Advances in Biomechanics
2017; 1(2): 34-41
http://www.sciencepublishinggroup.com/j/abm
doi: 10.11648/j.abm.20170102.12

Effects of the Extracts of *Euphorbia pulcherima* and *Atriplex nummularia* on the Infectivity of *Schistosoma haematobium* to *Bulinus truncatus* Snails

Fayez A. Bakry[1], Manal El-Garhy[2, *], Mahmoud Abd El-Atti[3], Marwa Th. Atwa[4]

[1]Theodor Bilharz Research Institute, Giza, Egypt

[2]Department of Zoology, Faculty of Science, Cairo University, Giza, Egypt

[3]Department of Zoology, Zagazig University, Zagazig, Egypt

[4]Zoology Departement, Faculty of Science, Fayoum University, Al Fayoum, Egypt

Email address:

man662017@gmail.com (M. El-Garhy)

[*]Corresponding author

To cite this article:

Fayez A. Bakry, Manal El-Garhy, Mahmoud Abd El-Atti, Marwa Th. Atwa. Effects of the Extracts of Euphorbia Pulcherima and Atriplex Nummularia on the Infectivity of Schistosoma Haematobium to Bulinus Truncates Snails. *Advances in Biomechanics*. Vol. 1, No. 2, 2017, pp. 34-41. doi: 10.11648/j.abm.20170102.12

Received: April 23, 2017; **Accepted**: May 8, 2017; **Published**: July 6, 2017

Abstract: Effects of extracts of *Euphorbia pulcherima* and *Atriplex nummularia* on infection rate and cercarial production of The *Bulinus truncatus* infected with *Schistosoma haematobium*, as well as on the free living stages of the parasite (miracidia and cercariae) besides biochemical parameters of the snail were studied. The results showed that the LC_{25} of the extracts of *E. pulcherima* and *A. nummularia* caused a considerable reduction in the infectivity of *S. haematobium* miracidia to the snail. It caused a reduction in the number of cercariae per snail during the period of cercarial shedding. The mortality rates of the miracidia and cercariae were elevated gradually by increasing the exposure period to the extracts of these plants. The results obtained also revealed that the glucose concentrations increased in haemolymph, while soft tissue glycogen and protein contents were decreased. The activities of Hexokinase, pyruvatekinase and lactate dehydrogenase were also significantly reduced in response to treatment, while adenosine triphosphatase (ATPase) activity of the tissue of the snails was significantly increased.

Keywords: *Bulinus truncatus*, *Schistosoma haematobium*, *Euphorbia pulcherima*, *Atriplex nummularia*, Glucose, Glycogen and Protein

1. Introduction

Schistosomiasis is a group of chronic parasitic diseases afflicting at least 240 million people all over 70 countries [1]. Almost, no country on the African continent is safe from these infections, as about 85% of the infected populations worldwide are Africans [2]. *Bulinus truncatus* snails are the snail host of *Schistosoma haematobium* with widespread distribution of the snails all over Egypt [3-5].

The use of molluscicides in the control of snails had a significant effect in reducing both incidence and prevalence of schistosomiasis brought about by a devastating reduction of the intermediate snail host population. Economic and ecological considerations increasingly the use of molluscicides that are selectively active, biodegradable, inexpensive and readily available in the affected areas. The imported synthetic molluscicides are high in their cost and toxicity for human, fish and domestic animals [6]. In view of these disadvantages, increasing attention is currently given to plant molluscicides hoping that they may prove safe, cheap, easily available and simply applicable agents.

The use of plants with molluscicidal properties appears to be a simple and inexpensive alternative to chemical molluscicides [7]. More than 1000 plant species have been screened for molluscicidal activity [8]. In Egypt, screening of local plants for molluscicidal activity has received increasing

attention [9-15] In Egypt, molluscicides of plant origin have received an increasing attention, so several plant species were screened in this concept [16-20].

The present work was planned to study the effect of sublethal concentrations of the extracts of *Euphorbia pulcherima* and *Atriplex nummularia* on infection of *Bulinus truncatus* with *S. haematobium* miracidia, in addition to cercarial production of infected snails, as well as on the free living stages (miracidia and cercariae). Besides biochemical parameters of the snail were studied.

2. Materials and Methods

2.1. Snails

The snails used in the present work were *Bulinus trncatus*, They were collected from irrigation canals not previously treated with any molluscicide nearby Abu–Rawash area, Giza province, Egypt. They were kept and examined for *S. haematobium* infection. Collected snails from the field (noninfected snails) were kept in glass or plastic containers (50 X 30 X 20 cm) with transparent covers as 100 snails per each aquarium for 3 weeks before use to accommodate the laboratory conditions.

2.2. Miracidia and Cercaria of Schistosoma Haematobium

Schistosoma haematobium miracidia and cercariae were obtained from Schistosome Biological Supply Centre, Theodor Bilharz Research Institute (SBSC/TBRI), Cairo, Egypt.

2.3. The Plants

The plants used in this study were *Euphorbia pulcherima* and *Atriplex nummularia* were obtained from Orman garden, Egypt. These plants were kindly identified by specialists in the Botany Department, Faculty of Science, Cairo University. The whole overground parts of these plants were left to dry in the air and then in an oven at 50°C and powdered by a mixer.

