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Full Length Research Paper

Impact of methanol extract of *Adenium obesum* plant on some biochemical and biological parameters of *Bulinus truncatus* snails

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The effect of methanol extract of *Adenium obesum* on reproduction, hatchability of eggs of *Bulinus truncatus* snails was studied. In addition, total protein contents and the activities of the transaminases (AST and ALT) and phosphatases (ACP and AKP) enzymes in hemolymph and tissues of snails treated with tested plant were determined. The present results showed that the molluscicidal activity of methanol extract of *A. obesum* plant was increased as the temperature was increase and the pH 7 was most promising results of the plants tested as molluscicides. It was found that, the effect of continuous exposure (4 week) to LC_{25} of the tested plant completely inhibited egg production after 2 weeks while LC_{10} of the tested plant stopped snails' egg laying after 3 weeks. All tested concentrations induced marked increases in the percentage of abnormal laid eggs compared to controls. The exposure of *B. truncatus* snails to such plant extract had led to a significant reduction in egg laying production and hatchability of eggs. The activities of the aminotransaminases [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)], acid phosphatase and alkaline phosphatase enzymes increased while protein content showed a decrease in hemolymph and the digestive gland-gonad (DGG) complex of the experimental snails than that of control snails. It was concluded that the application of sublethal concentration of methanol extracts of *A. obesum* may be helpful in snail control as it interferes with the snails' biochemistry and physiology.

Key words: *Bulinus truncatus* snails*, Adenium obesum*, plant mollucicides, transaminases, phosphatases enzymes.

INTRODUCTION

Bulinus truncatus snails are the snail host of *Schistosoma haematobium* with widespread distribution of the snails all over Egypt (Yousif et al., 1999; Bakry et al., 2004). *Schistosomiasis* is a parasitic disease that affects 200 million people in different countries (El-Ansary et al., 2000), use of molluscicides to eradicate the snail vector is considered the method of choice to eliminate *schistosomiasis* (WHO, 1985). For the past two decades, over 7000 chemicals have been screened as molluscicides for control of harmful snails (Singh et al., 1996). Due to low fatality rates, deleterious effects on the environment and the high cost of synthetic molluscicides (Cooppan et al., 1986), only one synthetic molluscicide, niclosamide (NIC) is commercially available and recommended by the World Health Organization (Webbe, 1987). All of these problems push the scientists to focus their attention on plant molluscicides which are less hazardous to the environment than their synthetic counterpart. A large number of plant products with molluscicidal activity have been identified (Hostettmann and Lea, 1987; Alard et al., 1991). In Egypt, molluscicides of plant origin have received an increasing attention, so several plant species were screened in this concept (Gawish et al., 2006; Hussein, 2005; Bakry, 2009). Molluscicides lacking capacity to kill snail eggs

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have to be re-applied at short time intervals in order to eradicate snails from infested water bodies (Duncan and Sturrock, 1987). In the laboratory, ovicidal effects are usually evaluated by scoring embryo deaths after short-term exposures to candidate molluscicidal compounds. As a rule, other endpoints of developmental toxicity such as teratogenicity and hatching delay are not examined (Sukumaran and Parashar, 2004; Adenusi and Odaibo, 2009; Rapado, 2010).

The amino transferases enzymes (AST and ALT) are the most frequently measured for hepatic diseases. The enzymes may be released from hepatocytes into the circulation by necrosis or by changes in cell membrane permeability that allows the enzymes to leak out of the cells (Fregia and Jenes, 1994). The phpsphatases enzymes are capable of hydrolyzing organic phosphate esters. Acid phosphatase (ACP) is a lysosomal enzyme concerning with digestion of foreign substances and bacteria inside the cells (Han and Gomak, 1979) and is involved in the defense mechanisms of both vertebrates and invertebrates (Cooper, 1976). The present work aimed to study the effect of methanol extract of *A. obesum* plant on egg normality and egg hatchability. In addition, total protein concentration and the activities of the transaminases (AST and ALT) and phosphatases (ACP and AKP) enzymes in haemolymph and tissues of snails treated with this plant were determined.

MATERIALS AND METHODS

Snails

Laboratory bred *B. truncatus* snails (2.5 to 10 mm in shell diameter) were obtained from Schistosome Biological Supply Program (SBSP), Theodor Bilharz Research Institute (TBRI), Egypt.

