Advances in Biological Chemistry, 2011, 1, 65-73 **ABC**
doi:10.4236/abc.2011.13009 Published Online November 2011 (http://www.SciRP.org/journal/abc/).

Biological and physiological parameters of *Bulinus truncatus* snails exposed to methanol extract of the plant *Sesbania sesban* plant

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Received 22 August 2011; revised 27 September 2011; accepted 8 October 2011.

ABSTRACT

The effects of sublethal concentrations of methanol extract of *Sesbania sesban* plant on survival rate, egg laying of *Bulinus truncatus* snails, hatchability of their eggs, infection rate with *Schistosoma haematobium* miracidia, cercarial production and certain physiological parameters of treated snails were studied. The sublethal concentrations of the tested plant extract (LC_0, LC_{10} and LC_{25}) caused considerable reduction in survival rates; egg production of *B. runcates* snails; hatchability of eggs as well as in the infectivity of *Schistosoma haematobium* miracidia to the snail. Also, the tested concentrations reduced the cercarial production per snail and the period of cercarial shedding. The glucose level in haemolymph of exposed snails was elevated while the glycogen, protein content and the activities of hexokinase (HK), pyruvatekinase (PK) and lactate dehydrogenase (LDH) showed a decrease in soft tissues when compared with the control group. It was concluded that the app lication of sublethal concentration of methanol extracts of *Sesania sesban* may be helpful in snail control as it interferes with the snails' biology and physiology.

Keywords: *Bulinus truncates*; *Schistosoma haematobium* Miracidia; *Susana sesban* Plant

1. INTRODUCTION

Schistosomiasis is a public health problem of social and economic importance in many developing countries calling for international cooperation [1]. There is no doubt that schistosomiasis is one of the major communicable diseases and it is second to the malaria with socio-economic and health importance in the developing world [2]. 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 [3].

There is a great interest in the use of molluscicides of plant origin by local communities in self-supporting system of schistosomiasis control program. Such molluscicides seem to be less expensive, readily available, rapidly biodegradable, have low toxicity to non-target organisms and probably easily applicable with simple techniques appropriate to developing countries [4,5].

The plant *Sesbania aegyptiaca* proved to have a molluscicial activity against *Biomphalaria alexandrina* snails as water suspensions from its dry powder [6]. Thereafter, *S. sesban* exhibited a marked toxic effect against the snail's *Biomphalaria pfeifferi* and *B. truncatus* [7]. The methanol extract of Datura innoxia showed a promising molluscicidal potency against *B. alexandrina*, *B. truncatus* and *Lymnaea caillaudi* snails [8]. In addition, methanol extract of *Datura stramonium* has a strong antifungal property against *Fusarium mangiferae* fungus [9].

In order to promote energy production gastropods categorize primarily carbohydrates, which are stored in certain tissues as glycogen and transported in the haemolymph as glucose [10]. The molluscicides greatly affect the metabolic activities of the snail intermediate hosts [11]. Also, they act on different enzymes chiefly those of respiration and carbohydrate metabolism [12].

The present work was planned to study the molluscicidal activity of methanol extract of *Sesbania sesban* plant against *Bulinus truncatus* snails. As well, the effects of sublethal concentrations of this extract on the snails mortality rates, longevity, egg laying , hatchability of their eggs, infection rate with *S. haematobium* miracidia, cercarial production and certain physiological pa-

rameters of treated snails were evaluated.

2. MATERIALS AND METHODS

2.1. Snails

Bulinus truncatus snails (6 - 8 mm) from laboratory bred colony in Medical Malacology Dept., Theodor Bilharz Research Institute (TBRI), and Giza, Egypt were used.

2.2. Miracidia

Schistosoma haematobium ova were obtained from *Schistosomiasis* Biological Supply Center (SPSC), TBRI. They were left in clean dechlorinated water for hatching under a desk lamp then fresh hatch miracidia were used in bioassay and infection tests.