2.4. Preparation of Plant Extracts

Groups of each plant powder were exhaustively extracted with methanol (70%) by soaking at room temperature (25 ±1-3°C) for one week. The solvent was distilled off under vacuum and the crude extract residues were assayed as aqueous solutions. The efficacy of the tested plant extract and water suspension against adult snails was determined according to the standard procedure recommended by WHO [21]. LC_{10}, LC_{25}, LC_{50} and LC_{90} were previously determined in the laboratory according to the method of Litchfield & Wilconxin [22].

2.5. Efficacy of Testing Plants on the Infection Rate of B. Truncatus with S. Haematobium Miracidia

The effects of the LC_{25} of the methanol extracts of *E. pulcherima* and *A. nummularia* on the infection rate of *B. truncatus* with *S. Haematobium* miracidia and cercarial production were examined by exposing a group for each plant of 50 noninfected snails individually to a dose of 10 miracidia/snail and maintained in each LC_{25} of concentration of the tested plant for 24 hours at room temperature (24 ± 2°C) and ceiling illumination. A control group of 50 snails was exposed to miracidia only without any treatment and maintained under the same conditions. Examination of snails for cercarial shedding was carried out twice weekly. After 20 days of miracidia exposure, surviving snails were individually examined for cercarial shedding in multidishes, artificial light for 3 hours (stimulant period) and 2ml of dechlorinated H_2o/snail. The product cercariae / snail was transferred to small pertridishes by Pasteur pipette, fixed in Boun´soln and counted under astereomicroscope. This examiner was repeated every 3 days until the end of shedding.

2.6. Efficacy of Testing Plants on

2.6.1. Miracidia

S. haematobium ova were exposed to desk lamp for hatching at 25-27°C. One ml of dechlorinated tap water containing 25 freshly hatched miracidia was mixed with 1 ml of double concentration (this means, we tested 500 ppm we use 1000 ppm actually, because the addition of the same volume of water containing the tested organism which dilute concentrations to half of each plant extracts; three replicates from each concentration were used. Under the same conditions, 2 ml of dechlorinated tap water containing 25 non treated freshly hatched miracidia was used as a control. During exposure period mobility and mortality of miracidia were recorded at intervals of 1/2, 1, 1.5, 2, 2.5, 3 hours using stereomicroscopic.

2.6.2. Cercariae

One ml of each concentration was added in a test tube to the same volume of dechlorinated tap water containing 50 freshly shed cercariae and mixed well (double concentration). The cercariae were observed for their mobility and mortality at different intervals. Two ml of dechlorinated tap water containing 50 fresh shed cercariae was observed as a control.

2.7. Physiological Studies

For studying physiological parameters of *B. alexandrina* snails (8 -10). Snails were randomly divided into 4 groups (50 snails each). The 1st, 2nd and 3rd groups were exposed to the LC_{25} of the methanol extracts from *E. pulcherima* and *A. nummularia* for one month, respectively. A fourth group of snails was left unexposed under the same laboratory conditions as a control. Surviving snails after exposure was used to study the effects of the plant extracts on protein and glycogen contents in their soft tissues and glucose level in their haemolymph. The activities of hexokinase (HK), pyruvate kinase (PK), lactate dehydrogenase (LDH) and adenosine triphosphatase (ATPase), were also, investigated in treated and untreated snails.

Haemolymph samples were collected according to Michelson [23] by removing a small portion of the shell and

inserting a capillary tube into the heart. Haemolymph was pooled from 10 snails and collected in a tube (1.5 ml) in an ice-bath. For preparation of tissues of snails, one gram of snails' soft tissues from each group was homogenized in 5 ml distilled water at pH 7.5. A glass homogenizer was used and the homogenate was centrifuged for 10 minutes at 3000 rpm, then the fresh supernatant was used.

Biochemical parameters were determined spectrophotometrically using kits purchased from BioMerieux Company, France. Total protein content was determined according to Lowry *et al*, [24]. Tissues glycogen was evaluated according to Carroll *et al.* [25], and haemolymph glucose concentration was determined according to the glucose oxidase method of Trinder [26].

Hexokinase (HK) was assayed according to the method of Uyeda and Racker [27]. Pyruvate kinase (PK) relative activity was measured spectrophotometrically by the method of McManus and James [28]. Lactate dehydrogenase (LDH) was measured according to the method of Cabaud and Wroblewski [29]. Adenosine triphosphatasae activity was assayed according to the modified method of Giles and Vanstone [30].

2.8. Statistical Analysis

Analysis of data was carried out applying student's *t*-test [31] (Spiegel, 1981).

3. Results

From the data of comparative effect of the tested extracts of *E. Pulcherima* and *A. Nummularia* plants on *Bulinus truncates* snails, recorded in Table 1, it was seen that *E. pulcherima* and *A. Nummularia* plants were more effective as molluscicides. (LC_{50} values were 43and 32 ppm, respectively).