Plants

The plant species used is *A. obesum* (Apocynaceae) from Sinai, Egypt (April to May 2008). Common names include Sabi Star, Kudu and Desert-rose. They were kindly identified by Botany Department, Faculty of Agriculture, Al-Azhar 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.

Methanol extract

The leaves of *A. obesum* plant were shade air-dried and in an oven at 50°C then powdered by an electric mill (Bakry et al., 2002). Each plant powder was then exhaustively extracted with methanol (70%) by soaking 250 g of the plant powder in 1 L of each solvent for 7 days at room temperature (25 $\pm$ 3°C). The filtrate was distilled off under vacuum and the residues were stored in dry clean dark glass bottle until use.

Bioassay tests

Molluscicidal screening

Stock solution of 1000 ppm based on content of the active ingredient of compounds was prepared on basis of weight/volume for methanol extracts of *A. obesum* plant. 10 snails were immersed in 1000 ml of the experimental concentrations for molluscicidal evolution. Three replicates were prepared for each concentration. The exposure periods for 24 h at different water temperature (16, 25 and 30°C) using water of different pH (4, 7 and 10) was prepared. Control snails were maintained under the same experimental conditions without exposure to the tested substances, followed by another 24 h of recovery. After recovery period for 24 h, snails were removed, washed thoroughly with dechlorinated tap water and transferred to containers with fresh dechlorinated tap water. Percentage of mortality was calculated against the concentration used. The concentrations (LC_0, LC_{10}, LC_{25}, LC_{50} and LC_{90}) of the tested materials were determined after 24 h of exposure at different water temperature (16, 25 and 30) using water of different pH (4, 7 and 10) against adult snails was determined according to the standard procedure recommended by WHO (1965). The efficiency of the tested plant extract against adult snails was determined according to the standard procedure recommended by WHO (1965). The LC_{50} and LC_{90} were previously determined in the laboratory according to Litchfield and Wilcoxon (1948) and Finny (1971) methods and using SPSS computer program under windows. Dilute solutions of reagent grade sodium hydroxide or hydrochloric acid were used to maintain the desired pH conditions. Adjustment in pH to the desired test pH conditions were made gradually over 24 h period and the snails were then maintained at the test pH for the desired exposure period (Salah, 1999). PH values (4, 7 and 10) were adjusted by pH meter.

Effect of sublethal concentration from plant's methanol extract on egg normality and egg hatchability

Healthy mature snails were exposed continuously at temperature (25°C) and pH (7) to LCo, LC_{10} and LC_{25} concentration of plant extract for 4 weeks. Control snails were maintained in clean dechlorinated water under the same experimental conditions. Molluscicidal solutions were changed every 24 h with new prepared ones to avoid the effect of storage. The containers of treated and untreated snails were provided by thin plastic sheets for egg deposition. In addition to lettuce, tetramine (fish food) was added twice weekly. The egg clutches were weekly collected. Examination of egg masses used in each experiment was done under microscope every week. Egg masses with deformed embryos, dead ones, masses containing different developmental stages in one egg mass, empty egg case or more than one embryo in each egg were considered as abnormal (Gawish, 1997). The egg clutches were transferred into container containing dechlorinated water and the control ones. The percentage of egg hatching was recorded every week.

Biochemical studies

Effect of sublethal concentration from plant's methanol extract on biochemical parameters in snails' hemolymph and tissues

Adult *B. alexandrina* snails (6 to 8 mm) were continuously exposed for 4 weeks to the concentrations LC_0, LC_{10} and LC_{25} from the tested plant (each of six replicates). For each concentration a group of 30 snails was used and another one was maintained in dechlorinated water (25±1°C) as control. The concentrations were renewed weekly.

Assay methods

Haemolymph samples were collected according to Michelson

Table 1. Effect of different pH values and temperature on the molluscicidal activity of methanol extract of *Adenium obesum* plant on *Bulinus truncates* snails.

