EFFECT OF ACTIVE MOLLUSCICIDAL COMPONENETS OF *ABRUS PRECATORIUS, ARGEMONE MEXICANA* AND *NERIUM INDICUM* ON CERTAIN ENZYMES IN THE NERVOUS TISSUE OF *LYMNAEA ACUMINATA*

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Abstract

In vivo and *in vitro* sublethal exposure of abrin, glycyrrhizin (active component of *Abrus precatorius* seed and root), sanguinarine, protopine (*Argemone mexicana* seed) and oleandrin (*Nerium indicum* leaf) inhibited the acetylcholinesterase, lactic dehydrogenase, acid and alkaline phosphatase activity in the nervous tissue of *Lymnaea acuminata*. The succinic dehydrogenase activity in the nervous tissue of *L. acuminata* was increased in *in vivo* treatment of these molluscicides whereas, with *in vitro* exposure, these molluscicides caused no significant change in succinic dehydrogenase activity.

Introduction

The snail *Lymnaea acuminata* is the vector of the liver flukes *Fasciola hepatica* and *F. gigantica* which cause endemic fascioliasis of cattle in the northern pert of India [1,2]. It has been observed that *A. precatorius*, *A. mexicana* and *N. indicum* are potential source of molluscicides [3,4]. The active moieties responsible for the molluscicidal activity are abrin and glycyrrhizin in *A. precatorius* seed and root, respectively; protopine and sanguinarine in *A. mexicana* seeds; oleandrin in *N. indicum* leaves [3,4]. Sublethal treatment of these molluscicides has caused a significant decrease in the levels of protein, free amino acids, DNA and RNA level in the nervous tissue of *L. acuminata* [5]. Except glycyrrhizin. all the above molluscicides caused a significant reduction in the phospholipid levels and simultaneous increase in the rate of lipid peroxidation [5]. The mechanism by which these natural compounds caused snail death is not exactly known. The present study is an extension of our previous study aimed at elucidating the *in vivo* and *in vitro* effects of the sublethal treatment of active moieties of the above three plants on various enzyme activities *viz.*, acetylcholinesterase (AChE), acid phosphatase (ACP), alkaline phosphatase (ALP), succinic dehydrogenase (SDH) and lactic dehydrogenase (LDH) in the nervous tissue of snail *L. acuminata*.

Keywords: *Abrus precatorius*; *Argemone mexicana*; *Nerium indicum*; Acetylcholine esterase; Lactate dehydrogenase; Succinic dehydrogenase

Experimental Section

In vivo Enzyme Assays

Adult *L. acuminata* (2.6±0.3 cm length) were collected from local ponds and allowed to acclimatize at 25°C for 72 h. Snails were maintained and treated with molluscicide according to the method of Singh and Agarwal [6,7]. Batches of ten snails were kept in glass aquarium, containing 3 l of dechlorinated water and left exposed to sublethal concentrations, 40% and 80% of 24 h LC_{50}, of abrin (consists of polypeptide chains, mol. wt. 63000-67000 Da; 0.14, 0.28 mg/l), glycyrrhizin

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($C_{42}H_{62}O_{16}NH_3$; 0.04, 0.08 mg/l), sanguinarine ($C_{20}H_{14}NO_4Cl$; 0.04, 0.08 mg/l), protopine ($C_{20}H_{19}NO_5$; 0.03, 0.06 mg/l), oleandrin ($C_{32}H_{48}O_9$; 0.28, 0.56 mg/l) (Sigma Chemical Co., USA) and purified *Nerium* bark (0.34, 0.69 mg/l) [4,8] for 24 h. These concentrations were based on LC_{50} values reported from earlier observations [4,8]. Six batches were prepared for each concentration. Control aquaria contained only dechlorinated tap water without treatment.

Fish, *Colisa fasciatus* was collected and treated with different active ingredients of plant products to see the *in vivo* biochemical changes in the nervous tissue. Ten experimental fishes were taken in 5 l of dechlorinated water. *In vivo* treatment of 24 h LC_{90}, (against *L. acuminata*) of abrin (4.92 mg/l), glycyrrhizin (0.21 mg/l), sanguinarine (0.27 mg/l), protopine (0.24 mg/l), oleandrin (2.81 mg/l) and purified *nerium* bark (1.86 mg/l) were given in aquarium water for 96 h. Control aquaria were placed aside without treatment.

