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Use of Some Plant Extracts to Control *Biomphalaria alexandrina* Snails with Emphasis on Some Biological Effects

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Abstract: The present work was carried out to evaluate, the molluscicidal activity of cold water, boiled water, methanol, ethanol, acetone and chloroform extracts of some plant species against *Biomphalaria alexandrina* snails. Preliminary screening tests on 10 plant species showed that the highest molluscicidal potency was recorded for *Euphorbia splendens, Atriplex stylosa* and *Guayacum officinalis*. Exposure of *B. alexandrina* snails to plant's methanol extracts led to a significant reduction in their survival and growth rates. In addition, newly hatched snails were susceptible to the tested plants? extract than older ones. LC_{25} of methanol extract from these plants caused a considerable reduction in the infectivity of *Schistosoma mansoni* miracidia to the snails. It caused a reduction in number of cercariae per snail during the patent period and in the period of cercarial shedding. The results, also, revealed that the glucose concentration in treated snails was increased in haemolymph, while soft tissue glycogen decreased. The activities of glycogen phosphorylase, succinate dehydrogenase (SDH), glucose-6-phosphatase and acetylcholinestrase (AchE) in homogenate of snail's tissues were s reduced ($P<0.001$) in response to treatment with plants methanol extract, while glucose-6-phosphate dehydrogenase (G-6-pD) activity increased ($P<0.001$). It was concluded that the application of LC_{25} of methanol extracts of *E. splendens, A. stylosa* and *G.officinalis* may be helpful in snail control as it interferes with the snails' biology and physiology.

Key words: *Biomphalaria alexandrina* · *Schistosoma mansoni* · Plant molluscicides

INTRODUCTION

Schistosomiasis remains as one of the world's most prevalent diseases. Despite more than a century of control efforts and the introduction of highly effective antischistosomal drug therapy in the 1980s, the disease just will not go away; more than 207 million of the world's poorest people are currently infected with schistosomiasis [1]. *B.alexandrina* is the intermediate host of *S. mansoni* in Egypt. In recent years, there are gaining increased attention for newly molluscicides as they may be highly effective, rapidly biodegradable, less expensive than synthetic molluscicides, readily available and probably easily applicable with simple techniques. Therefore, plant molluscicide could be appropriate for snail control measures against schistosomiasis in endemic areas [2-5]. These botanical molluscicides are of economic importance especially in developing countries with scarce hard currency [6]. Also, there is a continuous need to search for new plant species with ideal molluscicidal properties [7- 9].

The use of plants with molluscicidal properties appears to be a simple and inexpensive alternative to chemical molluscicides [10]. More than 1000 plant species and their extracts have been screened for molluscicidal activity [11]. In Egypt, screening of local plants for molluscicidal activity has received increasing attention [12-19].

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 [20]. The molluscicides greatly affect the metabolic activities of the snail intermediate hosts [12]. They act on different enzymes chiefly those of respiration and carbohydrate metabolism [21, 22].

The acetylcholinesterase (AchE) enzyme is responsible for the termination of cholinergic impulses by the hydrolysis of acetylcholine (Ach) released during synaptic transmission; inhibition of AchE thus permits accumulation of Ach at the synapses which concentration rises several folds in comparison to the normal levels leading first to paralysis and then eventually to death [23,24].

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The present study aimed to evaluate the molluscicidal and ovicidal properties of some promising plant extracts as monitored by determination of development stages of snails (*Biomphalaria alexandrina*), survival, growth rate and their rates of infection with *Schistosoma mansoni* miracidia. In addition the characteristic changes pattern in activities of some enzyme activities of this snails were investigated.

MATERIAS AND METHODS

Snails: Laboratory bred *Biomphalaria alexandrina* snails (5-6 mm) from laboratory bred stock in Medical Malacology Dep., Theodor Bilharz Research Institute (TBRI), were used.

Plants: Ten plant species were used in this study. They are *Guayacum officinalis* (Zygophyllaceae) from Fayoum governorate desert, Egypt (May 2005), *Euphorbia splendens* (Euphorbiacae), *Chenopodium murale* (chenopodiaceae), *Cestrum parqui* (Solanaceae), *Calatropis procera* (Asclepiadaceae), *Carissa carandus* (Apocynaceae), *Conyza dioscoridis* (Compositeae) and *Lantana camara* (Verbenaceae) from fields of Giza governorate during flowering stages (March-April 2005), *Atriplex stylosa* (Chenopediaceae) from Borg El-Arab, Mediterranean coast (May 2006). *Calligonum comosum* (Polygonaceae) from Sinai, Egypt (April-May 2006). These plants were kindly identified by Botany Department, Faculty of Science, Cairo University, Egypt.