Temperature	pH value	*Adenium obesum*				
		LC_{50}	LC_{90}	LC_{25}	LC_{10}	LC_0
16	4	21	31	12.6	6.8	2.1
	7	20	28	10.6	5.6	1.9
	10	26	34	12.1	6.2	2.6
25	4	17	26	9.8	4.8	1.7
	7	15	21	8.2	4.2	1.5
	10	21	28	10.1	6.1	2.1
30	4	19	29	8.5	5.2	1.9
	7	18	25	11	6.3	1.8
	10	24	33	12	7.5	2.3

(1966) by removing a small portion of the shell and inserting a capillary tube into the heart. The hemolymph pooled from 10 snails was collected in a vial tube (1.5 ml) and kept in ice-box. For preparation of tissue extracts of both exposed and unexposed snails, the shell was crushed and the soft body was removed. For preparation of tissue homogenates of both exposed and unexposed snails, 1 g 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 min at 3000 rpm then the fresh supernatant was used. All biochemical parameters determined in this study were analyzed spectrophometrically using reagent kits purchased from BioMerieux Company, France. Total protein concentration was determined according to Lowry et al. (1951). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in the heamolymph and DGG extracts of exposed and unexposed snails were determined according to the method of Rwitman and Frankel (1957). Acid phosphatase and alkaline phosphatase activities were determined according to Fishman and Ferner (1953) and Bessey et al. (1946) respectively.

Statistical analysis

Analysis of the obtained data was carried out by student's *t*-test for comparing the means of experimental and control groups (Spiegel, 1981).

RESULTS

The present results in Table 1 showed that the LC_{50} values were 15 ppm for tested plant after 24 h of exposure at pH level: 7 (25°C). The pH 7 showed the most promising results of the plants tested as molluscicides where their activities decreased under acidic and alkaline pH values (17 and 21 ppm). Also, data in Table 1 showed that the molluscicidal activity of tested plant was increased as the temperature increased. LC_{50} of tested plant was 20 ppm at 16°C and 18 ppm at 30°C respectively after 24 h at pH 7. The most effective application of methanol extract of *A. obesum* plant against snails after 24 h (LC_{50} value was 15 ppm, respectively) was obtained at pH 7 and 25°C. The present data in Table 2 showed that the least percentage of abnormal egg masses was seen in the case of control snails (11.8 %). The percentage of abnormal egg masses laid by treated *B. truncatus* snails with sublethal concentrations was more significantly (P<0.001) elevated in case of LC_0 (64.7%), LC_{10} (77.4%) and LC_{25} concentrations (82.6%) after 2 weeks of experiment in comparison with the control snails representing 19.6%. Greatest number of eggs (212± 0.342 egg/snails) was seen in case of control *B. truncatus* snails, however, this number was declined significantly (P<0.001) in case of all the treated snails compared to the control snails. The present investigation showed the effect of continuous exposure for 4 weeks to LC_{25} of the tested plant completely inhibited egg production after 2 weeks while LC_{10} of the tested plant stopped snail's egg lying after 3 weeks. All sublethal concentrations of tested plant induced marked increases in the percentage of abnormal laid eggs, compared to controls. The present investigation showed the molluscicding agents caused marked reduction in egg hatchability especially with LC_{25} (26.3%) while sublethal concentrations (LC_0 and LC_{10}) of the tested plant caused 31 and 20.2% hatchability reduction compared to controls (80.4%) after 2 weeks respectively.

The present results (Table 3, Figures 1 and 2) reveal that all tested biochemical parameters were altered in both hemolymph and tissues of *B. truncatus* as a consequence of exposure to sublethal concentrations of methanol extract of *A. obesum.* The protein content in haemolymph and tissues of treated snails (Table 3) was greatly reduced than in untreated control snails. However, transaminases and phosphatases enzymes were elevated in treated snails. Protein content was significantly (P<0.01) reduced in hemolymph and the tissues of snails treated with LC_0,LC_{10} and LC_{25} of

Table 2. Effect of continuous exposure for 4 weeks to sublethal concentrations of methanol extract of *Adenium obesum* plant on normality and egg hatchability of *Bulinus truncates* snails.