After 24 h, the treated snails, and after 96 h, the treated fishes were removed from the aquaria and rinsed with water. For *in vivo* enzyme assays, the nervous tissue in snails and fish were quickly dissected out and placed on ice-cubes. Afterwards, the nervous tissue was placed on filter paper to remove the adherent water and weighed. Enzyme assay were performed in treated as well as in control groups of both the test animals.

Acetylcholinesterase. AChE activity in the nervous tissue of *L. acuminata* was determined by the method of Ellman *et al.* [9] as modified by Singh and Agarwal [10]. The tissue was homogenized (50 mg/ml) in 0.1 M phosphate buffer (pH-8.0) and centrifuged (1000 g×30 min) at –4°C. The clear supernatant solution was taken as an enzyme source. Enzyme activity was measured in a 10-mm path-length cuvette using an incubation mixture consisting of 0.1 ml of enzyme source, 2.9 ml of 0.1 M buffer, 0.1 ml of chromogenic agent DTNB and 0.02 ml of freshly prepared ATChI solution in distilled water. The absorbance change at 412 nm was continuously monitored for 3 min at 25°C. Enzyme activity has been expressed as μ mole 'SH' hydrolyzed/min/mg protein.

Acid phosphatase. Assay for the activity of ACP was carried out by the method of Bergmeyer [11] as modified by Singh and Agarwal [7]. Tissue homogenate (2% w/v) was prepared in ice-cold 0.9% NaCl and centrifuged (5000 g×15 min) at 0°C. The supernatant was used as an enzyme source. To 0.2 ml of supernatant, 1 ml of pre-incubated (10 min) acid buffer substrate (0.41 g citric acid, 1.125 g sodium citrate and 165 mg disodium salt of p-nitrophenyl phosphate to 100 ml of double distilled water) was added. After mixing, the mixture was incubated for 30 min at 37°C and 4 ml of 0.1 N NaOH was added to it. The absorbance was made at 420 nm. The acid phosphatase activity has been expressed as μ mole substrate hydrolyzed/30 min/mg protein. Standard curves were drawn with p-nitrophenol.

Alkaline phosphatase. The enzyme source and standard curves were prepared by the same procedure adopted for ACP. For ALP, 0.1 ml of enzyme containing supernatant was added to 1 ml of pre-incubated (10 min) alkaline buffer substrate solution (375 mg glycine, 10 mg $MgCl_2.6H_2O$, 165 mg p-nitrophenyl phosphate in 42 ml of 0.1 ml NaOH and diluted with double distilled water to a total volume of 100 ml). The mixture was shaken gently and incubated for 30 min at 37°C. In the incubated mixture, 10 ml of 0.02 M NaOH was added. The enzyme activity was measured colorimetrically at 420 nm and the activity has been expressed as μ mole substrate hydrolyzed/30 min/mg protein.

Lactic dehydrogenase. The activity of LDH was measured according to Anon [12] as modified by Singh and Agarwal [13]. The tissue was homogenized (50 mg/ml) in 0.1 M phosphate buffer (pH-7.5) for 5 min and centrifuged (10000 g×30 min) at –4°C. To 0.01 ml of an enzyme source (supernatant), 0.5 ml of pyruvate substrate (10 mg NADH in 10 ml of 0.75 mM/l pyruvate buffer, pH-7.5) was added and kept for incubation for 45 min at 37°C. To this 0.5 ml of 2,4-dinitrophenyl hydrazine solution (0.2 g 2,4-dinitrophenyl hydrazine in 8.5 ml of concentrated HCl and volume made to 1 liter) was added and the mixture was left standing for 20 min at room temperature. Finally 5.0 ml of 0.4 N NaOH was added to the mixture and left for 30 min at room temperature. LDH activity was measured by monitoring the decrease in absorbance at 540 nm. Values were converted into LDH units and expressed as pyruvate reduced/min/mg protein.