2.3. Plants

The plant species used in this study are *Sesbania sesban* (Fabaceae) from from Al-Orman garden, Giza, Egypt (spring 2007). They were kindly identified by Botany Dept., Faculty of Agriculture, and Cairo University. Their leaves were shade dried, then powdered by an electric mill. The dry powder was stored in clean dry dark glass bottle till use in biological tests.

Plant's Extract

The dry powder of *S. sesban* plant was extracted by Soaking with 95% methanol (0.5 kg/liter) for seven days. Then the solvent was filtered and distilled off under vacuum and the crude extract residues were stored in clean dry dark vessel till use [13].

2.4. Bioassay Tests

2.4.1. Molluscicidal Screening

A series of concentrations that would permit the computation of LC_{50} and LC_{90} was prepared on the basis of weight/volume as water suspensions. Three replicates (each of 10 snails/L) were prepared. Another 3 replicates in dechlorinated water were used as control. Exposure and recovery periods were 24 hours each at 25˚C ± 1˚C [14,15]. Then, snails' mortality was recorded and corrected according to Abbots' formula [16]. The LC_0 was estimated as 1/10 LC_{50} [14].

2.4.2. Effect on Snails' Egg-Laying Capacity

For studying the mortality, longevity and egg laying of *B. truncatus*, specimens of 120 adult snails (8 - 10 mm) were randomly divided into 4 groups (30 snails each). Three groups were continuously exposed to LC_0, LC_{10} and LC_{25} of methanol extract of S. sesban plant, respectively. The fourth group was left unexposed under the same laboratory conditions as control. The tested plant solutions were changed every 24 hours with new prepared ones to avoid the effect of storage.

After each exposure period, snails were washed by dechlorinated tap water. The snails were daily fed on boiled lettuce leaves. Each aquarium was provided with polyethylene sheets for oviposition. The egg masses and eggs laid on these sheets were counted using a binocular steriomicroscope. Dead snails were removed daily from aquaria and the mortality rate was calculated [17].

2.4.3. Effect on Egg Hatchability

For studying egg hatchability of *B. truncatus* eggs of one, three and six days age were used to examine the effect of the tested plant on the different stages of egg development. Each group of eggs was continuously exposed to 100 ml of plant extract solution of LC_0, LC_{10} and LC_{25} in a Petri dish till hatching. Another group of 50 eggs was maintained in dechlorinated water as control.

2.4.4. Effect on Infection of *B. truncatus* Snails with *S. haematobium* Miracidia

The effects of these sublethal concentrations on infection rate of *B.truncatus* (5 mm - 7 mm in shell diameter) with *S. haematobium* miracidia and cercarial production were examined by exposing 3 groups, each of 50 snails, individually to *Schistosoma* miracidia with a dose of 10 miracidia/snail and maintained in each concentration of plant extract (LC_0, LC_{10} and LC_{25}) for 24 hours under room temperature (24˚C ± 1˚C) and ceiling illumination. After exposure to miracidia, snails were maintained in their corresponding sublethal concentrations. Another group of 50 snails was exposed to miracidia in the absence of the tested plant solutions and maintained under the same conditions (control group). Examination of snails for cercarial shedding was carried out twice weekly, 25 days post exposure, and the cercarial suspension was poured in a graduated Petri dish, then few drops of Bouin'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.

2.5. Effect on Biochemical Parameters in Snails' Hemolymph and Tissues

For studying the effect LC_0, LC_{10} and LC_{25} of *S. sesban* plant on some physiological parameters of *B. truncatus* snails, four identical groups of *B. truncatus* (each of six replicates) of which three groups were exposed to one month to LC_0, LC_{10} and LC_{25} of the tested plant, respectively. The fourth group was maintained as control under the same laboratory conditions, without exposure to plant extract.

2.5.1. Assay Methods

Hemolymph samples were collected according to Michelson [18] by removing a small portion of the shell and inserting a capillary tube into the heart. The haemo-

lymph pooled from 10 snails were collected in a vial tube (1.5 ml) and kept in an ice-box. For preparation of tissue homogenates of both exposed and unexposed snails, one gram of snails soft tissues from each group was homogenized in 5 ml distilled water pH 7.5. A glass homogenizer was used and the homogenate was centrifuged for 10 minutes at 3000 rpm, fresh supernatant was used .