Exposure period (weeks)		Control	LC_0	Lc_{10}	Lc_{25}
	Number of eggs	212± 0.342	160±0.43	100±0.56	82±1.32
	Number of abnormality eggs	25± 0.28	42±0.52*	56±0.43**	51±0.65**
week	Hatchability	180±1.23**	120±1.1*	38±0.73*	32±0.86
	Hatchability (%)	85%	75%	56%	39%
	Abnormality (%)	11.8%	26.3%	38%	62.2%
	Number of eggs	275±2.12	116±0.75	84±1.3	46±1.1
	Number of abnormality eggs	54±1.5*	75±0.3**	65±0.62***	38±0.8***
2 weeks	Hatchability	221±1.2***	36±1.3*	17±0.34*	10±0.43
	Hatchability (%)	80.4%	31%	20.2%	26.3%
	Abnormality (%)	19.6%	64.7%	77.4%	82.6%
	Number of eggs	182±1.6	45±0.93	26±0.67	0
	Number of abnormality eggs	38±1.7*	33±0.65**	22±0.55***	0
3 weeks	Hatchability	150±1.8***	13±0.45*	5±0.82*	0
	Hatchability (%)	82.4%	28%	19.2%	0
	Abnormality (%)	20.9%	73,3%	84.6%	0
	Number of eggs	96±1.9	35±0.46	0	0
	Number of abnormality eggs	20±0.87*	30±0.12***	0	0
4 weeks	Hatchability	77±0.66***	6±0.23*	0	0
	Hatchability (%)	80.3%	17.1%	0	0
	Abnormality (%)	20.8%	85.7%	0	0

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

extract plant than that of control snails with percentage reduction ranged 24.7 to 59.5% in hemolymph and 19.3 to 64.41% in tissues. The concentrations of these enzymes (AST and ALT) in hemolymph and tissues of *B. truncatus* snails treated with LC_0, LC_{10} and LC_{25} of extract plant were significantly (P<0.001) increased than that in control snails. The percentage of elevation ranged 43.8 to 90.1% for AST and 30.8 to 93.41% for ALT in hemolymph, and ranged 17.29 to 85% for AST and 50.26 to 85.8% for ALT in DGG complex. The present results indicated that there are significant (P<0.001) elevations in the level of acid phosphatasein hemolymph and tissues by exposure of snails with sublethal concentrations of the tested extract. This increase of alkaline phosphatase was ranged 38.9 to 83.33% in hemolymph and 31.25 to 93.75% in tissues of experimental snails than in controls. Also, there are significant (P<0.001) elevations in the level of alkaline phosphatase in hemolymph and tissues.

DISCUSSION

The present results showed that the molluscicidal activity of methanol extract of *A. obesum* plant was increased as the temperature was increase. The obtained results agree with those obtained by El-Sayed (1988), Abdel et al. (2002) and Mostafa (2002). The pH 7 showed the most promising results of the plants tested as molluscicides where their activities decreased under acidic and alkaline. The obtained results agree with Abdel et al. (2004) who found that the high molluscicidal activity of *Agave filifera, Agave attenuate* and *Calendula micrantha* at pH 7 and decreased in acidic and alkaline pH level against *B. alexandrina* snails. These results were supported by Abdel and Sharaf (2000). The present investigation showed the effect of continuous exposure to LC_{25} of methanol extract of *A. obesum* plant completely inhibited egg production after 2 weeks while Lc_{10} of the tested plant stopped snails egg laying after 3 weeks. All tested concentrations induced marked increases in the percentage of abnormal laid eggs compared to controls. This may be due to the active constituents present in *A. obesum* that might affect the internal mechanism of egg production (Abdel et al., 2005). This agree with Sakran and bakry (2005) who found that LC_{25} of methanol extracts of *Y. alaifolia* and *E. pseudocactus* showed good interruption in egg production of *Biomphalaria alesandrina* as the abnormal egg masses were increased. This long-term effect of such low concentrations are in accordance with Bakry et al. (2002)

Table 3. The protein content and the activities of the transaminases (AST and ALT) and phosphatases (ACP and AKP) enzymes in haemolymph and tissues of *Bulinus truncatus* snails treated with sublethal concentrations of methanol extract of *Adenium obesum* plant.