Table 1. *Molluscicidal activity of extract of Euphorbia pulcherima and Atriplex nummularia plants against Bulinus truncatus snails after 24 hours of exposure under laboratory conditions.*

Plant species	LC_{50}	Confidence limit	LC_{90}	LC_{25}	LC_{10}	LC_0	Slope
Euphorbia pulcherima	43	26-45	71	22	14	2.2	2.4
Atriplex nummularia	32	27- 40	78	28	15	2.8	2.51

The data are presented in tables 2 revealed that the exposure of *B. truncatus* to *S. haematobium* miracidia at LC_{25} of *E. Pulcherima* and *A. nummularia* extracts had a positive effect on infection rates. The infection rate of *B. truncatus* snails to S. *haematobium* miracidia exposed to LC_{25} of *E. Pulcherima* and *A. nummularia* extracts were 37.5% and 25%, respectively. These rates were significantly lower ($P<0.01$) than that of the control group (55%). The results in Table 2 indicated that, exposure of *B. truncatus* continuously to the tested plants extracts during miracidia exposure have shortened significantly ($P<0.05$), their prepatent period compared to their corresponding control. The prepatent periods of snail groups exposed to LC_{25} of *E. Pulcherima* and *A. nummularia* extracts were 38±0.64, and 31±0.34days, respectively, in comparison to 48 ±0. 52 days in the control group. Table 2 revealed that, treatment of snails with two tested plants during their exposure to *S. haematobium* miracidia resulted in a highly significant reduction of total cercarial production throughout its entire life span compared to the control ($P<0.001$). The total number of cercariae exposed to LC_{25} of *E. Pulcherima* and *A. nummularia* was 80±6.5and 52±4.4, respectively, compared to the control group 180±10.2 and duration of cercarial shedding was significantly decreased in all groups of *B. truncatus* tested with LC_{25} of the two tested plant extracts in comparison with the control group. The shortest period of cercarial shedding was observed for snails treated with LC_{25} of *E. Pulcherima* and *A. nummularia* being 2.6±0.16 and 2±0.13days compared to 4.5±0.41 days for the control group ($P<0.001$).

Table 2. *Effect of LC_{25} of extract of Euphorbia pulcherimaand Atriplex nummularia plants on the infection of Bulinus. truncates snails with Schistosoma haematobium.*

Concentration Ppm	Survival at 1st shedding		Infection rate of snails		Prepatent period of cercariae range (days)		Cercarial production Number of cercariae		Shedding duration of cercariae (days)	
	No. survival	%	No.	%	Range	Mean ± S. D	Range	mean ± S. D	Range	Mean ± S. D
Control	40	80%	22	55%	27-55	48 ±0.52***	92-210	180±10.2***	5-18	4.5±0.41***
Euphorbia pulcherima	24	48%	9	37.5%	25-43	38±0.64***	55-110	80±6.5***	3-6	2,6±0.16***
Atriplex nummularia	16	32%	4	25%	25-30	31±0.34	46-58	52±4.4	2-4	2±0.13

p<0.05, ** p<0.01, *** p<0.001. no=50

Data given in Tables 3, 4 indicated that, LC_{25} of the two plant extracts had a miracidia effect after one hour of exposure. However, at 100 ppm, *E. pulcherima* and *A. nummularia* plants exhibit a harmful effect (100%) after 90 min compared to 2% in the control group, while at 50 ppm *A. nummularia* plants give 100% mortality after 3 hours compared to 7% in the control group. As the concentration decreases, the harmful effect decreases. The most effective plant extracts as miracidia is on *E. pulcherima* and *A. nummularia.* The recorded LC_{50} value for 3 hours for *E. pulcherima* and *A. nummularia* extract on S. *haematobium* miracidia were 22 and 14 ppm, respectively (Table 5).

Table 3. *Effect of LC_{25} of extract of Euphorbia pulcherima plant on Schistosoma haematobium miracidia*

Conc. (ppm)	% mortality of miracidia after the following intervals (hours)						
	1/2	1	1.5	2	2.5	3	24
15 ppm	10	21	28	34	38	44	100
25 ppm	15	28	37	44	40	66	100
50 ppm	38	47	58	62	80	96	100
100 ppm	60	80	100	100	100	100	100
Control	0	1	2	3	4	5	

Table 4. *Effect of LC_{25} of extract of Atriplex nummularia plant on Schistosoma haematobium miracidia*

Conc. (ppm)	% Mortality of miracidia after the following intervals (hours)						
	1/2	1	1.5	2	2.5	3	24
15 ppm	12	26	33	38	44	52	100
25 ppm	22	32	43	48	56	62	100
50 ppm	46	52	62	70	88	100	100
100 ppm	75	85	100	100	100	100	100
Control	0	1	2	3	6	7	100

Table 5. *Miracidiacidal effects of Euphorbia pulcherima and Atriplex nummularia plants on Schistosoma haematobium miracidia after 3 hours of exposure in vitro.*

Plant name	LC_{50} ppm	LC_{90} ppm	Slope
Euphorbia pulcherima	22	44	2.1
Atriplex nummularia	14	33	2.2

The exposure of cercariae to the plant extracts (Tables 6, 7 and 8) at they started to die after 30 minutes of exposure to 15 ppm of *E. pulcherima* and *A. Nummularia* plant where 12%and 14% mortality took place, respectively. The mortality rate was increased gradually by increasing the exposure period. It became 50 and 70% for 15 ppm of *E. pulcherima* and *A. Nummularia* plant after 3 hours of exposure, respectively, compared with 17% for the control group. The high concentration (100 ppm) of *E. pulcherima* kills cercariae after 1.5 hours, while *A. nummularia* gives 100% mortality after 1 hour with comparison of control group (17%). The recorded LC_{50} value for *E. pulcherima* and *At. Nummularia* plant on *S. haematobium* cercariae were 16 and 12 for 3 hours, respectively (Table 8).