Biochemical parameters were determined analyzed spectrophometrically, using kits purchased from BioMerieux Company, France. Total protein content was determined according to Lowry *et al*. [19]. Determination of tissues glycogen was evaluted according to Carrol *et al*. [20]. Haemolymph glucose concentrations were determined according to the glucose oxides method of Trinder [21]. Hexokinase (HK) was assayed according to the method of Yueda & Racker [22] in which glucose-6-phosphate formed by the hexokinase reaction is measured by adding glucose-6-phosphate dehydrogenase and NADP and following NADPH formation.

2.5.2. Pyruvate Kinase (PK)

PK relative activity was measured spectrophometrically by the method of McManus and James [23]. Lactate dehydrogenase (LDH) as measured spectrophotometrically according to Cabaud and Wroblewski [24].

2.6. Satistical Analysis

Snails' mortality and infection rates were analyzed by Chi-square values of contingency tables [25]. The mean values of prepatent and patent periods, cercarial production/snail and life span of infected snails in the tested and control groups were compared using student "t" test [26]. Statistical analysis was performed with the aid of the SPSS computer program (version 13.0 windows).

3. RESULTS

The molluscicidal activity of methanol extract of *S. sesban* plant on *B. truncatus* snails after 24 hours of exposure is presented in (**Table 1**). The data indicate that LC_{50} and LC_{90} values for plant extract were 18 ppm and 31 ppm respectively. The sublethal concentrations (LC_0, LC_{10} & LC_{25}) were found to be 1.8, 8 and 14 ppm respectively.

The present results in **Table 2** showed that a rapid increase in mortality rate of exposed snails to sublethal concentrations of *S. sesban* methanol extract which is significantly higher than that of control group. The data revealed that no *B. truncatus* snails could survive more than 40, 28 and 22 days in groups maintained at LC_0, LC_{10} and LC_{25} respectively with a mean life span of 22.5 ± 6.2, 15.6 ± 6.4 and 11.8 ± 4.2 days respectively (**Table 3**). The death rate of *B. truncatus* snails in groups treated with LC_0 was highly significant as compared with those in groups treated with LC_{10} and LC_{25} ($p < 0.01$).

Regarding the egg production of *B. truncatus* snails exposed continuously to the sublethal concentrations of *S. sesban* extract, **Table 4** showed that control snail's proeeded the experimental ones in egg lying by 2 days. Snails stopped egg lying after 28 days being exposed to LC_{25} of plant extract, while snails treated with LC_0 and LC_{10} ceased to deposit eggs after 18, 9 days respectively. The total mean number of eggs laid by treated snails with LC_0, LC_{10} and LC_{25} of plant extract throughout their life span were 21.07, 10.39 & 5.62 versus 81.19 (No. of eggs/snail) in the control group. Regarding the net reproductive rate (Ro), the results showed that all experimental groups showed highly significant reduction ($p < 0.01$) in reproductive rate. This reduction was 74.05%, 87.2% and 93.08% for snails exposed to LC_0, LC_{10} and LC_{25} of plant extract respectively.

The eggs hatchability of snails exposed to sublethal concentrations of *S. sesban* extract was decreased by increasing their age after deposition (**Table 5**). Thus, hatchability rates of eggs exposed to LC_{25} of the tested plant were 36%, 28% & 20% for 1, 3 and 6 days old eggs respectively. The hatchability of eggs in the different groups treated with sublethal concentrations was significantly lower than that of control group ($p < 0.001$).

The effect of the tested sublethal concentrations of of *S. sesban* extract on infection of *B. truncatus* with *S. haematobium* miracidia was presented in (**Table 6**). The infection rate was significantly lower than that of control snails (75%), being 47.37%, 35.71% and 30% for snails exposed to LC_0, LC_{10} and LC_{25} respectively with a reduction rate 36.84%, 52.39% and 60% respectively.

Table 1. Molluscicidal activity of methanol extract of *Sesbania sesban* on *Bulinus truncatus* snails after 24 hours of exposure under laboratory conditions.