Miracidia: *Schistosoma mansoni* miracidia were obtained from Schistosomiasis Biological Supply Center (SBSC) TBRI.

Extract Preparations from the Plant's Dry Powders: The whole overground parts of the tested plants were left to dry in air and then in an oven at 50°C and powdered by a mixer [15].

Cold Water Extract: A stock extract was prepared by sooking 100 grams of the plant powder in 500 ml dechlorinated water for 7 days at room temperature (25±1°C). The filtrate was used to prepare a series of concentrations to calculate the LC_{50} and LC_{90} values.

Bioled Water Extract: This was prepared by boiling 100 grams of the plant powder in 500 ml dechlorinated water for 15 minutes, Then left to cool at room temperature with adjusting the final water volume to 500 mL. The filtrate was used to prepare concentrations for calculation LC_{50} and LC_{90}.

Extracts with Organic Solvents: Methanol, ethanol, acetone and chloroform extracts were prepared by soaking 250 grams of the plant powder in one litre of each solvent for 7 days at room temperature. The filtrate was distilled off under vaccum and the residues were stored in clean dark glass till use.

Bioassay Tests

Molluscicidal Screening: A series of concentrations was prepared from each experimental plant powder on the basis of weight/volume as water suspensions. Another series of concentrations from either cold water extract, boiled water extract or organic solvents was prepared [25]. For each experimental concentrations 3 replicates were prepared, each of 10 snails /hour. Another 3 replicates were prepared in declorinated water as control. Exposure and recovery periods were 24 hours each. Mortality rates were recorded and corrected according to Abbot's formula (1925), then Litchfield and Wilcoxon [26] and Finny[27] methods and using SPSS computer program under windows.

Due to the promising effect of methanol extract from the plants *G.officinalis, A.stylosa* and *E. splendens* against *B. alexandrina* snails, they were selected for comprehensive tests as follows:

Effect of LC_{90} on Various Stages of Snails and Their Eggs: Snail's eggs (3 days old), newly hatched snails (0.4-0.8mm),Young snails (3.5-5 mm) and mature ones (8-10 mm) were exposed to LC_{90} of methanol extract from the three selected plants for 24 hours and recovery for another 24 hours, then mortality rates were recorded. Control snails stages and eggs were maintained in dechlorinated water.

Effect of Prolonged Exposure to LC_{25} on Growth of Snails: The snails (3-5mm) were continuously exposed to LC_{25} from methanol extract for one month and the concentration was renewed every 3 days. Mortality rates of snails were recorded weekly and the shell diameter of the survived ones was measured once a week by acaliper [28].

Effect of LC_{25} from Methanol Extract on Infectivity of *S. Mansoni* Miracidia to *B. Alexandrina* Snails: Snails were exposed for 24 hours to LC_{25} from plant'methanol

extract during their exposure to miracidia (10 miracidia /snail).Thereafter; they were removed and continuously maintained in their concentration for one month (25±1°C). Three replicates, each of 10 snails /L, were prepared. The methanol extract concentration was renewed every 3 days. Another 3 replicates were prepared in dechlorinated water, whereas snails were exposed to miracidia (Control). The snails were daily fed leltuce leaves and dead ones were removed. Twenty days post miracidial exposure, the survived snails were individually examined for cercarial shedding (3hours/3days). The cercarial production/ infected snails was recorded [29].

The Physiological Effects of LC_{25} from Plant' Methanol Extract: 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 LC_{25} of methanol extract from *G. officinalis*, *A. stylosa* and *E. splendens*, for one month. A fourth group of snails was left unexposed under the same laboratory conditions as control. Surviving snails were subjected to withdrawal of their hemolymph. Haemolymph samples were collected [30] by removing a small portion of the shell and inserting a capillary tube into the heart. The haemolymph pooled from 10 snails was collected in a vial tube (1.5ml) and kept in 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 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.

All physiological parameters were determined spectrophometrically, using kits purchased from BioMerieux Company, France.

