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Environmental Toxicology and Pharmacology 15 (2004) 87–93
ENVIRONMENTAL TOXICOLOGY AND PHARMACOLOGY ETAP
www.elsevier.com/locate/etap

Toxic effect of two common Euphorbiales latices on the freshwater snail *Lymnaea acuminata*

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Received 16 June 2003; accepted 7 November 2003

Abstract

The aqueous and serially purified latex extracts of plants *Euphorbia pulcherima* and *Euphorbia hirta* (family Euphorbiaceae) have potent molluscicidal activity. Sub-lethal doses (40 and 80% of LC_{50}) of aqueous and partially purified latex extracts of both the plants also significantly alter the levels of total protein, total free amino acid, nucleic acid (DNA and RNA) and the activity of enzyme protease and acid and alkaline phosphatase in nervous tissue of the snail *Lymnaea acuminata* in time and dose dependent manner. *E. pulcherima* and *E. hirta* are common medicinal plants of family Euphorbiaceae. *E. pulcherima* is useful for a variety of conditions, such as rheumatism, snakebite, asthma, obstipation, and skin-diseases. While, *E. hirta* is also used in cough, asthma, colic, dysentery, and genito urinary diseases.

Keywords: *Lymnaea acuminata*; *Euphorbia pulcherima*; *Euphorbia hirta*; Latex; DNA and RNA; Metabolism

1. Introduction

The heavy uses of synthetic pesticides and synthetic pyrethroids affect the aquatic environment due to some properties such as high toxicity to target and non-target organism and long-term persistence in the environment (Sastry and Shukla, 1993; Devi, 1997; Singh and Deepak, 2002). Their hazardous nature has prompted the scientists, to find out non-disruptive, suitable, and newer options for the control of weed and aquatic harmful pest. In recent time, the use of plant/natural pesticides has gained more popularity all over the world. These plant products are a focus of attention as a suitable alternative to synthetic pesticides due to some ideal properties such as low cost, easy availability, and biodegradability in nature (Marston and Hostettmann, 1985; Verma and Dubey, 1999).

Many plant pesticides are used in aquatic ecosystem for the control of aquatic weeds, pest, and harmful snails (Kinghorn and Evans, 1975; Belot et al., 1991; Mohapatra and Nayak, 1998 and Yadav and Singh, 2002). Plants such as *E. schimperiana*, *E. milli*, *E. splendens*, *C. tiglium*, *E. tirucalli*, *E. continifolia* and others of the family Euphorbiaceae have high molluscicidal potency (Souza et al., 1997; Schall et al., 1998; AI-Zanbagi et al., 2001; Yadav et al., 2002). *Euphorbia pulcherima* and *Euphorbia hirta* are common medicinal plants of the family Euphorbiaceae. *E. pulcherima* is useful for a variety of conditions, such as rheumatism, snakebite, asthma, obstipation, and skin-diseases (Vlietink, 1987). While, *E. hirta* is also used in cough, asthma, colic, dysentery, and genito urinary diseases (Wealth of India, 1985; Jain, 1996; Bhatnagar et al., 2000).

The aim of the present communication is to present biochemical alterations, caused by the aqueous and serially purified latex extracts of both the plants in nervous tissue of the fresh water snail *Lymnaea acuminata*, which is the intermediate host of *Fasciola hepatica* and *Fasciola gigantica*, which causes endemic fascioliasis in cattle and livestock in northern part of India.

2. Materials and methods

Latex of *E. pulcherima* and *E. hirta* were collected from the Botanical garden of Deen Dayal Upadhyay Gorakhpur University, Gorakhpur (UP) India. White latex produced by these plants was drained into glass tubes following cutting of the stem apices and lyophilized at −40 °C and the lyophilized dry powder was used for further study. The wet weight of 1 ml of latex of *E. pulcherima* and *E. hirta* was

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doi:10.1016/j.etap.2003.11.001

Table 1
Sub-lethal doses of latex extracts of *E. pulcherima* and *E. hirta* used for biochemical studies in the freshwater snail *L. acuminata*

Plants	Doses (mg/l)			
	40% of LC_{50} (24 h)	80% of LC_{50} (24 h)	40% of LC_{50} (96 h)	80% of LC_{50} (96 h)
E. pulcherima	0.04	0.08	0.02	0.04
E. hirta	0.52	1.04	0.24	0.48

875 and 815 mg and the dry weight was 385 and 405 mg, respectively.