LC_{50} ppm (Confidence limit)	LC_{90} ppm	Slope function	Sublethal Concentrations		
			LC_0	LC_{10}	LC_{25}
18 (15 - 21.6)	31	1.5	1.8	8	14

Table 2. Longevity of *Bulinus truncatus* snails exposed continuously to sublethal concentrations of methanol extract of *Sesbania sesban.*

Concentration Ppm	Longevity Bulinus truncatus snails (days)	
	Range	Mean
LC_0	2 - 40	22.5 ± 6.2
LC_5	2 - 28	15.6 ± 6.4
LC_{10}	2 - 22	11.8 ± 4.2
Control	2 - 75	52.6 ± 11.5

Table 3. Mortality percentage of *Bulinus truncatus* snails exposed continuously to sublethal concentrations of methanol extract of *Sesbania sesban* plant.

Duration of experiment (days)	Sublethal concentrations of methanol extract of *Sesbania sesban* (ppm)						Control	
	LC_0		LC_{10}		LC_{25}			
	Cumulative number of dead snails	Cumulative % mortality	Cumulative number of dead snails	Cumulative % mortality	Cumulative number of dead snails	Cumulative % mortality	Cumulative number of dead snails	Cumulative % mortality
0	0	0	0	0	0		0	0
2	4	8	6	12	10	20	0	0
4	8	16	12	24	15	30	1	2
6	10	20	15	30	18	36	2	4
9	14	28	18	36	24	48	4	8
13	18	36	28	56	32	64	5	10
18	22	44	36	72	42	84	6	12
22	26	52	44	88	50	100	8	16
28	30	60	50	100			10	20
32	36	72			-	-	14	28
36	42	84	-	-			16	32
40	50	100					18	36
44		-					20	40
48							22	44

Table 4. Egg production of *Bulinus truncatus* snails exposed continuously to sublethal concentration of methanol extract of *Sesbania sesban*.

6	Mean eggs number/snails						Control	
	LC_0 ppm		LC_{10} ppm		LC_{25} ppm			
	No. of surviving snails	Mean No. of eggs/snail	No. of surviving snails	Mean No. of eggs/snail	No. of surviving snails	Mean No. of eggs/snail	No. of surviving snails	Mean No. of eggs/snail
0	50	0	50	0	50	0	50	3.1
2	48	2.1	44	1.2	40	2.8	50	10.8
4	42	3.8	38	2.1	35	3.8	49	12
6	40	5.8	35	4.5	32	1.2	48	8.6
9	36	4.2	32	2.2	26	0	46	10.2
13	32	2.1	22	1.2	18		45	15.2
18	28	2.2	14		8		44	7.2
22	24	1.2	6		50		42	6.5
28	20	0	50				40	4.2
32	14						36	4.2
36	8						34	4.7
40	0						32	
Total		21.4		11.2		5.62		87.7
% Reduction		75.6%		87.23%		93.6%		

Table 5. Effect of methanol extract of *Sesbania sesban* on hatchability % of *Bulinus truncatus* ggs.

6	Mean eggs number/snails						Control	
	LC_0 ppm		LC_{10} ppm		LC_{25} ppm			
	No. of surviving snails	Mean No. of eggs/snail	No. of surviving snails	Mean No. of eggs/snail	No. of surviving snails	Mean No. of eggs/snail	No. of surviving snails	Mean No. of eggs/snail
0	50	0	50	0	50	0	50	3.1
2	48	2.1	44	1.2	40	2.8	50	10.8
4	42	3.8	38	2.1	35	3.8	49	12
6	40	5.8	35	4.5	32	1.2	48	8.6
9	36	4.2	32	2.2	26	0	46	10.2
13	32	2.1	22	1.2	18		45	15.2
18	28	2.2	14		8		44	7.2
22	24	1.2	6		50		42	6.5
28	20	0	50				40	4.2
32	14						36	4.2
36	8						34	4.7
40	0						32	
Total		21.4		11.2		5.62		87.7
% Reduction		75.6%		87.23%		93.6%		

Table 6. Effect of sublethal concentrations of methanol extract of *Sesbania sesban* on infectivity of *Schistosoma haematobium* miracidia to *Bulinus truncatus* snails.