Succinic dehydrogenase. The method of Arrigoni and Singh [14] as modified by Singh and Agarwal [15] was used to determine the SDH activity. The tissue homogenate (50 mg/ml) was prepared in 0.5 M phosphate buffer (pH-7.6) and centrifuged (10000 g×30 min) at –4°C. Supernatant, 0.1 ml was pre-incubated with 0.05 ml of 0.5 M succinate for 7 min at 37°C. Afterwards, 0.05 ml of this solution was added to a cuvette containing 0.2 ml of 0.5 M buffer, 0.1 ml of KCN solution, 0.2 ml 4 mM of $CaCl_2$, 0.3 ml DCIP, 0.1 ml of 0.5 M succinate, 2 ml of distilled water, 0.05 ml of 2% PMS. The decrease in absorbance at 600 nm was continuously monitored for 3 min. The enzyme activity has been expressed as μ mole dye reduced/min/mg protein.

In vitro Enzyme Assay

In vitro experiment were performed by dissolving the

molluscicides in ether and an appropriate volume was added to 10-mm path-length cuvette to maintain the doses of molluscicide as given in Table 5. Ether, containing molluscicide, was then allowed to evaporate following which it was pre-incubated with an enzyme source for 15 min at 25°C. Enzyme activities were determined using the same assay procedure as mentioned in *in vivo* experiments. Control cuvette contained only ether.

Every estimation was replicated six times and values were expressed as mean±SE. Data were analyzed using Student's t-test [16].

Results and Discussion

In vivo inhibition of enzymes

In vivo 24 h exposure of 40% and 80% of 24 h LC_{50} of the abrin, glycyrrhizine, sanguinarine, protopine, oleandrin and purified *Nerium* bark caused significant dose-dependent changes in AChE, LDH, acid and alkaline phosphates activity in the nervous tissue of *L. acuminata* (Table 1-3). Maximum inhibition of AChE (48% of control) was observed when snails were exposed to 80% of 24 h LC_{50} of protopine followed by glycyrrhizin (61.33% of control), sanguinarine (69.33% of control), oleandrin (72% of control), abrin (76% of control) and purified *Nerium* bark (78.66% of control) (Table 1). Maximum reduction in the LDH (73.72% of control) was observed at 80% of 24 h LC_{50} of sanguinarine (Table 2). Maximum decrease in ACP activity (51.70% of control) was observed in snails exposed to 80% of 24 h LC_{50} of protopine (Table 3). Maximum decrease in ALP activity (56.57% of control) was observed at 80% of 24 h LC_{50} of oleandrin (Table 3). Sublethal exposure of all the above treatment caused a significant increase in the SDH activity in the tissue of *L. acuminata* (Table 2).

In vivo 24 h exposure of 24 h LC_{90} (against *L. acuminata*) of abrin, glycyrrhizine, sanguinarine, protopine and purified *Nerium* bark did not cause significant changes in AChE, ACP and ALP activity in the nervous tissue of fish, *Colisa fasciatus* (Table 4). Treatment of 24 h LC_{90} of oleandrin caused 100% mortality in fish, *C. fasciatus.*

In vitro inhibition of enzymes

In vitro preincubation of different concentrations (μg) of abrin, glycyrrhizin, sanguinarine, protopine, oleandrin and purified *Nerium* bark caused a significant dose-dependent decrease in AChE, LDH, ACP, ALP activity in the nervous tissue of *L. acuminata* (Fig. 1, 2 and Table 5, 6). Treatment of 0.14 μg of sanguinarine caused maximum reduction (48.05% of control) in AChE activity in the nervous tissue of *L. acuminata* (Fig. 1). SDH activity in the nervous tissue of *L. acuminata* was 67.29 μmol dye reduced/min/mg protein. There was no significant change in SDH activity after 15 min preincubation of the same treatment. *In vitro* preincubation of 0.14 μg protopine caused a maximum decrease (80.52) in the LDH activity in the nervous tissue of *L. acuminata* (Fig. 2). Preincubation of 0.14 μg glycyrrhizine, and 1.2 μg purified *Nerium* bark caused a maximum reduction in acid and alkaline phosphatase activity, respectively (Table 5, 6).

