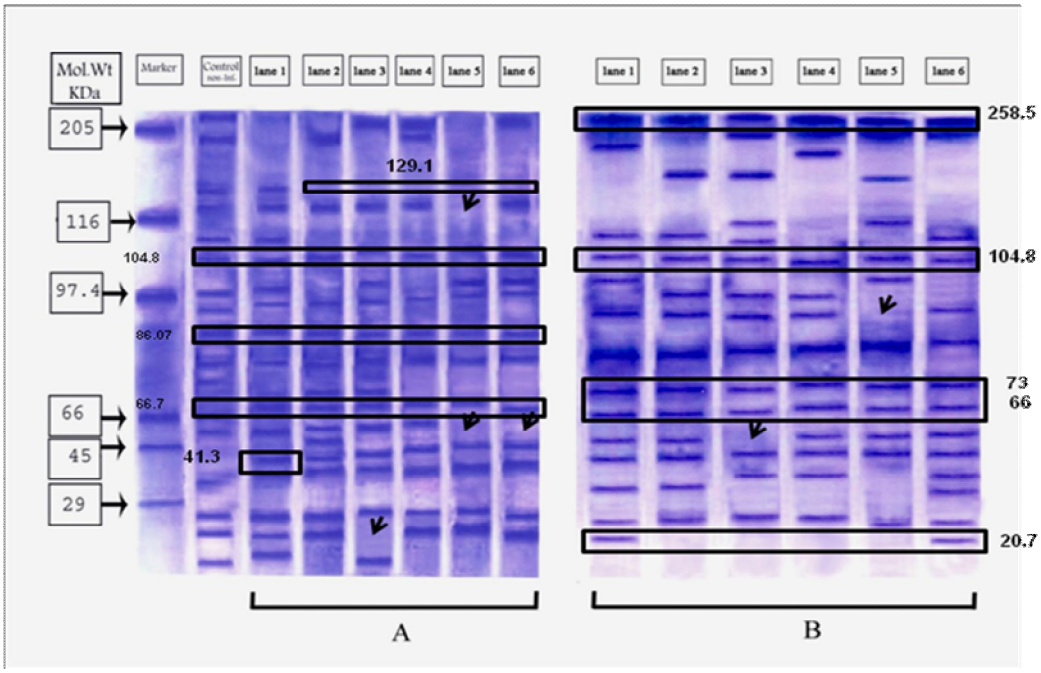

Figure (3): SDS-PAGE profiles of proteins stained with CBB extracted from hermaphrodite- digestive glands of: (A) *B. alexandrina* snails treated with Mirazid. (B) *B. alexandrina* snails infected with *S. mansoni* and treated with Mirazid. Lanes (1-6): treated infected snails at time intervals of 24, 48 h, 1st, 2nd, 3rd and 4th week post exposure, respectively.

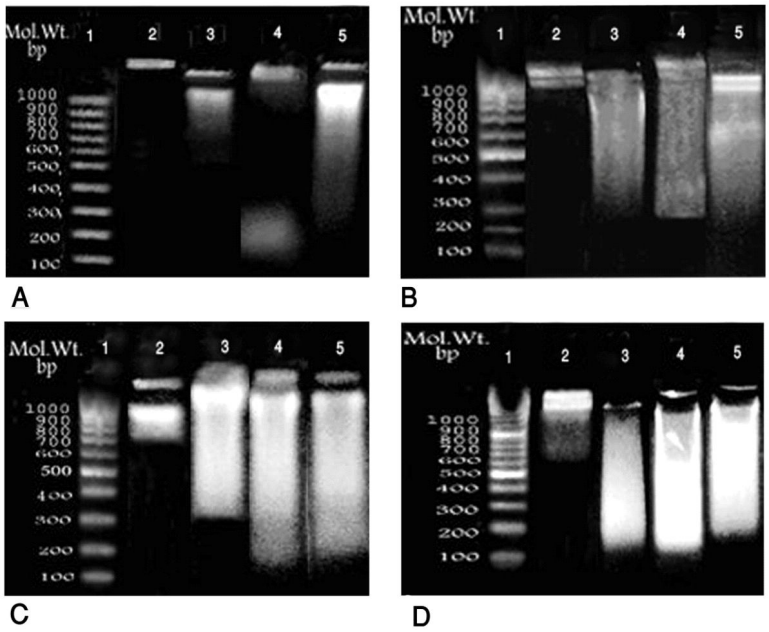

Figure (4): DNA electrophoretic patterns of hermaphrodite-digestive glands of non-infected and *S. mansoni* infected *B. alexandrina* snails treated with Mirazid during experimental periods 24 h (A), 48 h (B), 1 week (C) and 4 weeks (D). Lane (1) DNA ladder, lane (2) Control snails, lane (3) Infected snails, lane (4) Non-infected-treated snails, and lane (5) Infected-treated snails.