Treatment	Number of exposed snails	Survived snails at first shedding		Infected snails		% Reduction
		Number	%	Number	%	
Control	50	46	92	36	75	
LC_0 (3ppm)	50	38	76	18	47.37	36.84*
LC_{10} (12ppm)	50	28	56	10	35.71	52.39**
LC_{25} (18ppm)	50	20	40	6	30	60***

*p < 0.05, **p < 0.01, ***p < 0.00.

Prepatent period (**Table 7**) of exposed snails to LC_0, LC_{10} and LC_{25} of tested plant was prolonged to be 29.5 + 1.6, 30.4 + 1.1and 32.1 + 4.2 days compared to 30.8 + 2.1days for the control group. Meanwhile, the duration of cercarial shedding was significantly shortened among these snails, being 11.6 + 2.2, 9.2 + 3.4 and 6.2 + 2.6 days for LC_0, LC_{10} and LC_{25}, respectively, compared with 18.2 + 5.8 days for control snails. Highly significant reductions of total cercarial production per snails and per stimulant were also detected in experimental snails in comparison with the control group.

The results in (**Table 8**) show a significant reduction (24.5 ± 2.2*, 16 ± 2.6** and 11 ± 2.8 mg/g tissue) in the protein content in snails exposed to LC0, LC10 and LC25, respectively of the tested plant, compared to their corresponding control (38.23 ± 4.12 mg/g tissue). The glycogen contents in tissues of the treated snails were

Table 7. Effect of sublethal concentrations of methanol extract of *Sesbania sesban* on cercarial production of *Schistosoma haematobium* from infected *Bulinus truncatus* snails.

Concentration (ppm)	Number of cercariae/snail	Prepatent period (days)	Duration of shedding (days)
LC_0 (3ppm)	411.23 ± 651.1*	29.5 ± 1.6	11.6 ± 2.2**
LC_{10} (12ppm)	284.11 ± 6,3**	30.4 ± 1.1	9.2 ± 3.4**
LC_2 (18ppm)	216.2 ± 14.2***	32.1 ± 4.2*	6.2 ± 2.6***
Control	111.2 ± 320	30.8 ± 2.1	18.2 ± 5.8

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

Table 8. Effect of a month continuous exposure to methanol extract of *Sesbania sesban* on glucose level in haemolymph, total protein and glycogen content in soft tissues of *Bulinus truncatus*.

		In soft tissue	In haemolymph
Sublethal concentrations (ppm)	Protein content (mg/g tissue)	Glycogen content (mg/g tissue)	Glucose level in (mg/ml)
Control	38.23 ± 4.12	30 ± 2.4	27 ± 4.1
LC_0 (ppm)	24.5 ± 2.2*	21 ± 1.2*	32 ± 1.2*
LC_{10} (ppm)	16 ± 2.6**	18 ± 2.6**	38 ± 1.8**
LC_{25} (ppm)	11 ± 2.8***	12 ± 3.5***	44 ± 2.5***

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

significantly lower (p < 0.01) than in the control group. On the other hand, glucose levels increased in snails exposed to these concentrations of the tested plant in comparison with control ones. The results in (**Table 9**) showed that the levels of Hexokinase (HK), Pyruvatekinase (PK) and lactate dehydrogenase (LDI) in the soft tissues of normal and treated snails were also significantly reduced in repose to treatment with these concentrations of the tested plant. The HK activity in snails exposed for one month was 18.21 u/mg ± 2.2 u/mg, 14.14 u/mg ± 1.4 u/mg & 10.12 u/mg ± 2.1 u/mg tissue respectively. Such reduced values were significant than those of the corresponding controls (22 u/m ± 1.4 u/mg tissue).