Table 1. *In vivo* effect of 24 h sublethal exposure to abrin, glycyrrhizin, sanguinarine, protopine, oleandrin and purified bark of *Nerium indicum* on the acetylcholinesterase activity (AChE) in the nervous tissue of *Lymnaea acuminata*

Treatment	AChE	
	40% 24 h LC_{50}	80% 24 h LC_{50}
Control	0.075±0.001 (100)	0.075±0.001 (100)
Abrin	0.064±0.0019^{+} (85.33)	0.057±0.0022^{+} (76.00)
Glycyrrhizin	0.056±0.0022^{+} (74.66)	0.046±0.0015^{+} (61.33)
Sanguinarine	0.066±0.0016^{+} (88.00)	0.052±0.0019^{+} (69.33)
Protopine	0.059±0.0017^{+} (78.66)	0.036±0.0015^{+} (48.00)
Oleandrin	0.060±0.003^{+} (80.00)	0.054±0.0017^{+} (72.00)
Purified bark	0.067±0.0015^{+} (89.33)	0.059±0.0014^{+} (78.66)

Values are mean±SE of six replicates. Values in parentheses indicate percent enzyme activity with control taken as 100%. Concentrations (w/v) have been expressed as final concentration in aquarium water.

+: significant ($P<0.05$) difference between control and treated groups when t-test was used for locating differences between experimental and control groups of animals.

Acetylcholinesterase activity: μmole SH hydrolyzed/min/mg protein.

Table 2. *In vivo* effect of 24 h sublethal exposure to abrin, glycyrrhizin, sanguinarine, protopine, oleandrin and purified bark of *Nerium indicum* on the lactic dehydrogenase (LDH) and succinic dehydrogenase (SDH) activity in the nervous tissue of *Lymnaea acuminata*

Treatment	LDH	SDH

	40% 24 h LC_{50}	80% 24 h LC_{50}	40% 24 h LC_{50}	80% 24 h LC_{50}
Control	342.68±3.33 (100)	342.68±3.33^{+} (100)	47.15±0.74^{+} (100)	47.15±0.74^{+} (100)
Abrin	294.59±2.82^{+} (85.96)	276.58±1.82^{+} (80.71)	51.29±0.41^{+} (108.78)	56.54±0.83^{+} (119.91)
Glycyrrhizin	299.64±1.37^{+} (87.44)	286.98±1.45^{+} (83.74)	53.79±0.57^{+} (114.08)	58.45±0.25^{+} (123.96)
Sanguinarine	260.67±1.06^{+} (76.06)	252.65±1.16^{+} (73.72)	54.10±0.75^{+} (114.74)	61.42±0.32^{+} (130.90)
Protopine	296.70±2.43^{+} (86.58)	283.76±1.85^{+} (82.78)	53.96±0.86^{+} (114.44)	62.45±0.74^{+} (132.44)
Oleandrin	312.19±2.88^{+} (91.10)	301.59±1.37^{+} (88.00)	55.57±0.90^{+} (117.85)	59.01±0.45^{+} (125.15)
Purified bark	300.62±1.50^{+} (87.72)	287.31±3.94^{+} (83.84)	51.43±0.63^{+} (109.07)	59.46±0.77^{+} (126.10)

Values are mean±SE of six replicates. Values in parentheses indicate percent enzyme activity with control taken as 100%. Concentrations (w/v) have been expressed as final concentration in aquarium water.
+: significant ($P<0.05$) difference between control and treated groups when t-test was used for locating differences between experimental and control groups of animals.
LDH activity: μ mole pyruvate reduced/min/mg protein, and SDH activity: μ mole dye reduced/min/mg protein.

Table 3. *In vivo* effect of 24 h sublethal exposure to abrin, glycyrrhizin, sanguinarine, protopine, oleandrin and purified bark of *Nerium indicum* on the acid phosphatase (ACP) and alkaline phosphates (ALP) activity in the nervous tissue of *Lymnaea acuminata*