Determination of tissues glycogen [31], haemolymph glucose concentrations (the glucose oxidase method) [32]. glycogen phosphorlase [33], glucose-6-phosphatase (G-6-Pase) [34], succinate dehydrogenase (SDH) [35]; glucose-6-phosphate dehydrogenase activity (G-6-pD) [36] were carried out.

Acetylcholinestrase activity was measured in nerve tissue of treated and control snails using the method of Ellman *et al.* [37] modified by Singh and Singh [24]. Soft parts of snails were dissected out from shells after gentle crushing, the cerebral ganglia around the buccal mass were dissected and collected in isotonic saline and freezed for-15°C.Nerve tissue (50 mg), The supernatant was used as enzyme source.

Statistical Analysis: The infection and mortality rates were analyzed by Chi-square values of contingency tables [38]. Incubation period, duration of shedding and cercarial production of the infected snails in the experimental groups and the control were compared using student "t" test [39].

RESULTS

It is clear from Table 1 that among the plants tested as dry powder water suspension, *E. splendens* was the most active one against *B. alexandrina* snails (LC_{90}=73 ppm). Three plants showed a moderate molluscicidal activity (*A. stylosa, G.officinalis* and *C.procera*), with $LC_{90's}$ ranging from 180 to 360 ppm. The low toxic plants were *C.parqui, L.camara, C. dioscoridis* and *C.murale*, with $LC_{90's}$ ranged from 1600 to 5200 ppm. Meanwhile, *C. comosum* and *C.carandur* have no toxic effect against *B.alexandrina* snails after 24 hours of exposure.

The most active plant *E.splendens* and two of the moderate toxic ones (*A.stylosa and G.officinalis*) were selected for preparation of different extracts from their dry powder to evaluate their effect against *B.alexandrina* snails.

Data in Table 2 show that plant's methanol extract was more toxic to the snails than the other tested extracts.Chloroform and cold water extracts showed a low molluscicidal activity. However, ethanol, acetone and boiled water extracts exhibited a moderate harmful effect. Also, it is, seen that *E.splendens* methanol extract was the most active ones with LC_{90} of 27 ppm followed by those of *A. stylosa* and *G. officinalis* (LC_{90}= 54 and 62 ppm,respectively).

Due to the promising effect of plant's methanol extract, it was chosen for the following comprehensive tests.

From Fig. 1. it is seen that the larger snails, the more tolerant to the toxic effect of the tested plant's methanol extract.Thus, LC_{90} of *E.splendens* against mature snails was 1.2 and 2.9 times that of young and newly hatched ones,r, being 29, 24 and10 ppm,respectively.

It is, also, seen that the ovicidal activity of the tested plant's methanol extract was approximately similar to that of young snails. Thus, LC_{90} values of *G. officinalis* and *A.stylosa* for young snails were 53 and 49 ppm and those for snails'eggs were 55 and 51 ppm, respectively.

The results in Fig. 1 confirm those in Table 2 that *E.splendens* was more harmful to the snails and their eggs than *G.officinalis* and *A.stylosa*.

Table 1: Molluscicidal activity of different plants as a cold water suspension of the dry powder, against *Biomphalaria alexandrina* snails after 24 hours of exposure under laboratory conditions

Plant species	LC_0	LC_{10}	LC_{25}	LC_{50}	LC_{90}	Slope
Guayacum officinalis	15	62	97	120	210	1.57
Atriplex stylosa	9.2	38	46	94	180	1.67
Calligonum comosum	0	0	0	0	0	0
Calotropis procera	24.3	98	134	243	360	2.24
Euphorbia splendens	4	22	29	40	73	1.22
Lantana camara	123	520	820	1230	2400	2.54
Chenopodium murale			1100	2450	5200	2.94
Conyza dioscoridis	300	2300	2600	3000	4100	0
Carissa carandus	0	0	0	0	0	0
Cestrum parqui	86	422	620	860	1600	2.61

Table 2: Molluscicidal activity of different plant extracts against *Biomphalaria alexandrina* snails after 24 hours of exposure under laboratory conditions