4. DISCUSSION

The methanol extract of the plant *S. sesban* showed considerable molluscicidal effect against *B. truncatus*. The LC_{50} and LC_{90} were found to be 18 ppm & 31 ppm respectively. This agrees with the findings of Shoeb *et al.* (1994) on the high toxicity of V. tinus against *B. alexandrina*, *B. truncatus* and *L. caillaudi* snails. The plant *S. sesban* exhibited and acceptable toxic effect to *B. alexandrina* snails according to WHO, [1] recommendations on plant molluscicides. This is coinciding with the activity of *S. sesban* against *B. pfeifferi* and *B. trancatus* snails [7]. This effect could be due to the presence of 3 glucuronide derivatives of oleanolic acid in this plant

Table 9. Effect methanol extract of *Sesbania sesban* on hexokinase (HK), Pyruvate Kinase (PK) and lactate dehydrogenase (LDH) in the soft tissues of *Bulinus truncatus* snails.

	PK (U/mg tissue) × ± SD	HK (U/mg tissue) × ± SD	LDH (U/mg tissue) × ± SD
Control	2.15 ± 2.9	22 ± 1.4	1.8 ± 2.14
LC_0 (ppm)	1.22 ± 2.40*	18.21 ± 2.2*	1.15 ± 0.21*
LC_{10} (ppm)	0.82 ± 1.4**	14.14 ± 1.4**	0.72 ± o.14**
LC_{25} (ppm)	0.62 ± 3.1***	10.12 ± 2.1***	0.21 ± 0.81***

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

species that have a high molluscicidal activity [6,28]. As well, methanol extract from *D. innoxia* exhibited a remarkable toxic effect against the snails *B. alexandrina*, *B. trancatus* and *L. caillaudi*, and this could be due to the presence of a compound from the coronaridine glycoside derivatives [8].

The results showed that there was a significant increase in the mortality rates of snails exposed to sublethal concentrations of the tested plant compared to the control group. This finding agrees with those of Rawi *et al*. [29,30], Bakry and Sharaf El-Din [31,32], Gawish *et al*. [33]. They showed marked reduction in the survival rate of snails treated with sublethal concentrations of different plant species compared to the control.

Concerning the effect of sublethal concentrations of the tested plant on egg production, it was found that *B. truncatus* snails exposed to LC_0, LC_{10} and LC_{25} of the tested plant laid few eggs, then they stopped egg laying from 28 to 9 days of experiment. Similar observations were recorded on suppressing egg laying capacity of *B. alexandirna* snails after 4 weeks of continuous exposure to the dry powder of the plants *Solanum nigrum* and *Dizygotheca kerchoveana* [5].

The authors attributed this to severe histological damages to the snail's hermaphrodite gland cells and evacuations of some of its tubules from various gametogenetic stages. The same phenomenon was stated on the plants *Agave filifera* and *Agave attenuate* [34] *Ambrosia maritime* [35], *Lantana camara* and *Salvia officinalis* [36] and *Spirolina platensis* [17] against *B. alexandrina* snails.

Moreover, the present study showed that hatchability of *B. truncatus* eggs exposed to sublethal concentrations of the tested plant was decreased by increasing their age and the plant concentrations. This is in agreement with Gawish [17] who reported that the older embryonic stages of *B. truncatus* eggs were more susceptible to the experimental molluscicides than freshly laid ones. This may be due to the thicker yolk layer surrounding the embryo in freshly laid eggs than in older ones. This layer and the egg membrane act as a mechanical barrier against the molluscicides or other pesticides that could not penetrate these barriers [37].

The present result also showed that LC_{25} of the tested plant was significantly more effective against the hatchability of all developmental stages of eggs than LC_0 and LC_{10}. This result agrees with that reported by Tantawy *et al*. [38] who found that hatchability of *B. alexandrina* eggs exposed to sublethal concentrations of *Solanum dubium* was decreased by increasing its concentrations.