		Control	LC0		LC10		LC25	
		Mean±SD	Mean±SD	Change	Mean±SD	Change	Mean±SD	Change
Haemolymph (Mg/m)	Protein content	38±3.1	28.6±1.4*	24.7	21±1.8**	-44.7	15.4±3.1***	59.5
	Aspartate aminotransferase (AST) (U/mg protein)	20.3±1.8	29.2±2.3**	43.8	33±2.3***	62.6	38.6±3.1***	90.1
	Alanine aminotransferase (ALT) (U/mg protein)	18.2± 2.1	23.8± 1.4*	30.8	30.5±6.2***	67.58	35.2±1.3***	93.41
	Acid phosphatase (ACP) (U/mg protein)	0.018±0.03	0.023±0.031*	27.8	0.030±0.06***	66.7	0.035±0.014***	94.4
	alkaline phosphatase (ALKP) (U/mg protein)	0.18±0.06	0.25±0. 5	38.9	0.29±0.03**	61.1	0.33±0.15***	83.33
DGG comlex (Mg/g)	Protein content	44.4±2.1	35.8±2.6*	19.3	25.2±3.2**	43.4	15.8±2.4***	64.41
	Aspartate aminotransferase (AST) (U/mg protein)	42.8±5.8	50.2±7.3	17.29	66.6±1.4**	55.6	79.2±1.6***	85
	Alanine aminotransferase (ALT) (U/mg protein)	38.8± 2.7	58.3± 4.3	50.26	62.8±1.8**	61.85	72.1±4.2**	85.8
	Acid phosphatase(ACP) (U/mg protein)	0.32±0.07	0.41±0.03	28.1	0. 48±0.05**	50	0.61±0.03***	90.6
	alkaline phosphatase (ALKP) (U/mg protein)	0.016±0.07	0.021±0.03	31.25	0.026±0.02**	62.5	0.031±0.03***	93.75

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

and Sakran (2004) who found that low concentrations of both chemical and natural molluscicide substances decreasing the total numbers of eggs laid by the exposed snails in comparison with normal snails.

Abdel and Sharaf (2000) and Abdel et al. (2005) studied the interruption of several synthetic and natural molluscicides on egg production, egg abnormality and egg masses of *B. alexandrina*. They found that the long term exposure to low concentrations of different molluscicide agents markedly induced inhibition in egg production and increased abnormal eggs and egg masses rates. The present investigation showed the molluscicide agents caused marked reduction in egg hatchability. This agree with Abdel et al. (2005) who found that the molluscicide agents caused marked reduction in eggs 'hatchability

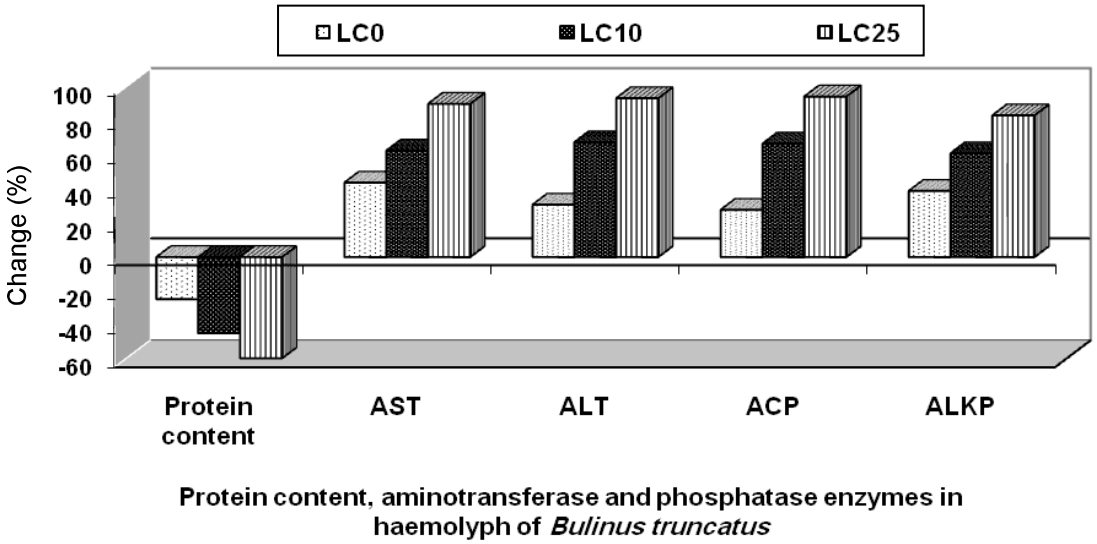

Figure 1. Changes (%) of total protein and the activities of AST, ALT, ACP and ALkP enzymes in heamolymph of *Bulinus truncatus* treated with sublethal concentrations of methanol extract of *Adenium obesum* plant.