Treatment	**ACP**		**ALP**	
	40% 24 h LC_{50}	80% 24 h LC_{50}	40% 24 h LC_{50}	80% 24 h LC_{50}
Control	2.94±0.03 (100)	2.94±0.03 (100)	2.51±0.02 (100)	2.51±0.02 (100)
Abrin	2.08±0.06^{+} (70.74)	1.76±0.01^{+} (59.86)	1.70±0.26^{+} (67.72)	1.44±0.02^{+} (57.37)
Glycyrrhizin	2.31±0.04^{+} (78.57)	1.85±0.01^{+} (62.92)	1.81±0.04^{+} (73.30)	1.52±0.07^{+} (60.55)
Sanguinarine	2.33±0.03^{+} (79.25)	1.96±0.06^{+} (66.66)	1.86±0.11^{+} (74.10)	1.57±0.06^{+} (62.54)
Protopine	2.04±0.01^{+} (69.38)	1.52±0.07^{+} (51.70)	1.96±0.02^{+} (78.08)	1.73±0.16^{+} (68.92)
Oleandrin	2.06±0.04^{+} (70.06)	1.65±0.07^{+} (56.12)	2.06±0.02^{+} (82.07)	1.42±0.02^{+} (56.57)
Purified bark	2.44±0.06^{+} (82.99)	2.02±0.09^{+} (68.70)	2.21±0.04^{+} (88.04)	1.65±0.07^{+} (65.73)

Values are mean±SE of six replicates. Values in parentheses indicate percent enzyme activity with control taken as 100%. Concentrations (w/v) have been expressed as final concentration in aquarium water.
+: significant ($P<0.05$) difference between control and treated groups when t-test was used for locating differences between experimental and control groups of animals.
ACP and ALP activity: μ mole substrate hydrolyzed/30 min/mg protein.

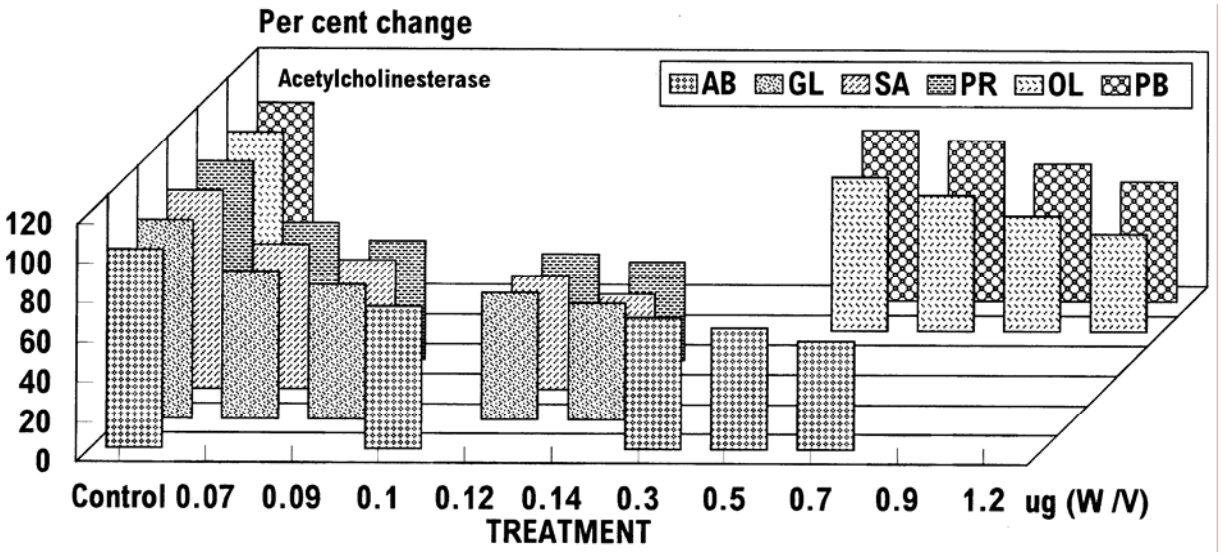

Figure 1. Histogram showing *in vitro* effect of different concentrations (μg) of abrin (AB), glycyrrhizin (GL), sanguinarine (SA), protopine (PR), oleandrin (OL) and purified bark (PB) of *Nerium indicum* on the acetylcholinesterase activity in the nervous tissue of *L. acuminata*. Concentrations (w/v) have been given as final concentration in the incubation mixture present in the cuvette. There was a significant ($P<0.05$) difference between control and treated groups when t-test was applied.