Plant extracts		Cold water	Bioled water	Methanol	Ethanol	Acetone	Chlorofom
Guayacum officinalis	LC_0 ppm	11	9	3.5	7.8	14	10
	LC_{10} ppm	78	48	17	42	52	52
	LC_{25} ppm	88	62	24	57	61	65
	LC_{50} ppm	110	90	35	78	84	100
	LC_{90} ppm	230	140	62	120	140	180
	Slope	1.65	1.63	1.72	1.43	1.52	1.66
Atriplex stylosa	LC_0 ppm	5.3	4.2	3.1	9.8	6.6	7.4
	LC_{10} ppm	32	22	14	42	42	54
	LC_{25} ppm	39	30	21	49	53	62
	LC_{50} ppm	53	42	31	62	66	74
	LC_{90} ppm	88	56	54	98	110	130
	Slope	1.44	1.5	2.51	1.35	1.49	1.37
Euphorbia splendens	LC_0 ppm	3.2	2.1	1.1	3	4.4	5.2
	LC_{10} ppm	13	11	6.2	18	22	30
	LC_{25} ppm	21	15	8.4	23	31	41
	LC_{50} ppm	32	21	11	30	44	52
	LC_{90} ppm	54	28	27	48	62	78
	Slope	1.72	1.52	1.2	1.38	1.58	1.39

Table 3: Mean shell diameter (mm) of *Biomphalaria alexandrina* snails continuously exposed to methanol extract (LC_{25}) of the tested plant species

	Biomphalaria snails exposed to LC_{25} of methanol extract							
	Euphorbia splendens		*Atriplex Stylosa*		*Guayacum officinalis*		Untreated snails (Control)	
Observation period (weeks)	L_x	Mean diameter S.D	L_x	Mean diameter S.D	L_x	Mean diameter S.D	L_x	Mean Diameter S.D
0	1	3.±0.31	1	3±0.12	1	3±0.15	1	3±0.31
1	0.60	3.6.±0.15	0.780	3.8±0.41	0.82	4.2±0.24	0.92	5.2±0.12
2	0.46	4.2.±0.32	0.56	4.4±1.22	0.68	4.8±0.38	0.90	6.2±0.14
3	0.32	5. 2.±0.54	0.40	5.8±0.33	0.52	6.2±0.16	0.86	7.4±0.56
4	0.20	5.6.±0.34	0.32	6.2±0.41	0.42	6.8±0.85	0.82	7.8±0.34
5	0.8	6.1.±0.61	0.16	6.6±0.21	0.32	7.2±0.47	0.76	8.8±0.18
6	-	-	0.4	7.2±0.45	0.28	6.8±0.62	0.68	9.4±0.46
7			-	-	0.08	7.8±0.18	0.56	10±0.21
8					-	-	0.48	10.2±0.36

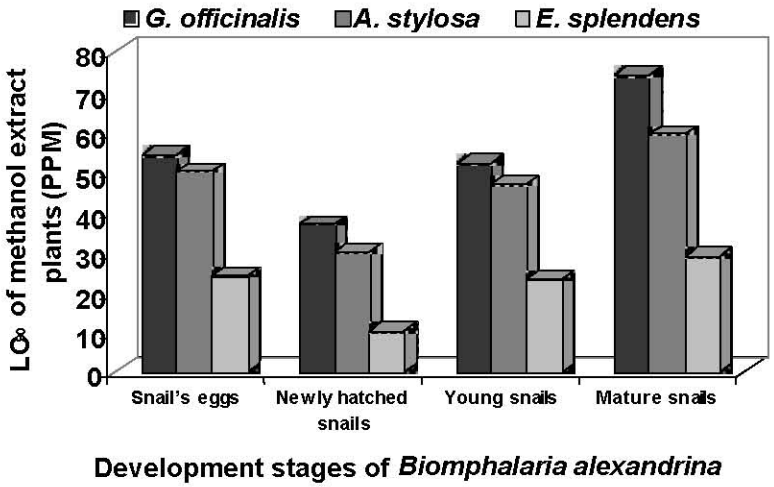

Fig. 1: LC_{90} of methanol extract of some plants on various developmental stages of *Biomphalaria alexandrina* after 24 hours of exposure under laboratory conditions

Table 4: Growth rate (%) of *Biomphalaria alexandrina* under continuous exposure to methanol extract (LC_{25}) of the tested plant species