Table 6. *Effect of LC_{25} of extract of Euphorbia pulcherima plants on Schistosoma haematobium cercariae.*

Conc. (ppm)	% mortality of cercariae after the following intervals (hours)						
	1/2	1	1.5	2	2.5	3	24
15 ppm	12	25	35	40	45	50	100
25 ppm	20	35	50	55	75	80	100
50 ppm	35	60	70	75	100	100	100
100 ppm	70	85	100	100	100	100	100
Control	4	7	9	9	12	17	100

Table 7. *Effect of LC_{25} of extract of Atriplex nummularia plants on Schistosoma haematobium cercariae.*

Conc. (ppm)	% mortality of cercariae after the following intervals (hours)						
	1/2	1	1.5	2	2.5	3	24
15 ppm	14	33	45	60	65	70	100
25 ppm	25	40	55	65	80	90	100
50 ppm	40	60	75	85	100	100	100
100 ppm	65	100	100	100	100	100	100
Control	3	6	10	12	15	17	100

Table 8. *Cercariacidal effect of Atriplex nummularia and Euphorbia pulcherima plants after 3 hours of exposure in vitro.*

Plant name	LC_{50} ppm	LC_{90} ppm	Slope
Euphorbia pulcherima	16	34	2.5
Atriplex nummularia	12	23	2.1

The results (Table 9) showed that also, a significant reduction in the total protein content of the soft tissues throughout the exposure period to *E. pulcherima* and *A. nummularia* plant. The rates of reduction were 28.4%and -51% after one month of exposure. The glycogen content in tissues of the treated snails was also significantly reduced. The rates of reduction were -29.4%and -52% after one month of exposure to LC_{25} of *E. pulcherima* and *A. nummularia* plant, respectively. On the other hand, the glucose concentration in haemolymph of the snails exposed to the tested extracts showed a significant increase in comparison with the control group (12±1.6ml /ml). The values recorded were 18.2±1.7and 21.2±1.4ml / ml in haemolymph of snails exposed to LC_{10} of *E. pulcherima* and *A. nummularia* plant, respectively.

Table 9. *Effect of one month exposure to LC_{25} of Euphorbia pulcherima and Atriplex nummularia extracts on glucose level in haemolymph, total protein and glycogen content in Bulinus truncates.*

	In soft tissue				In haemolymph	
	Protein content (mg/g tissue)	% change	Glycogen content (mg/g tissue)	% change	Glucose level (mg/ml)	% change
Control	25.3±1.4		21.4±1.5		12±1.6	
Euphorbia pulcherima plants	18.1 ±1.6**	-28.4%	15.1±1.2**	-29.4%	18.2±1.7**	51.7%
Atriplex nummularia	12.4±1.4***	-51%	10.3±1.8***	-52%	21.2±1.4***	76.7%

X±SD mean of 4 experiments. *p< 0.05,**p< 0.01 & ***p< 0.001

The present study showed that the LC_{25} of *E. pulcherima* and *A. nummularia* extracts induced a significant increase in the activities of ATPase being 0.95±0.08 (55.7%) and 1.1 ±0.07 (80.3%) µmoles pi /min/g wet tissue respectively in *B. truncatus* tissues (Table 10). The activities of hexokinase (HK), pyruvate kinase (PK), lactate dehydrogenase (LDH), in

the soft tissue of normal and treated snails displayed in Table 10. The LDH activity values in snails exposed to LC_{25} of *E. pulcherima* and *A. nummularia* extracts for one month decreased by 39.7% and 62.1%, respectively. Such values were statistically significant as compared to the corresponding control value (5.8±1.2). The maximal effect however was attained in hexokinase activity; the values detected were decreased by 42.3% and 53.8 % as compared to the control level.

Table 10. *Effects of one month exposure to LC_{25} of Atriplex nummularia and Euphorbia pulcherima extracts on some enzyme activities in tissues of Bulinus truncates.*

Plant Species	PK (µ/mg tissue)	% change	(LDH) µ mol pyruvate reacted /min/g tissue	% change	HK (µ/NADH oxidized/min/g tissue)	% change	ATPase activity (µ mol pi /min/ g wet tissue)	% change
Control	2.3 ±0.72		5.8±1.2		2.6± 0.52		0.61±0.007	
Euphorbia pulcherima plants	1.5±0.81 ***	-34.8%	3.5±0.689**	39,7%	1.5±0.06**	-42.3%	0.95±0.08**	55.7%
Atriplex nummularia	1.2±0.80***	47.8%	2.2±0.68***	62.1%	1.2±0.04***	-53.8%	1.1±0.07***	80.3%

X±SD mean of 4 experiments. *p< 0.05,**p< 0.01 & ***p< 0.001

4. Discussion

Screening of the molluscicidal activity of two selected plants was worked out, namely *E. pulcherima* and *A. nummularia* plant. The results indicated that *A. nummularia*, had molluscicidal activities against *B. truncatus* higher than *E. pulcherima*. The high molluscicidal activity of *A. nummularia* apparently attributed to the high concentration of active constituent. This is supported by Shoeb *et al*. [32]; Mansour *et al*. [33]; Bakry *et al*. [34]; Abdel Kader [35]. This finding, however, may be attributed to several factors, including plant specific differences in active gradients, type of the natural products, differences in their mode of action, method of penetration and the behavioral characteristic effect on the snails [4, 5].