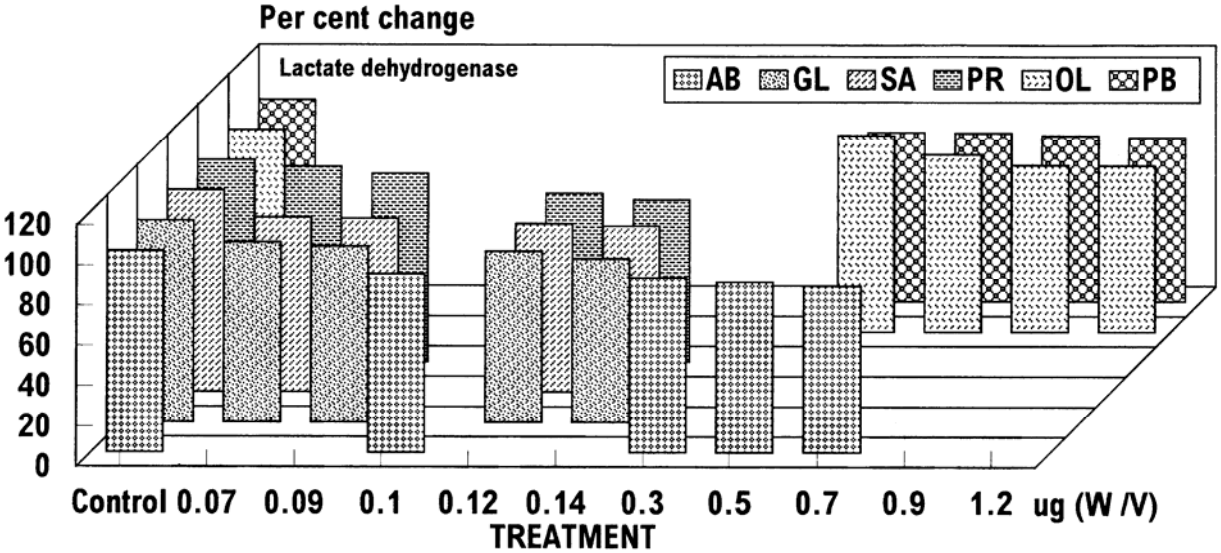

Figure 2. Histogram showing *in vitro* effect of different concentrations (μg) of abrin (AB), glycyrrhizin (GL), sanguinarine (SA), protopine (PR), oleandrin (OL) and purified bark (PB) of *Nerium indicum* on the lactate dehydrogenase activity in the nervous tissue of *L. acuminata*. Concentrations (w/v) have been given as final concentration in the incubation mixture present in the cuvette. There was a significant ($P<0.05$) difference between control and treated groups when t-test was applied.

Data presented in the previous section clearly demonstrate tat *in vivo* treatment of 40% and 80% of 24 h LC_{50} of the active components of *A. precatorius* (abrin, glycyrrhizin), *A. mexicana* (sanguinarine,

protopine), *N. indicum* (oleandrin and purified *Nerium* bark) caused a significant inhibition of AChE, LDH, ACP, ALP and increase in SDH activity in the nervous tissue of *L. acuminata*. The *in vitro* treatment of these active components indicate that the activity of AChE. LDH, ACP and ALP were significantly inhibited. However, *in vitro* treatments of abrin, glycyrrhizin, sanguinarine, protopine, oleandrin and purified *Nerium* bark caused no significant change in the SDH activity. It seems that the increase in SDH activity in *in vivo* treatment may be due to the interaction of other reactions. *In vitro* inhibition of AChE, LDH, ACP and ALP activity by abrin, glycyrrhizin, sanguinarine, protopine, oleandrin and purified *Nerium* bark indicates that they act directly on the enzymes. Alterations in the enzyme activities by the above mentioned active components which change the important biochemical reactions, may be the cause of their molluscicidal activity.