	Biomphalaria snails exposed to LC_{25} of methanol extract							
	Euphorbia splendens		*Atriplex Stylosa*		*Guayacum officinalis*		Control snails	
Growth phase	Period (week)	Growth rate (%) week	Period (week)	Growth rate (%)	Period (week)	Growth rate (%)	Period (week)	Growth rate (%)
First	3	73.3	3	93.3	3	106.7	3	146.7
Second	2	17.31	3	24.13	4	25.8	5	37.8

Table 5: Effect of LC_{25} methanol extract of *Guayacum officinalis*, *Atriplex stylosa* and *Euphorbia splendens* plants on the infectivity of *S.mansoni* miracidia to *B. alexandrina* snails

	Survival at 1st shedding		Infection of snails		Prepatent period (days)		Cercarial production/ infected snail		Shedding Duration (days)	
Plant species	No. survival	%	No.	%	Range	mean±S.D	Range	mean± S.D	Range	mean±S.D
Guayacum officinalis	11	36.6%	7	23.3%	22- 30	28.7±0.32	62- 94	840 ±11.1***	3-6	4.6±0.73***
Atriplex Stylosa	10	33.3%	5	16.6%	21-28	27.5±4.5	31-88	850±6.2***	3-5	3.8±0.166***
Euphorbia splendens	8	26.6%	4	13.3%	21-27	27 ±0.62	27-62	520±8.5***	3-4	2,8±0.11***
Control	22	73.%	14	46.6%	26-30	30±0.21	115-266	2221± 37	4- 20	12± 0.33

*P <0.05, ** P<0.01, ***P <0.001

Table 3 shows that there is a rapid decline in survival rate (L_x) of snails exposed to LC_{25} of methanol extract of *E. splendens*, *A. stylosa and G. officinalis* plants.Thus, the survival rates after 5 weeks of experiment were 8, 16 and 32%, respectively which are lower than that of control group (76%, P< 0.001). Snails exposed to methanol extract of *E. splendens*, *A. stylosa and G.officinalis* plants died after 6, 7 and 8 weeks respectively. Moreover, methanol extract of the plants caused reduction (P<0.01) in growth rate of exposed snails. The snail'shell diameter was 5. 2, 5.8 and 6.2 mm at the 3rd week for snails exposed continuously to LC_{25} of methanol extract of *E. splendens*, *A. stylosa* and *G.officinalis* plants, respectively, which are lower (P<0.001) than that of control group (7.4 mm).

The data obtained (Table 4) indicate that there are two phases of snail growth, the first phase is characterized by a fast growth rate (3 weeks). The growth rates in this period for snail groups exposed to LC_{25} of methanol extract of *E. splendens*, *A. stylosa* and *G.officinalis* plants were 73.3, 93.3 and 106.7% respectively which were lower than that of control group (146.7%, P<0.001). The second phase of growth is characterized by a slower growth rate, whereas the rate was 17.31% for snails exposed for 2 weeks to LC_{25} of methanol extract of *E. splendens.*

The infection rate of snails by *S. mansoni* miracidia (Table 5) was lower than that of the control snails. The rates were 13.3, 16.6 and 23.3% for snails exposed to

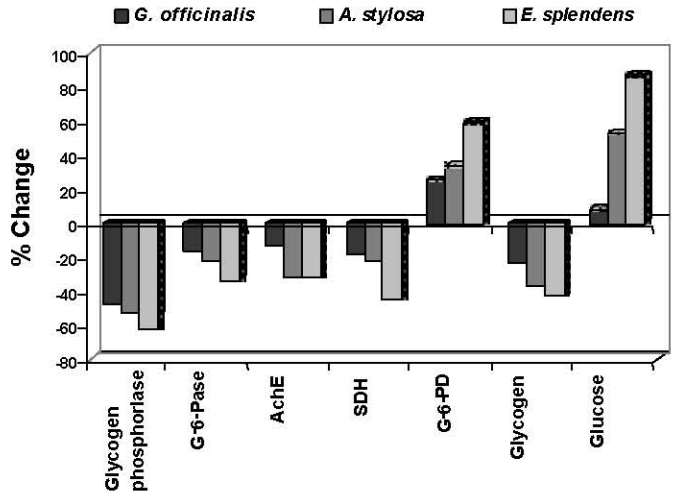

Fig. 2: Changes(%) of activities of some enzymes,glucose and glycogen content in *Biomphalaria alexandrina* exposure to LC_{25} of methanol plant extracts for one month