2.1. For aqueous extracts

The freeze-dried powder was mixed with an appropriate volume of distilled water to obtain the desired concentrations (40 and 80% of LC_{50} of 24 h) as given in Table 1.

2.2. For serial purification

The lyophilized latex powder was extracted serially with 5 ml each of chloroform → carbon tetrachloride → acetone → diethyl ether → ethyl alcohol. Centrifugation for 20 min at 2000 × *g* was carried out in a refrigerated centrifuge, at −4 °C, after each extraction. The solvent fraction was decanted and the solvent allowed to evaporate. The dried soluble fraction was re-dissolved in water for further experiments.

Adult snail *L. acuminata* (2.6 ± 0.3 cm in shell height) were collected from Ramgarh Lake, Gorakhpur, 273 009 and used as test animals. The snails were maintained and treated with aqueous and partially purified extracts of latices of the test plants according to Singh and Agarwal (1990). Adult *L. acuminata* were kept in glass aquaria containing 3 l of de-chlorinated tap water. Atmospheric and water temperature was ranging from 30.5 to 31.5 °C and 27.0–28.0 °C, respectively, pH of water was 7.3–7.5, while dissolved oxygen, free carbon dioxide, and bicarbonate alkalinity were ranging from 6.8 to 7.6, 4.4 to 6.5 mg/l and 105.0 to 109.0 mg/l, respectively, during the experiments. Each aquarium contained 20 experimental animals.

After completion of treatment the test animals were removed from the aquaria and washed with tap water. The nervous tissue of *L. acuminata* was excised and used for biochemical analysis. Control animals were held in similar conditions without treatment. Each experiment was replicate six times and the values have been expressed as mean ± SE of six replicates. Student's *t*-test and analysis of variance were applied to locate significant changes (Sokal and Rohlf, 1973).

2.3. Biochemical estimation

2.3.1. Protein

Protein levels were estimated according to the method of Lowry et al. (1951) using bovine serum albumin as standard. Homogenates (5 mg/ml, w/v) were prepared in 10% TCA.

2.3.2. Total free amino acids

Estimation of total free amino acid was made according to the method of Spices (1957). Homogenates (10 mg/ml, w/v) were prepared in 95% ethanol, centrifuged at 6000 × *g* and used for amino acid estimation.

2.3.3. Nucleic acids

Estimation of nucleic acid (DNA and RNA) was performed, by the methods of Schneider (1957) using diphenylamine and orcinol reagents, respectively. Homogenates (1 mg/ml, w/v) were prepared in 5% TCA at 90 °C, centrifuged at 5000 × *g* for 20 min and the supernatant was used for the estimation of nucleic acids.

2.3.4. Protease

Protease activity was estimated by the method of Moore and Stein (1954). Homogenate (50 mg/ml, w/v) was prepared in cold distilled water. Optical density was measured at 570 nm. The enzyme activity was expressed in μ mol of tyrosine equivalent/mg protein/h.

2.3.5. Acid and alkaline phosphatase

The activity of acid and alkaline phosphatase in the nervous tissue was determined, according to the method of Anderson and Szcypinski (1947) as modified by Bergmeyer (1967) and Singh and Agarwal (1983). Tissue homogenates (2% w/v) were prepared in ice-cold 0.9% sodium chloride solution and centrifuged at 5000 × *g* at (0 °C) for 15 min. Optical density was measured at 420 nm against a blank, prepared simultaneously. The enzyme activity has been expressed as the amount of ρ-nitrophenol formed/30 min/mg protein in the supernatant.

3. Results

Data of sub-lethal (40 and 80% of LC_{50}) exposure of the freshwater snail *L. acuminata* against aqueous and serially extracted latex of *E. pulcherima* and *E. hirta* for 24 and 96 h on nitrogenous metabolism in nervous tissue are given in Tables 2–5.