Sinha [17] has demonstrated that oral administration of 50% ethanol extract of *A. precatorius* seeds (250 mg/kg) for 30 days caused significant inhibition of acid phosphatase and succinic dehydrogenase activity in testis of albino rats. Sanguinarine is reported to inhibit the acetylcholinesterase, butyrylcholinesterase and cholineacetyl transferase [18]. The structural similarity of sanguinarine and acetylcholine appears to explain the esterase inhibitor. However, this explanation does not agree with the kinetic study, indicating that the sanguinarine binding site differed from the active site of AChE. This different effect might be due to interactions with sulphydryl group of enzyme [18]. Inhibition of AChE activity in snail nervous system by sanguinarine may be one of the causes of its toxicity. Tandon *et al.* [19] have reported that *Argemone* alkaloid sanguinarine showed a dose-dependent inhibition of Na^+, K^+-ATPase activity in rats, in non-competitive manner which ultimately inhibited the active transport sodium pump across the membrane. Vavreckova *et al.* [20] tested the susceptibility of the keratinocytes for the action of the quaternary benzophenanthridine alkaloid, sanguinarine, on plasma membrane integrity, which resulted in a two-fold increase in the lactic dehydrogenase activity. The release of LDH is commonly used as an indicator of plasma membrane damage [20]. The release of LDH into the serum of Lohmann broiler chicks fed *A. precatorius* reflected the hepatic damage in chicks [21]. In the present study, decrease in LDH activity in the nervous tissue of snail, *L. acuminata* exposed to abrin, glycyrrhizin, sanguinarine, protopine and purified bark indicate that they are inhibiting the anaerobic metabolism [13] of snail.

The inhibition of alkaline phosphatase may result in the reduction of protein levels [7,22], as it plays critical role in protein synthesis [23] and other secretory activities [24] in gastropods. Feeding *A. precatorius* produced increased serum acid phosphatase activities in Lohmann broiler chicks [21]. In the present study, treatment of abrin, glycyrrhizin, sanguinarine, protopine and purified *Nerium* bark caused significant inhibition of acid phosphatase. Previously, Singh and Singh [5] have reported that there is a significant reduction in the protein level in the nervous tissue of *L. acuminata* exposed to abrin, glycyrrhizin, sanguinarine, protopine, oleandrin and purified *Nerium* bark. Reduction in the activity of acid phosphatase, the lysosomal enzyme known to cause protein breakdown indicates that it is not activated for the breakdown of protein.

Treatment of 24 h LC_{90} (against *L. acuminata*) of abrin, glycyrrhizin, sanguinarine, protopine and purified *Nerium* bark did not cause mortality and significant alteration in the AChE, ACP and ALP activities in the nervous tissue of *C. fasciatus*. It clearly indicates that, except for oleandrin, these treatments are not harmful to the fish, *C. fasciatus* which shares the same habitat with snails.

Table 4. *In vivo* effect of 96 h sublethal exposure (24 h LC_{90}) of abrin, glycyrrhizin, sanguinarine, protopine, oleandrin and purified bark of *Nerium indicum* on the acetylcholinesterase, acid phosphatase (ACP) and alkaline phosphatase (ALP) activity in the nervous tissue of *Colisa fasciatuas*

Treatment	AChE	ACP	ALP
Control	0.636±0.05 (100)	5.73±0.41 (100)	4.82±0.33 (100)
Abrin	0.633±0.034 (99.52)	5.50±0.09 (95.81)	4.95±0.10 (95.22)
Glycyrrhizin	0.632±0.057 (99.37)	5.56±0.08 (97.03)	4.70±0.04 (97.51)
Sanguinarine	0.630±0.046 (99.05)	5.28±0.19 (91.98)	4.48±0.14 (92.94)
Protopine	0.626±0.049 (98.42)	5.23±0.29 (91.27)	4.40±0.08 (91.28)
Oleandrin	–	–	–
Purified bark	0.617±0.024 (97.01)	5.58±0.15 (97.38)	4.41±0.16 (91.49)

Values are mean±SE of six replicates. Values in parentheses indicate percent enzyme activity with control taken as 100%. Concentrations (w/v) have been expressed as final concentration in aquarium water.

+: significant ($P<0.05$) difference between control and treated groups when t-test was used for locating differences between experimental and control groups of animals.

Acetylcholinesterase activity: μ mole SH hydrolyzed/min/mg protein; ACP and ALP activity: μ mole substrate hydrolyzed/30 min/mg protein.