The infectivity of *S. haematobium* miracidia was greatly reduced by *A. nummularia* and *E. pulcherima* extracts. This may be interpreted as the LC_{50} of extracts of the two tested plants have weakened the ability of the miracidia to penetrate the tissues of the snails. This agreed with Bakry *et al.* [11] observation on *B. alexandrina* snails infected by *S. mansoni* miracidia and subjected to LC_{25} methanol extracts of *Euphorbia lacteal*. El-Emam *et al.* [36] reported that 50 ppm of *Calendula micrantha* decreased the infection rate. Tantawy *et al*. [37] using *Solanium dubium* plant, Barky *et al.* [34] using *A. franzosinii*, Mohamed *et al*.[38] using *Abamectin,* Sharaf El-Din *et al.*[39] using *Zygophyllum simplex* plant and Bakry *et al.* [4] using methanol extracts of *O. reticulatum* and *Furcraea selloea* revealed similar conclusions. These results also were in accordance with many investigations that used various chemical and plant molluscicides (Mahmoud [40]; Rawi *et al*. [10]; Gawish [41]; El-Ansary *et al*. [42]). Thus, El-Ansary *et al*. [42] reported that *A. maritima* caused a remarkable decrease in cercarial shedding and cercarial production in *B. alexandrina* snails treated with this plant powder. Sharaf El-Din *et al*. [39] obtained similar reduction in cercarial shedding and cercarial production from *B. alexandrina* treated with sublethal concentrations of aqueous suspension of *Zygophyllum simplex*.

Regarding the prepatent period, the present results showed that the prepatent period for snails exposed to LC_{50} of the tested plants during their exposure to miracidia has been shorted. This was in accordance with Mahmoud [40] using sublethal concentrations of *Abamectin*, Tantawy *et al*. [37] using *S. dubium* and Bakry *et al.* [34] using *A. franzosinii*.

The present results showed that duration of cercarial shedding was shorter for testing snails than their corresponding control. This shortening in cercarial shedding period may be due to the rapture of snail's tissues through miracidia penetration that caused an increase in the harmful effect of these treatments. Such stress could disturb the physiological activities of treated snails and result in a shorter life span and shedding period in comparison with their control groups [41]. Similar findings have been reported by Mahmoud [40], Mohamed [38] and Barky et al. [34]. The authors found that, the period of cercarial shedding in snails treated with experimental molluscicides during their exposure to miracidia is significantly shorter than that in control snails. It is also agreed with Badawy [43]. Who found that, exposure of *B. alexandrina* to 50 ppm from *F. cretica* during exposure to *S. mansoni* miracidia decreased the duration of cercarial shedding.

The present data claimed, that the mean number of cercariae produced by each infected *B. truncatus* previously tested with plants throughout its life span was significantly less than that revealed by Badawy [43] on *B. alexandrina* snails treated with *F. cretica* at the early prepatent period. El-Emam et al. [36] found that, 0.050g/L from the dry powder of *C. micrantha* for 24hours highly reduced the cercarial production from infected *B. alexandrina* treated as early and late prepatent period. Adewunmi et al. [44] recorded that the active compound aridanin isolated from *T. tetraptera* at 0.25 mg/L reduced *S. mansoni* cercarial shedding. The same result was observed by Ahmed and Ramzy [45]; Tantawy [37]; Bakry et al. [34]; Mostafa and Tantawy [46]; Sharf El-Din et al. [39] and El-Ansary et al. [47]. The authors used the plants *S. nigrum, S. dubium*, *A. arvensis*, *A. franzosinii*, *Z. simplex* and *P. harmula*, respectively against *S. mansoni* cercarial production inside the soft tissues of *B. alexandrina*.

From the previous results, it was clear that, *B. truncatus* snails exposed to LC_{25} of *E. pulcherima* and *A. Nummularia*

plants were less susceptible to the infection with *S. haematobium* miracidia. Also, the cercarial production decreased during the entire life of the infected snails treated with the extracts than those infected and untreated. These results declared that, such sublethal concentrations when used in the field could suppress the development of *S. haematobium* inside snails and shorten the duration of cercarial shedding. It also could induce decrease in the number of cercariae produced by infected snails and thus, can interrupt parasite transmission.

Concerning the effect of sublethal concentrations of extracts of the tested plants against *S. haematobium* miracidia and cercariae, the present results showed that mortality rates of *S. haematobium* miracidia and cercariae were increased by increasing sublethal concentrations of the tested plants as well as by the period of exposure. Therefore, when these plants are used in snail control, they will kill the free-living larval stages of *Schistosoma*.

In the present investigation, a significant decrease has been recorded in the tissue protein in snails treated with the LC_{25} of the extracts of the tested plants. This decrease may be due to interference of the active substance of the tested plants in protein metabolism by inhibiting protein synthesis. Similar results were obtained by Abdel Kader and Tantawy [48]. *Agave fififera* and *Agave attenuate* plant and Bakry *et al* [11] using *Calotropisprocera*, *Euphorbianubia* and *Atriplex halimus* plant.

Regarding the sources of energy for snails, the LC_{25} of the extracts of the tested plants significantly decreased the glycogen content of soft tissues while the increased glucose level in haemolymph. This may be attributed to the effect of the active substance of the tested plants that impedes oxygen consumption of snails, thus inducing anaerobic respiration. To restore its energy requirements, the snail has to increase the rate of glycolysis thus bringing about a reduction of the glycogen content and increase glucose level in the haemolymph. This finding agrees with the results of similar experiments applying *Euphorbia pseudocactus*, *Yaccaalaifolia* and *Portalaccaoleracca* methanol extracts [5].