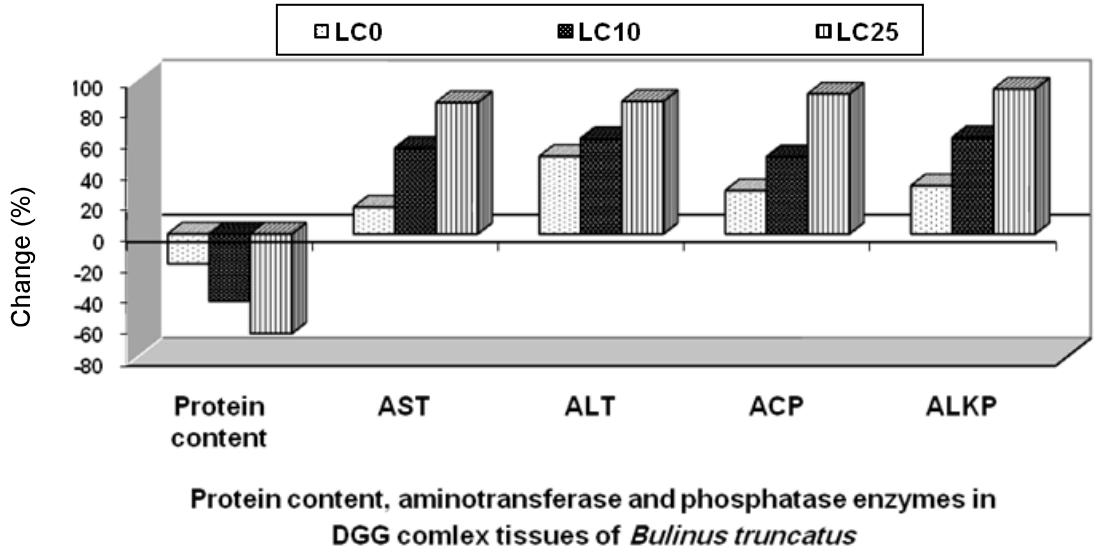

Figure 2. Changes (%) of total protein and the activities of AST, ALT, ACP and ALkP enzymes in DGG comlex tissues of *Bulinus truncatus* treated with sublethal concentrations of methanol extract of *Adenium obesum* plant.

Especially with Bayluscide and Uccmaluscide (0 and 18% respectively). *A. filifera* and *A. attenuate* plants caused 21 and 15% hatchability reduction compared to controls (73 and 53%) after 4 and 5 weeks respectively. The present result indicated a reduction of total protein concentrations in the hemolymph and tissues of *B. truncatus* snails exposed to the tested plant. This decrease may be due to interference of saponins in the plant extract in protein metabolism by inhibiting protein synthesis. Similar observations were noticed in the lymph and tissues of *L. natalensis* (Abdel-Megeed, 1999), *B. alexandrina* and *L. carinatus* (Eissa, 2002) snails treated with plants of *C. micrantha* and *E. peplus* as molluscicial agents. This harmful effect could be attributed to enhancement of energy utilization and/or destruction of cells' organelles of treated snails that may lead to inhibition of protein synthesis (Abdel-Megeed, 1999; Eissa, 2002). The aminotransaminases [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] represent an important link between carbohydrate and amino acid

metabolic pathways (Christic and Michelson, 1975; Nevo, 1978). Also, these enzymes are considered good sensitive tools for detection of any variations in the physiological process of living organisms (Nevo et al., 1978; Tolba, 1997).

The concentration of these enzymes (AST anfALT) in hemolymph and tissues of experimental *B. truncates* snails were significantly increased than that in control snails. The elevation in the activities of these enzymes could be due to a variety of conditions including muscle damage, intestinal and hepatic injury and toxic hepatitis (Farkas et al., 2004).

REFERENCES

Abdel kA, 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: 841-858.

Abdel KA, Mostafa BB, Ragab FMA, El-Said KM, Tantawy AA, El-Khayat HMM (2002). Influence of some natural and synthetic substances on certain fresh water organisms and the snail vectors of Schistosomiasis and Fascioliasis. Abst. Int. Conf. TBBi, Cairo Egypt. pp. 27.