Table 1. Effect of the sublethal concentration of Mirazid on biological activities of non-infected and *S. mansoni* infected *B. alexandrina* snails during 4 weeks of continuous exposure

Exposure period (week)	Biological parameters	Experimental groups			
		Control snails		Mirazid-treated snails	
		Non-infected	Infected	Non-infected	Infected
1	Survived snails	10 ± 0.0	9.7 ± 2.2	8.33 ± 2.1	8 ± 1.8
	Egg masses/snail/week	1.9 ± 0.24	0.8 ± 0.09 *	0.3 ± 0.1*	0.6 ± 0.9 *
	Eggs/snail/week	25.8 ± 2.8	15.2 ± 1.2 *	3.4 ± 0.8 *	1.7 ± 0.5 *
	Shell diameter (mm)	8.7 ± 0.4	8.4 ± 0.4	8.6 ± 0.4	8.6 ± 0.4
2	Survived snails	9.7 ± 2.4	8.7 ± 2.1	6.5 ±1.4	6 ± 1.1
	Egg masses/snail/week	1.9 ± 0.1	1.1 ± 0.3 *	0.5 ± 0.0	0.7 ± 0.1
	Eggs/snail/week	33.6 ± 2.7	14.3 ± 2.5*	6.2 ± 1.2 *	2.8 ± 0.6 *
	Shell diameter	9.0 ± 0.5	8.6 ± 0.4	8.6 ± 0.3*	8.7 ± 0.4
3	Survived snails	9.3 ± 2.3	7.7 ± 1.9	4.7 ± 1.2	4 ± 1
	Egg masses/snail/week	1.6 ± 0.3	1.6 ± 0.3 *	0.5 ± 0.1	0.6 ± 0.1 *
	Eggs/snail/week	31.9 ± 2.4	15.1 ± 1.5 *	6.4 ± 1.6*	3.8 ± 0.7 *
	Shell diameter	9.7 ± 0.4	9.3 ± 0.3	8.9 ± 0.3	8.9 ± 0.3
4	Survived snails	9 ± 2	6.6 ± 1.6	3 ± 0.8	2 ± 0.5
	Egg masses/snail/week	1.8 ± 0.2	0.9 ± 0.2 *	0.5 ± 0.1*	0.3 ± 0.1 *
	Eggs/snail/week	33.6 ± 3.0	18.4 ± 1.6 *	2.8 ± 0.6 *	2.7 ± 0.5 *
	Shell diameter	10.5 ± 0.4	9.3 ± 0.4	9 ± 0.4	9 ± 0.2

Data are expressed as mean ± SD, n = 30. * indicates significant difference when $p < 0.05$, compared to control non-infected snails

Table 2. Optical density of DNA fragments in hermaphrodite-digestive glands of non-infected and *S. mansoni* infected *B. alexandrina* snails treated with Mirazid during 24, 48 h, 1 and 4 weeks of exposure

Visualized DNA	After 24 h				After 48 h				After 1 week				After 4 weeks			
	Control	Infected	Treated	Treated infected	Control	Infected	Treated	Treated infected	Control	Infected	Treated	Treated infected	Control	Infected	Treated	Treated infected
Intact DNA	102.9	7.71	30	167.1	61.31	70	36.8	160	200.7	199.2	170	111.8	32.71	130.6	50.13	70.2
800 bp	2.24	6.42	6.24	30.7	23.1	3.45	30.1	70	21.20	107.3	105.5	112.5	35.73	61.7	69.6	35.72
600 bp	3.9	9.2	13	23.52	8.7	3.6	17.8	25	15.41	44.71	54.41	65.13	40.24	65.44	81.51	38.61
400 bp	2.13	6.15	51.71	9.93	1.14	3.75	18.9	15.35	4.91	19.19	38.3	52.8	35.61	51.4	85.22	35.9
200 bp	1	6.35	56.72	2.94	7.11	22.4	7.73	6.3	17.14	39.5	4.6	10.4	8.45	4.24	16.2	4.95

4. Discussion

The present study demonstrated that Mirazid had molluscicidal activity against adult *B. alexandrina* snails and larvicidal activity against *S. mansoni* aquatic larval stages (Miracidia and Cercariae). Mirazid (3 ppm) caused reduction in survival rates among adult *B. alexandrina* snails during the experimental period (4 weeks). The previous results are in agreement with those obtained by Massoud and Habib (2003) who indicated that the sublethal concentration of Myrrh (20 ppm) caused death of *B. alexandrina* snails after 24 h of exposure and the number of dead snails increased with prolongation of exposure time. Abdel-Kader et al. (2005) stated that exposed *B. alexandrina* snails to plant molluscicides led to metabolic disorders on survival rates, egg laying, egg hatchability, lack of smooth transmission at nerve junction, loss of muscular coordination and convulsions then snails' death. The present study indicated that miracidia are more tolerant to the toxic effect of tested materials than cercariae. This may be due to the presence of heavy ciliated tegument in miracidia, which could reduce the harmful effect of the tested materials (Rizk, 1998 and Rizk et al., 2001).