In the present study, the glycolytic enzymes, hexokinase (HK), pyruvate kinase (PK), lactate dehydrogenase (LDH) in the snail tissues showed a significant decrease. The depletion in HK activity in the soft tissues caused an alteration of glycolytic mechanism which in turn induced a state of anoxia. A similar effect was detected by Bakry *et al*. [49] using *Euphorbia lactea* plant and Bakry [4] using *Lampranthus. spectabilis* and *Furcraea gigantean* plants The activities of PK of treated *B. alexandrina* showed significant reductions. This reduction may be due to the toxic effect of the tested extract plants that minimizes the ATP level by disturbing the enzymatic pathways contributing to ATP generation and hence causes depression of the snails energy metabolism. This finding agrees with that of Bakry *et al*. [11] using methanol extracts of *Calotropisprocera*, *Euphorbianubia* and *Atriplex halimus*.

The present study also showed a significant decrease in LDH activity in the tissue extracts of *B. truncatus* in response to treatment with LC_{25} of the tested plant extracts. The decrease in LDH activity of *B. truncatus* tissues may be due to the release of the enzyme from the tissues as a result of cellular damage caused by the toxic effect of molluscicides. It was reported that the tissue damage followed by the release of cellular enzymes such as LDH [50, 51]. Several authors have reported a significant decline in LDH activity of tissues of various mollusks in response to some molluscicides [5, 11, 52, 53].

The present study also showed that the LC_{25} of the two tested plant extracts induced a significant increase in the activity of ATPase in treated *B. truncatus*. This result is in agreement with Ismail *et al.* [54] who reported that urea and magnesium sulfate stimulate ATPase activity in tissue homogenate of *B. truncatus*. This increase in ATPase activity reflects the drastic rise in ATP requirements due to the toxic insult of the molluscicides and induced anoxia [55].

5. Conclusion

In conclusion, in the present study, *E. pulcherima* and *A. nummularia* extracts are the most natural products. They have a miracicidal and cercaricidal activity The results showed that LC_{25} of extract of *E. pulcherima* and *A. nummularia* caused a considerable reduction in the infectivity of *S. haematobium* miracidia to the snail. It caused a reduction in the number of cercariae per snail during the patent period and the period of cercarial shedding. The mortality rates of miracidia and cercariae were elevated gradually by increasing the exposure period to extract of these plants. The present data could be concluded that the depletion in tissue protein, glycogen, and activity of some enzymes of energy metabolism (HK, PK, LDH, ATPase) of *B. truncatus* snails treated with LC_{25} of extract of test plants.

References

[1] Larson, M. K.; Bender, R. C. and Bayne, C. J. (2014): Resistance of *Biomphalaria glabrata*13-16-R1 snails to *Schistosoma mansoni* PR1 is a function of haemocyte abundance and constitutive levels of specific transcripts in haemocytes. Int. J. Parasitol., 44 (6): 343-353.

[2] World Health Organization (2010) World health report on Schistosomiasis. *Wikipedia, the Free Encyclopedia MHT*.

[3] Youssef, A. A. (2010) Studies on the impact of some pesticides and Egyptian plants on some biological and physiological parameters of *Biomphalaria alexandrina* snails and their susceptibility to infection with *Schistosoma mansoni* miracidia. *M. Sc. Thesis, Faculty of science, Al-Azhar University, Egypt.*

[4] Bakry, F. A, F. A; Ismail, S. M and 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. Vol. 8, No, 4: 313-446.

[5] Bakry F. A, Ismail S. M., Abd El-Atti M. S. (2015). Glyphosate herbicide induces genotoxic effect and physiological disturbances in Bulinus truncatus snails. Pesticide Biochemistry and Physiology 123: 24–30.

[6] Mousa, A. H., Yousif, F. and El-Ghayeb, F. M. (1968-1969): Studies on low concentrations of molluscicides. I- Effect of low concentrations of molluscicides on the survival and egg production of the snail intermediate hosts of schistosomiasis in UAR. Bull. Zool. Soc. Egypt, 22: 105-116.

[7] Perrett, S. and Whitfield, PJ. (1996): In vitro cercaricidal activity of schistosomiasis. J. Parasitol., 52: 617.

[8] El-Bolkiny, YE.; Salem, ML.; Attia, WY. and Al-sharkawi, I. M. (1997). Toxicological study of *Ammi majus* as a plant molluscicide on the hemolytic. J. Egypt. Grt. Soc. Zool., 23 (A): 379-401.

[9] Mohamed, A. M.; El-Fikii, S. A.; El-Sawy, M. F. and El-Wakil, H. (1981): Effect of prolonged exposure of *Biomophalaria alexandrina* to low concentration of some molluscicides. 1- Effect on longevity, growth rate, fecundity and susceptibility to Schistosoma infection. J. Egy. Soc. Parasitol., 11 (2): 295-309.

[10] Rawi, S. M.; El-Gindy, H.; Haggag, A. M.; Abou El Hassan, A. & Abdel Kader, A. (1995): Few possible molluscicides from calendula *Micrantha officinalis* and *Ammimajus* plants. I. Physiological effect on *B. alexandrina* and *B. truncatus*. *J.* Egypt. Ger. Soc. Zool. 16: 69-75.

[11] Bakry, F. A; Ragab, F. M. A and Sakran, A. M. A. (2002a): Effect of some plant extracts with molluscicidal properties on some biological and physiological parameters of *Biomphalaria alexandrina* snails. J. Ger. Soc. Zool. 38: 101- 111.

[12] Sakran, AM. A. (2004): Biological and physiological studies on *Biomphalaria alexandrina* snails exposed to two herbicides. Egypt. J. Zool. 42: 205-215.