Abdel KA, Ramzy MT, Tantawy A (2004). Evaluation of the molluscicidal and in vitro schistosomicidal activity of butanol extract of the plant Agave filifera. Egypt. J. Biomed. Sci., 16: 53-67.

Abdel KA, Sharaf EL-Din A (2000). Potency of Copper Sulphate, Bayluscide, *Calendula micrantha officinalis* and *Ammi majus* Flowers on egg masses of *Biomphalaria alexandrina* and *Bulinus truncatus*. Egypt. J. Schistosomiasis Infect, Endem Dis., 22: 159-173.

Abdel-Megeed KN (1999). Studies on the molluscicidal activity of *Calendula micrantha officinalis* (Compositae) on fascioliasis transmitting snails. J. Egypt Soc. Parasitol., 29: 183-92.

Adenusi AA, Odaibo AB (2009). Effects of varying concentrations of the crude aqueous and ethanolic extracts of *Dalbergia sissoo* plant parts on *Biomphalaria pfeifferi* egg masses, Afr. J. Tradit. Complement. Altern. Med., 6: 139–149.

Alard F, Geerts S, Trisst ETL (1991). Toxicite d'Ambrisia maritime L. Plant molluscicide,surles organisms aquatiques noncibles, Toxicon., 29: 745–750.

Bakry F A, Ragab F M A, Sakran AMA (2002). Effect of some plant extracts with molluscicidal properties on some biological and physiological parameters of *Biomphalaria alexandrina* snails, J. Egyptian German Soc. Zool. 38: 101–111.

Bakry FA (2009). Use of some plant extracts to control *Biomphalaria alexandrina* snails with emphasis on some biological effects. Pesticide Biochem. Physiol., 9: 159–165

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

Bessey WH, Lowry OH, Brock MJ (1946). A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum, J. Biol. Chem., 16**:** 321-329.

Christic JH, Michelson EH (1975). Transaminase levels in the digestive gland-gonad of Schistosoma mansoni infected *Biomplalaria alexandrina*. Comp. Biochem. Physiol., 50: 233-236.

Cooper EL (1976). Comarative immunology. Prentice-Hall, INC., Englewood Cliffs, N. J., p. 338.

Cooppan RM, Schutte CHJ, Mayet FGH, Dingle CE, Van Deventer GM G, Mosese PG (1986). Morbidity from urinary schistosomiasis in relation to intensity of infection in the Natal Provine of South Africa, Am. J. Trop. Med. Hyg., 35: 765–776.

Duncan J, Sturrock RF (1987). Laboratory evaluation of potential plant molluscicides. In: K.E. Mott, Editor, Plant *Molluscicides*, John Wiley & Sons New York. pp. 251–265.

Eissa SH, Rizk ET, Abou-Shafey AE, Mona MH (2002) Atlum A. Toxicological effect of Euphorbia peplm water suspension on heamocytes of the fresh water snails (B. alexandrina and Lanistus carinatus. Proc, I.C.B.S., 2: 417-447.

El-Ansary A, Sammour AM, Mohamed AM. (2000). Susceptibility of *Biomphalaria alexandrina* to infection with *Schistosoma mansoni*: correlation with the activity of certain glycolytic enzymes. J. Egyptian Soc. Parasitol., 30: 547–560.

El-Sayed KM (1988). Studies on the chemical constituents of *Anagallis latifolia* (primulaceae) and *Atriplex halimus* (chenopodiaceae) and their applications as plant molluscicides, Ph.D. of Science, Department of chemistry, Faculty of Science, Al-Azhar University.

Farkas J, Farkas P, Hyde D (2004). Liver and gastroenterology tests. Ini Basic skills in interpreting laboratory data. Mary Lee 3rd Ed. Amer. Soc. Helth-Syst. Pharmacists, Bethesda, Maryland, USA, pp. 330-336.

Finney DJ (1971). Probit Analysis (3rd edition). Cambridge University Press, Cambridge, UK. ISBN 052108041X. OCLC 174198382.

Fishman WH, Ferner F (1953). A method for estimating serum acid phosphatase of prostatic origin. Biol J. Chem., 200: 89-97.