Table 5. *In vitro* effect of different concentrations (μg) of abrin, glycyrrhizin, sanguinarine, protopine, oleandrin and purified bark of *Nerium indicum* on the acid phosphatase activity in the nervous tissue of *Lymnaea acuminata*

	Acid phosphatase activity					
Treatment (μg)	**Abrin**	**Glycyrrhizin**	**Sanguinarine**	**Protopine**	**Oleandrin**	**Purified bark**
Control	5.36±0.08 (100)	5.36±0.08 (100)	5.36±0.08 (100)	5.36±0.08 (100)	5.36±0.08 (100)	5.36±0.08 (100)
0.07		3.78±0.06$^+$ (70.52)	4.73±0.07$^+$ (88.24)	4.09±0.07$^+$ (76.30)		
0.09		3.62±0.01$^+$ (67.53)	4.40±0.07$^+$ (82.08)	3.88±0.04$^+$ (72.38)		
0.12		3.46±0.05$^+$ (64.55)	4.11±0.20$^+$ (76.67)	3.56±0.04$^+$ (66.41)		
0.14		3.30±0.01$^+$ (61.56)	3.72±0.14$^+$ (69.40)	3.45±0.07$^+$ (64.36)		
0.1	4.41±0.06$^+$ (82.27)					
0.3	4.09±0.06$^+$ (76.30)					
0.5	3.77±0.08$^+$ (70.33)				4.58±0.14$^+$ (85.44)	4.89±0.14$^+$ (91.23)
0.7	3.45±0.08$^+$ (64.36)				4.26±0.13$^+$ (79.47)	4.74±0.21$^+$ (88.43)
0.9					3.94±0.13$^+$ (73.50)	4.21±0.16$^+$ (78.54)
1.2					3.78±0.06$^+$ (70.52)	3.89±0.11$^+$ (72.57)

Values are mean±SE of six replicates. Values in parentheses are percent change with control taken as 100%. Concentrations (w/v) have been given as final concentration in the incubation mixture present in the cuvette.

+: significant ($P<0.05$) difference between control and treated groups when student's t-test was applied between treated and control group.

Acid phosphatase activity: μ mole substrate hydrolyzed/30 min/mg protein.

Table 6. *In vivo* effect of different concentrations (μg) of abrin, glycyrrhizin, sanguinarine, protopine, oleandrin and purified bark of *Nerium indicum* on the alkaline phosphatase activity in the nervous tissue of *Lymnaea acuminata*

	Alkaline phosphatase activity					
Treatment (μg)	**Abrin**	**Glycyrrhizin**	**Sanguinarine**	**Protopine**	**Oleandrin**	**Purified bark**
Control	2.99±0.11 (100)	2.99±0.11 (100)	2.99±0.11 (100)	2.99±0.11 (100)	2.99±0.11 (100)	2.99±0.11 (100)
0.07		2.88±0.08 (96.32)	2.53±0.18 (84.61)	2.83±0.04 (94.64)		
0.09		2.68±0.11 (89.63)	2.46±0.05$^+$ (82.27)	2.78±0.08 (92.97)		
0.12		2.37±0.10$^+$ (79.26)	2.35±0.03$^+$ (78.59)	2.63±0.10 (87.95)		
0.14		2.04±0.03$^+$ (68.22)	2.29±0.10$^+$ (76.58)	2.57±0.09$^+$ (85.95)		
0.1	2.68±0.11 (89.63)					
0.3	2.51±0.04$^+$ (80.60)					
0.5	2.14±0.07$^+$ (71.57)				2.73±0.14 (91.30)	2.42±0.09$^+$ (80.93)
0.7	1.96±0.06$^+$ (65.55)				2.63±0.10 (87.95)	2.26±0.07$^+$ (75.58)
0.9					2.41±0.04$^+$ (80.60)	2.09±0.04$^+$ (69.89)
1.2					2.31±0.09$^+$ (77.25)	1.89±0.03$^+$ (63.21)

Values are mean±SE of six replicates. Values in parentheses are percent change with control taken as 100%. Concentrations (w/v) have been given as final concentration in the incubation mixture present in the cuvette.

+: significant ($P<0.05$) difference between control and treated groups when student's t-test was applied between treated and control group.

Alkaline phosphatase activity: μ mole substrate hydrolyzed/30 min/mg protein.

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