In this study, the infectivity of *S. haematobium* miracidia to *B. truncatus* was greatly reduced by the tested sublethal concentrations of *S. sesban*. The reduction of

infection rate was found to increase with the increase of sublethal concentrations. These results are in once accords with many authors working on various chemical and plant molluscicides [11,12,32,39,40]. This was recorded by Ibrahim *et al.* [5] on significant reduction of *B. alexandrina* infection rates with *S. mansoni* miracidia post their exposure to LC_{25} of *P. repens* dry powder. Comparable results were obtained by Bakry [41] using the plants *Euphorbia splendens*, *Atriplex stylosa* and *Guayacum officinalis* and by Gawish *et al*. [33] on the plant *Callistemon citrinus* against *B. truncatus* snails infected with *S. haematobium*. The low production of cercariae from snails treated with the present plants could attribute, also, to initiation of certain components in their internal defense system. This was recorded by El-Emam and Ebid [42] who found that treatment of *B. alexandrina* with the plant *Calendula micrantha* officinalis increased the activity of acid phosphatase in snail's tissues, that has an important role in their defense system and may has a negative reflect on cercarial production. The same phenomenon was recorded by Mahmoud and El-Sayed [43] on increasing AcP activity in tissues of *S. mansoni* infected *B. alexandrina* snails treated with the molluscicide niclosamide.

However, there was no significant difference between the prepatent period of the snails exposed to LC_{25} of methanol extract of the tested plants and the control. Despite that, a highly significant reduction in the duration of cercarial shedding and total cercarial production per infected snails were detected. 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 those molluscicides which increased the harmful effects of these plants on the subsequent development of the parasite within snail's tissues [44]. These observations are in accordance with many authors using different plant species as molluscicides. Thus, El-Ansary *et al*. [35] reported that A. maritime caused a remarkable decrease in cercarial shedding and cercarial production in *B. alexandrina* snails treated with this plant powder. Sharaf El-Din *et al*. [32] obtained similar reduction in cercarial shedding and cercarial production from *B. alexandrina* treated with sublethal concentrations of aqueous suspension of *Zygophyllum simplex*.

In the present investigation, a significant decrease has been recorded in the tissue protein in snails treated with the concentrations of plant extract. This decrease may be due to interference of the plant active substances with protein synthesis. Similar results were reported by Abdel-Kader and Tantawy [34] and Bakry *et al*. [45] in *B. alexandrina* using plant molluscicides.

The present study also indicated that LC_{25} of the tested plant significantly decreased the glycogen content in soft tissues while the glucose in hemolymph increased. Consequently, the snail tries to obtain its energy requirements through increasing the rate of glycolysis that resulted in reduction of the glycogen and increase the glucose in hemolymph. This finding was reported by Bakry *et al*. [46] using Agave franzosini plant against *B. alexandrina*, and Bakry [41] using Furcraea gigantean and Lampranthus spectabilis plants.

In the present study the glycolytic enzymes, hexokinase (HK), pyruvate kinase (PK) and lactate dehydrogenase (LDH) in the snails tissues showed variable decrease between significant and highly significant on applying the tested plant. The depletion in HK activity in the soft tissues causes an alteration of glycolytic mechanism which in turn induces a state of anoxia .A similar effect was detected by Bakry *et al.* [45] using plant extract and Mohamed *et al.* [47] using Abamectin as a molluscicide. Another explanation for the reduction in PK activity is may be due to the toxic effect of the tested plant that minimizes ATP level by disturbing the enzymatic pathways contributing to ATP generation and hence depression of energy of the snails, metabolism .This agreed with Bakry *et al.* [45] using plant extract. The decrease in LDH activity in the soft tissues of *B. alexandrina* exposed to LC_{25} of tested plant may be attributed to the release of the enzyme from the tissues as a result of cellular damage caused by the toxic of the tested plant. A similar result was detected by Aboul-Zahab & El-Ansary [48].

From the above data, it can be concluded that the depletion in tissue protein, glycogen, and activity of glycolytic enzymes (HK, PK & LDH) of *B. truncatus* snails treated with the tested plant is mostly responsible for reduction of egg production and the high rate of mortality in treated snails. Thus, the application of sublethal concentrations of the methanol extract of *Sesbania sesban* plant may play an important role in reducing the use of chemical molluscicides without severe degree of environmental damage.

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