[13] El-Sayed MM, Abdel-Hameed ES, El-Nahas HA, El-Wakil EA. 2006. Isolation and identification of some steroidal glycosides of *Furcraea selloa*. Pharmazie. 61 (5): 478-482.

[14] Sakran A. M. A and Bakry F. A (2005): Biological and physiological studies on *Biomphalaria alexandrina* snails exposed to different plant molluscicides. J. Egypt. Ger. Soc. Zool, Vol (48A). 237-256.

[15] Bakry F. A and Hamdi S. A. H.(2007): Molluscicidal activity of latex Aqueous solution of *Euphorbia acetonitril* and *Euphorbia granulate* against the intermediate hosts of Schistosomiasis and Fascioliasis. J. Union. Arab. Biol. 27: 101-126.

[16] Gawish, F. A. and El-Bardicy, S. (2002) Effect of the plants *Solanumnigrum* and *Ambrosia maritima* on susceptibility of *Biomphalariaglabrata* snails and albino mice to Peurto Rican strain of *Schistosoma mansoni*. Abs. Int. Conf. TBRI, Cairo, Egypt, 26.

[17] Rawi, S. M.; El-Gindy, H & Abdel Kader, A. (1996): New possible molluscicides from calendula *Micrantha officinalis* and *Ammi majus*. Molluscicidal, physiological and egg laying effects against *Biomphalaria alexandrina* and *Bulinus truncates.* J. Eco. Toxical. Environmental Safety, 35: 261.

[18] Bakry, F. A. (2009) Impact of Some Plant Extracts on Histological Structure and Protein Patterns of *Biomphalaria alexandrina* Snails. Globul J. Molecul. Sci. 4(1): 34-41.

[19] Bakry F. A., Eleiwa E. M., Taha S. A., and Ismil S. M. (2016a). Comparative toxicity of Paraquat herbicide and some plant extracts in *Lymnaea natalensis* snails Toxicology and Industrial Health. 32: 143-153

[20] Bakry F. A., El-Hommossany K., Ismil S. M. (2016b). Alterations in the fatty acid profile, antioxidant enzymes and protein pattern of *Biomphalaria alexandrina* snails exposed to the pesticides Basudin and Selecron. Toxicology and Industrial Health, 32: 666–676.

[21] WHO (1965): Molluscicide screening and evaluation. *Bull. WHO. 33: 567-581.*

[22] Litchfield, J. T. and Wilconxon, F. (1949): A simplified method of evaluation dose-effect experiments. J. Phamacol. Exp. Therep., 96: 99-113.

[23] Michelson, EH. (1966). Specificityof hemolymph antigens in taxonomic discrimination of medically important snails. J. Parasitol., 52**:** 466-472.

[24] Lowry, OH.; Rose Brough, NJ.; Farr, AL. & Randall, RJ. (1951). Protein measurement with thefolin-phenol reagent. J. Biol. Chem., 2: 265-275.

[25] Carroll, N. V.; Longley, R. W. & Roe, J. H. (1956) The determination of glycogen in the liver and muscles by use of anthrone reagent. *J. Biochem.* 220: 583-593.

[26] Trinder, P. (1969) Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem*,* 6: 24-27.

[27] Uyeda, K. and Racker, E. (1965) Regulatory mechanisms in carbohydrate metabolism. VII. Hexokinase and phosphofructokinase. J. Biol. Chem. 240: 4682-4688

[28] McManus, D. P. and B. L. James (1975) Anaerobic glucose metabolism in the digestive gland of *Littorinasaxatitis* rudis (Maton) and the daughter sporocysts *Microphallussimilis* (Tag). Comp. Biochem. Physiol., 51B" 293-297

[29] Cabaud, P. & Wroblewski, F. (1958) Colorimetric measurement of lactic dehydrogenase activity of body fluids. *Am. J. Clin. Pathol.* 3 0: 234.

[30] Giles, A. and Vanstone, W. E. (1976) Changes in ouabain-sensitive adenosine triphosphatase activity in gills of cohosalmon (*Oncorhynchus kisutch*) during parrsmolt transformation. J. Fish. Res. Bd. Can; 33: 54-62.

[31] Spiegel, R. M. (1981): Theory and problems of Statistics, Schaum's outline series. McGraw-Hill, Singapore.

[32] Shoeb, H. A.; El-Emam, and Osman, N. S. (1982) The molluscicidal activity of Eupherbiaceae- *Euphorbia peplus* Egypt. J. Bilh. *9: 41-55.*

[33] Mansour, S. A.; Ibrahim, A. M. and Abdel-Hamid, H. F. (2002): Botonical Biocides. 8- impact of some plant extracts on Biomphalaria alexandrina snails and on Schistosoma mansoni miracidia and cercariae. Egy. J. Schisto. Infect. Endem. Dis., 24: 81-99.

[34] Barky, F. A.; Tantawy, A. A. and Ragab, F. M. A. (2001): Effect of sublethal concentrations of *Agava franzosinii* plant (Agavaceae) on the infectivity of *Schistosoma mansoni* to *Biomphalaria alexandrina* snails. J. Egypt. Ger. Soc. Zool., 33 (D): 129-141.

[35] Abdel Kader, A.; Hamdi, SAH.; Rawi, SM. (2005). Biological and biochemical studies on *Biomphalaria alexandrina* snails, treated with low concentrations of certain molluscicides (synthetic and of plant origin). J. Egypt. Soc. Parasitol., 35 (3): 841-858.