Fregia A, Jenes D (1994). Evaluation of abnormal liver tests, Comp. Ther. 1994, 20: 50-54.

Gawish FM (1997). Evaluation of combination of certain molluscicide against *Biomphalaria alexandrina* and the free living stages of *Schistosoma mansoni*. Ph.D. Thesis, Zoology Dep. Girls Coll. For Arts, Sci. and Education, Ain Shams University.

Gawish F, El-Bardicy S, Tadros MM, Mahmoud K, Yousief F (2006). Biocidal activity of twenty eight plant species from two families, Myrtaceae and Agavaceae on *Biomphalaria alexandrina* snails and *Sichistosoma mansoni* free larval stages. J. Egypt Ger. Soc. Zool., 50: 89.

Han AW, Gomak DH (1979). Histology. 8th ed. J.B. Lippincott Company, Philadelphia and Toronto, pp. 764.

Hostettmann K, Lea PJ (1987). Biologically Active Natural Products, Oxford Science Publication, Oxford.

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

Litchfield JT, Wilcoxon F (1948). A simplified method of evaluating dose effect experiments. J. Pharmacol. Exper., 98: 99–113.

Lowry OH, Rosebrough NG, Farr EL, RandallR G (1951). Protein measurement with Folin phenol reagent. J. Biol. Chem., 193: 265–275.

Michelson EH (1966). Specificity of haemolymph antigens in taxonomic discrimination of medically important snails. J. Parasitol., 52: 466-472.

Mostafa BB, Abdel Kader A, Ragab FMA, El-Said KM, Tantawy AA, El-Khayat HMM (2002). Studies on different treatment of copper sulphate with certain plant molluscicides and adjuvants on both snails (Biomphalaria alexandrina and Lymnaea natalensis) and the zooplankton (Daphnia pulex), Abst Int Conf Tbbi Cairo Egypt, p.19.

Nevo E, Shimony T, Libin M (1978). Pollution selection of allozyme polymorphisms in barnacles. Experentia, 26: 1562-1564.

Rapado LN, Nakano E, Ohlweiler FB, Kato MJ, Yamaguchi LF, Pereira CA, Kawano T (2010). Molluscicidal and ovicidal activities of plant extracts of the Piperaceae on *Biomphalaria glabrata* (Say, 1818), J. Helminthol., pp. 1-7

Rwitman S, Frankel S (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., pp. 28, 56.

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

Sakran AMA, Bakry FA (2005). Biological and physiological studies on *Biomphalaria alexandrina* snails exposed to different plant molluscicides. J. Egypt Ger. Soc. Zool., 48: 237-256.

Salah El-Din MA (1999). Toxicological and physiological effects of ammonia on Grass Carp Ctenopharyngodon idella at different pH levels, J. Zool., 33: 219.

Singh A, Singh DK, Misra TN, Agarwal RA (1996). Molluscicides of plant origin, Biol. Agric. Horti., 13: 205–252.

Spiegel IRM (1981). Theory and problems of Statistics, *Schaum's outline series.* McGraw-Hill, Singapore.

Sukumaran D, Parashar BD (2004). Gupta A K, JeevaratnamK, Prakash S. Molluscicidal effect of nicotinanilide and its intermediate compounds against a freshwater snail *Lymnaea luteola*, the vector of animal schistosomiasis, Mem. Inst. Oswaldo Cruz, 99: 205–210.

Tolba MR, Mohammed B, Mohammed A (1997). Effect of some heavy metals on respiration, mean enzyme activity and total protein of the pulmonate snails *Biomphalaria alexandrina* and *Bulinus truncatus*, J. Egypt. Ger. Soc. Zool., 24: 17-35.

Webbe G (1987). The toxicology of molluscicide screening and evaluation, Bull. World Health Organization, 33: 567–581.

WHO (1965). Molluscicide screening and evaluation, Bulletin of the World Heath Organization, 33:567–581.

WHO (1985). Tech. Rep. 728. Geneva, Switzerland: WHO; The control of Schistosomiasis.

Yousif F, El-Emam MA, Abdel KAA, Sharaf El-Din AK, El-Hommossany K, Shiff C (1997) Schistosomiasis in newly reclaimed areas in Egypt 1-Patterns of transmission. J. Egypt Soc. Parasitol., 29: 635.