Table 6: Effect of one month exposure to LC_{25} of methanol plant's extracts on glucose level in haemolymph and glycogen content in soft tissues of *Biomphalaria alexandrina* snails (mean±S.D)

	Soft tissue		Haemolymph	
Plant species	Glycogen content (mg/g tissue)	% reduction	Glucose level (mg/ml)	% increase
Guayacum officinalis	24.8±3.1**	22.7%	24.4±1.6*	8%
Atriplex Stylosa	20.6±1.2***	35.8%	34.5±2.3**	52.7%
Euphorbia splendens	18.4±1.82***	42.7%	42.1±3.2***	86%
Control	32.1±2.4		22.6±1.1	

*P < 0.05,** P< 0.01 & *** P< 0.001

Table 7: Effect of one month exposure to LC_{25} of methanol plant extracts on some glycolytic enzymes in haemolymph and soft tissues of *Biomphalaria alexandrina* snails

	In soft tissues								In Haemolymph	
Parameters / Treatment	Glycogen Phosphorlase	% change	glucose-6-phosphatase (G-6-Pase)	% change	AchE (μMSH hydrolyzed /mg protein)	% change	Succinate dehydrogenase (S(SDH)	% change	G-6-PD (mμ/ml)	% change
Guayacum officinalis	4.4±0.34	47%	0.61±0.043***	15.3%	0.062±0.04*	12.7%	0.43±0.03**	17.3	154.3±1.02**	25%
Atriplex Stylosa	4.0±0.35***	52.2%	0.56±0.07***	22.2%	0.049.3±0.03**	31%	0.41±0.056**	21.2%	165.5±5.3***	34.1%
Euphorbia splendens	3.2±0.51***	61.65%	0.48±0.0.33***	33.3%	0.038±0.02***	46.5%	0.29±0.032***	44.2%	196.7±7.1***	59.4%
Control	8.3±0.47		0.72±0.05**		0.071±0.008		0.52±0.041*		123.4 ±1.2	

*P < 0.05,**P < 0.01 & ***P < 0.001

LC_{25} of methanol extract of *E. splendens*, *A. stylosa* and *G.officinalis* plant's extract, respectively compared to 46.6% for control group. There is no significant difference between prepatent period of the snails exposed to LC_{25} of the methanol extracts and the control group. The duration of cercarial shedding for snails treated with LC_{25} of methanol extract of *E. splendens*, *A. stylosa* and *G.officinalis* plants decreased to 2,8, 3.8 and 4.6 days, respectively, compared to 12± 0.33 days for the control snails. A marked (P<0.01) reduction of total cercarial production by treated snails in comparison with control group was, also, observed.

The results in Table 6 and Fig. 2 show a clear reduction (P<0.001) in the glycogen content in soft tissues of snails exposed LC_{25} of methanol extracts from *G. officinalis, A. stylosa* and *E. splendens,* respectively compared to control group. The reduction rates were 22.7, 35.8 and 42.7%, respectively. On the other hand, glucose concentration in hemolymph of treated snails showed a maked increase (P< 0.001) in comparison with the control group. The rates of increase were 8, 52.7 and 86% for *G. officinalis, A. stylosa* and *E. splendens* extracts, respectively.

The results (Table. 7 & Fig.2) showed that maintainence of *Biomphalaria* snails in LC_{25} of methanol extract of *E. splendens*, *A. stylosa* and *G. officinalis* plants for one month induced a clear inhibitory effect in activities of glycogen phosphorylase, G-6-Pase, AchE and SDH in snail'soft tissues. Meanwhile, it increased the activity of G-6-pd in the heamolymph. The glycogen phosphorlase activity in tissues of snails exposed for one month to *E.splendens* extract was 3.2 u/mg compared to 8.3 u/mg for control group ($P<0.001$).

The reduction rates in activities of AchE, G-6-Pase and SDH in tissues of snails exposed to *E.splendens* extract were 46.5, 33.3 and 44.2%, respectively.

Meanwhile, G-6-PD activity in hemolymph of snails exposed to LC_{25} of methanol extract from *G. officinalis, A. stylosa* and *E. splendens* extract showed an increase ($P< 0.001$) in comparison with the control group. The rates of increase were 25, 34.1 and 59.4%, respectively.