[36] El-Emam, M. A., Shoeb, H. A., Ebid, F. A. and Refai, L. A. (1986) Snail control by *Calendula micrantha officinalis*. J. Egypt. Soc. Parasitol., 16 (2): 563-571.

[37] Tantawy, A. A.; Sharaf El-Din, A. T. and Bakry, F. A. (2000): Molluscicidal effect of Solanum dubium (Solanaceae) against Biomphalaria alexandrina snails under laboratory conditions. Proc. Int. Conf. Biol. Sci., 1 (2): 307-318.

[38] Mohamed, A. M., Bakry, F. A. and Heiba, F. N. (2000) Molluscicidal effects of Abamectin on *Biomphalaria alexandrina* and its inflection with *Schistosoma mansoni*. J. 1st Inter. Cong. Biolg. Sci., 1 (2): 207-216.

[39] Sharaf El-Din, A. T. and El-Sayed, K. A. (2001) Alteration in glucose, glycogen and lipid content in Biomophalaria alexandrina snails post-exposure to Schistosoma mansoni and Echinostoma liei miracidia. J. Egy. Ger. Soc. Zool., 36 (D): 103-113.

[40] Mahmoud, M. B. (1993) Effect of certain pesticides on *Biomphalariaalexandrina* and the intramolluscan larval stages of *Schistosoma mansoni*. M. Sc. Thesis, Fac Sci Cairo Univ

[41] Gawish, F. A. (1997) Evaluation of combination of certain molluscicides against *Biomphalaria alexandrina* and the free living stages of *Schistosoma mansoni*. Ph. D. Thesis, Girls College for Arts, Sci. & Education, Ain Shams Univ., Egypt.

[42] El-Ansary, A., El-Baricy, S., Soliman, M. S., Zayed, N. (2000): Sublethal concentration of *Ambrosia maritima* (Damsissa) affecting compatibility of *Biomphalaria alexandrina* snails to infection with *Schistosoma mansoni* through distributing the glycolytic pathway. J. Egypt. Soc. Parasitol. Dec; 30 (3): 809-19.

[43] Badawy, A. M. S. (1991): Control of snail vectors of bilharziasis by using some plants. MSc. Thesis, Monoufia Univ., Egypt.

[44] Adewunmi, C. O. and Furu, P. (1989) Evaluation of Aridanin, a glycoside and Aridan an aqueous extract of *Tetraptera* fruit on *Schistosoma mansoni* and *S. bovis*. J. Ethnopharmacol., 27 (3): 277-283.

[45] Ahmed, A. H. and Ramzy, R. M. (1997): Laboratory assessment of the molluscicidal and cercaricidal activities of the Egyptian weed, Solanum nigrum L. Ann. Trop. Med. Parasitol., 91 (8): 931-937.

[46] Mostafa, B. B. and Tantawy, A. A. (2000) Bioactivity of *Anagallis arvensis* and *Calendula micrantha* plants treated with ammonium nitrate superphosphate and potassium sulphate fertelizers on *Biomphalaria alexandrina*. J. Egypt. Soc. Parasitol., 30 (3): 929-942.

[47] EL-Ansary, A., Sammour, E. M.; Soliman, M. S. and Gawish, F. A. (2001): Invivo, attenuation of schistosome cercarial development and disturbance of egg laying capacity in Biomphalaria alexandrina using sublethal concentrations of plant molluscicides. J. Egy. Soc. Prasitol., 31 (3): 657-669.

[48] Abdel Kader, A. and Tantawy, AA. (2000). Effect ofAgave *filifera* and *Agaveattenuata* on *Biomphalariaalexandrina* snails, the vector of *Schistosomamansoni* in Egypt. Egypt. J. Schist. Infect. Endem. Dis., 22: 173-185.

[49] Bakry, FA; Sakran, AA and Ismail, NMM (2002b). Molluscicidal effect of fungicide, herbicide and plant extract on some biological and physiological parameters of *Biomphalaria alexandrina.* J. Egypt. Soc. Parasitol., 32 (3): 821-835.

[50] Paul, J.; Bekker, A. Y. and Duran, W. N. (1990). Calcium entry prevents leakage of macromolecules induced by ischemia-reperfusion in skeletal muscle. Circ. Res., 66: 1636-1642.

[51] Prasad. M. R; Popeseu, L. M; Moraru, I. I.; Liu, X.; Maity, S.; Engelman, R. M. and Das, D. K. (1991). Role of phospholipase A and C in myocardial ischemic repefusion injury. Am. J. Physiol., 29: 877-H 883.

[52] Singh, D. K. and Agarwal, R. A. (1991). Action sites of cypermethrin asynthetic pyrethroid in the snail *Lymnaea acuminate*. Acta Hydrochim. Hydro. Biol., 19 (4): 425-430.

[53] Aboul-Zahab, A. O. & El-Ansary, A. (1992): Biological aspects of controlling snail hosts of bilharziasis using wild and cultivated plant extracts. Ann. Agric. Sci. Moshtohor, 30 (3): 1531-1539.

[54] Ismail, M; Farrag, F. and Hosny, A. (1996). Variation of Cholinesterase and adenosine triphosphatase activity in snails treated with different fertilizers. J. Union Arab Biol., 5A: 355-360.

[55] Fahmy S. R., Abdel-Ghaffar F., Bakry F. A., Sayed D. A. (2014). Ecotoxicological Effect of Sublethal Exposure to Zinc Oxide Nanoparticles on Freshwater Snail *Biomphalaria alexandrina*. Arch Environ Contam Toxicol (2014) 67: 192–202.