The present study showed that the cercarial production of *S. mansoni* infected *B. alexandrina* snails were completely stopped under the effect of sublethal concentration of Mirazid. Massoud and Habib (2003) reported that shedding of *S. mansoni* cercariae from infected *B. alexandrina* snails stopped at 1 ppm of Myrrh treatment and suppressed at 0.8 ppm and the emerged cercariae from treated snails incapable of infecting humans. Massoud et al. (2004) studied the susceptibility of *B. alexandrina* snails to infection with *S. mansoni* after exposure to sublethal concentrations of Myrrh ($LC_{10, 20}$). They reported that sublethal concentration of Myrrh dramatically reduced the compatibility of *B. alexandrina* to *S. mansoni* infection. Auffray (2007) suggested that *C. myrrha* essential oil is an antioxidant as a singlet oxygen quencher. He proved that *C. myrrha* essential oil gave the best protection for the skin against singlet oxygen and Zelck and Von Janowsky (2004) stated that killing of intramolluscan *S. mansoni* stages (miracidia, sporocysts and cercariae) is mediated by (ROS). Generation of ROS resulted in increased levels of antioxidant enzymes of schistosomes in interaction with susceptible snail hosts in which they survive. Therefore, Mirazid may be retarding the development of the parasite inside the snails.

The present results revealed a reduction of the growth rate of *B. alexandrina* snails treated Mirazid. This result has been reported by several investigators, that reduction of host growth rate during trematode infection is a common phenomenon in the long-term of infection (Gerard and Theron, 1995, Ibrahim, 2006). In the present study, prolonged exposure of the snails to Mirazid led to remarkable changes in egg laying capacity. The exposure of *B. alexandrina* snails to sublethal concentrations of plant molluscicides caused a significant decrease in the number of egg masses and eggs (Rizk, 1998; and El-Ansary et al., 2001).

Results of the present investigation recorded impaired hatchability of *B. alexandrina* eggs under the effect of Mirazid. These results are in accordance with those obtained by Massoud and Habib (2003) who proved that one day-old egg masses were susceptible to ovicidal effect of Myrrh (60 and 80 ppm) and are killed. Similar results were obtained in treatment of *B. arabica* snails' eggs with *C. molmol* (Al-Mathal and Fouad, 2006).

In the present investigation, the damaging effect of prolonged exposure of non-infected and *S. mansoni* infected *B. alexandrina* snails to Mirazid on their histological construction was studied. In the digestive gland, Mirazid treated and treated-infected *B. alexandrina* snails the digestive and secretory cells degenerated which may be explain the reduction in the growth rate of treated and treated-infected snails. In the hermaphrodite glands, the acini become almost empty of gametes. Thus, complete destruction of gametogenic cells and sever damage of hermaphrodite gland may explain the reduction in egg laying capacity of treated and treated-infected snails. Rawi et al. (2011) investigated the histological effect of some natural plants (*Agave filifera*, *Ammi majus* and *Canna indica*) as molluscicidal agents on the hermaphrodite gland of B. alexandrina snails.

Regarding the effect of prolonged exposure of *B. alexandrina* snails to Mirazid and/or *S. mansoni* infection for 4 weeks caused decrease in tissue proteins. This decrease in protein content may be due to interference of the tested material with protein synthesis. Massoud et al. (2001) studied the electrophoretic analysis of total protein, lipo-proteins and glycoproteins of *Culex pipiens* larvae treated with oil and oleo-resine extracts of Myrrh. They mentioned that the plant extracts have inhibitory effect on protein contents and explained that as loss of certain enzymes which affect the metabolic processes.

In the present study, there were sever DNA damage. Similar results obtained by Singh et al. (2004) who used two Euphorbiales plant extracts on *Lymnaea acuminata* and mentioned that both plants decreased the levels of DNA in the nervous tissue of the snail. They added that inhibition of DNA synthesis. Metwally et al. (1990) proved that the significant decrease in total protein in *S. mansoni* infected mice is mainly due to increase in messenger RNA degradation. DNA damage explained by Wang et al. (1984) who stated that Schistosoma parasites unable to synthesis purines by itself and rely on host supplies of bases or nucleosides and a network of reactions converts these nucleic acids into the nucleotides required for DNA and RNA synthesis and other processes for the parasite.

The obtained results revealed that treatment, *S. mansoni* infection or both led to presence of apoptotic fragments in DNA during the whole experimental period. DNA double-strand cleavage occurs in the linker region

between nucleosomes and produces DNA fragments that are multiples of 180 base pairs (Wyllie, 1995). This process is known as apoptosis (organized DNA damage) and these fragments can be demonstrated by agarose gel electrophoresis (Hassab El-Nabi, 2004b). He added that necrosis, in contrast, is accompanied by random DNA breakdown, with diffuse smear in agarose gels.

In conclusion, Mirazid had molluscicidal and larvicidal activities against both adult *B. alexandrina* snails and *S. mansoni* larvae. Consequently, the tested material is recommended as a molluscicide for the control program of schistosomiasis.

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