DISCUSSION

A among the ten plants tested, *E. splendens* had the highest molluscicidal activity against *B. alexandrina* snails, followed by A. *stylosa* then *G. officinalis* plants. The high molluscicidal activity of *E. splendens*, *A.stylosa* and *G. officinalis* plants are apparently attributed to the high concentration of active constituents (saponins & flavonoid). This is finding were previously recorded [9, 21, 40, 41,42,]. In addition, the tested plants showed slight ovicidal effect, but newly hatched snails were more susceptible to the tested plant's methanol extract than the older stages. This is in agreement with Sakran and Bakry [21] who found that the young snails are generally more susceptible to molluscicides than older stages.

Prolonged exposure of the snails to LC_{25} of methanol extract of *E. splendens*, *A.stylosa* and *G. officinalis* reduced their growth rate, This finding agrees with Bakry and Hamdi[43] when the snails were exposed to of methanol extract of *Agave celsii* plant and Mohamed *et al.* [44] for snails exposed to sublethal concentrations of Abamectin.

The reduction in growth of treated snails may be due to interference of molluscicides with the physiological activities of these snails [15,45, 46].This was confirmed by the present data on the interruption in the activities of enzymes in tissues and hemolymph of treated snails. Moreover, treated snails suffered from high mortality rates that associated with a negative effect on their growth [47, 44].

The infectivity of *S. mansoni* miracidia to *B. alexandrina* was greatly reduced by LC_{25} of methanol extract of the tested plants. Comparable results were obtained in literature [43, 48-50] using the plants *S. lubium, Z.simplex*, *O. reticulum, F. selloea* and *A. celsii*. 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 reported. 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 [50]. These observations are in accordance with many authors using different plant species as molluscicides. Thus, El-Ansary *et al.* [51] 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.* [49] obtained similar reduction in cercarial shedding and cercarial production from *B. alexandrina* treated with sublethal concentrations of aqueous suspension of *Zygophyllum simplex*.

Regarding the sources of energy for snails, LC_{25} of the tesed plants significantly decreased the glycogen content in soft tissues of treated snails, while the glucose level in haemolymph increased. This may be attributed to the activity of the tesed plants that impedes oxygen consumption of snails, thus inducing anaerobic respiration. Glycogen is the primary source for serum glucose, known to be the most important anaerobic energy source in anoxic-tolerant mollusks [52]. The decrease in tissue glycogen may be due to increased glycogenolysis and it is known that hypoxic or anoxic conditions which induced by the action of pesticides normally increase glycogenolysis and thus increased blood glucose [53]. 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*, *Yacca alaifolia* and *Portalacca oleracca* methanol extracts [21] and niclosamide [44].

In the present study, the levels of glycogen phosphorylase and glucose-6-phosphatase (G-6-Pase) in the soft tissues of normal and treated snails were also

significantly reduced in response to treatment with the tested plants. With respect to G-6-Pase as glycogenolytic enzyme, it showed reduced activity in treated snails which was attributed to either synthesis and/ or degradation of glycogen [54] increasing the glucose concentration stimulated glycogen synthesis and decreased the activity of glycogen phosphorylase. Glucose was incorporated into glycogen during period of net glycogen breakdown and vice versa; glycogen degradation occurred during periods of net glycogen synthesis which depends on glucose concentration [55].

The present results showed a significant decrease in SDH activity together with a concomitant increase in G-6-PD activity level in comparison with control. Succinate dehydrogenase is an important active regulatory enzyme of the tricarboxylic acid cycle (TCA), the common pathway for carbohydrates. While,G-6-PD is the key enzyme that catalyses the oxidative irreversible step of the alternative route of glucose metabolism via hexose monophosphate shunt (HMP).The increase in G-6-PD activity indicated the mobilization of glucose through pathways other than glycolysis-Kerbs'cycle axis and indicates the high rates of pentose phosphate pathway.The inhibition of SDH activity in the present study suggested the increased activity of G-6-PD to reflect the differential effects of pollution stress and can be considered as a biomonitoring tool in the assessment of environmental pollution [56]. HMP shunt stress conditions have been reported by Gonzaler and Tejedor [57].

It is evident that the application of LC_{25} of methanol extracts of *E. splendens*, *A.stylosa* and *G. officinalis* may be helpful in snail control as it interferes with the snails' biology and physiology. However, further investigations seem necessary in order to solve the problem of how to apply consistent low doses of these plants in semifield and field conditions.

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