3.1. Euphorbia pulcherima

Exposure of snails to 40 and 80% of LC_{50} of aqueous latex extracts of *Euphorbia puichrima* for 24 or 96 h caused significant alterations in nitrogenous metabolism in nervous tissue of the freshwater snail *L. acuminata* (Tables 2 and

Table 2
Changes in total protein, total free amino acids, nucleic acid (DNA and RNA) (μg/mg), protease (μg mol of tyrosine equivalents/mg protein/h), and acid and alkaline phosphatase (μ mol substrate hydrolyzed/30 min/mg protein) level in nervous tissue of *L. acuminata* after exposure to 40 and 80% of LC_{50} of aqueous and serially purified latex extracts of *E. pulcherima* for 24 h

Parameters	Nature of latex	Control	40% of LC_{50}	80% of LC_{50}
Protein	A	65.12 ± 0.30 (100)	34.45 ± 0.38[a] (53)	24.05 ± 0.38[a] (37)
	B	65.45 ± 0.82 (100)	33.80 ± 0.72[a] (52)	26.65 ± 0.58[a] (41)
Amino acid	A	34.75 ± 0.40 (100)	47.26 ± 0.50[a] (139)	51.34 ± 0.74[a] (151)
	B	34.25 ± 0.70 (100)	46.24 ± 0.78[a] (136)	49.30 ± 1.03[a] (145)
Nucleic acid	DNA			
	A	74.95 ± 0.95 (100)	59.20 ± 0.75[a] (80)	52.54 ± 0.68[a] (71)
	B	75.55 ± 1.12 (100)	61.50 ± 0.95[a] (82)	54.75 ± 0.95[a] (73)
	RNA			
	A	60.25 ± 0.80 (100)	52.80 ± 0.60[a] (88)	41.40 ± 0.49[a] (69)
	B	61.75 ± 0.90 (100)	52.20 ± 0.39[a] (87)	43.92 ± 0.92[a] (72)
Protease	A	0.375 ± 0.075 (100)	0.433 ± 0.058[a] (114)	0.484 ± 0.042[a] (129)
	B	0.380 ± 0.052 (100)	0.441 ± 0.065[a] (116)	0.483 ± 0.043[a] (127)
Acid phosphatase	A	0.194 ± 0.0006 (100)	0.171 ± 0.0004[a] (88)	0.147 ± 0.0004[a] (76)
	B	0.194 ± 0.0012 (100)	0.175 ± 0.0003[a] (90)	0.155 ± 0.0005[a] (80)
Alkaline phosphatase	A	0.385 ± 0.0016 (100)	0.339 ± 0.0005[a] (88)	0.250 ± 0.0004[a] (65)
	B	0.388 ± 0.0013 (100)	0.349 ± 0.0006[a] (90)	0.248 ± 0.0005[a] (64)

Values are mean ± SE of six replicates. Values in parenthesis are percentage change with control taken as 100%. A: Supernatant of aqueous solution of latex. B: Latex serially extracted through chloroform, carbon tetrachloride, acetone, diethyl ether and ethyl alcohol.
[a] Significant ($P < 0.05$) Student's *t*-test was applied between control and treated groups.

3). Total protein levels were reduced to 53 and 37% of controls, respectively after exposure to 40 and 80% of LC_{50} (24 h) of aqueous latex extract. The maximum decrease in protein level (30% of control) was observed in snails treated with 80% of LC_{50} (96 h) of aqueous latex extract. DNA level was reduced to 80 and 71% of controls after treatment with 40 and 80% of LC_{50} (24 h), respectively. The maximum decrease in DNA (34% of control) was observed in snails treated with 80% of LC_{50} (96 h) of aqueous latex extract. RNA level was reduced to 88 and 69% of control

Table 3
Changes in total protein, total free amino acids, nucleic acid (DNA and RNA) (μg/mg), protease (μg mol of tyrosine equivalents/mg protein/h), and acid and alkaline phosphatase (μ mol substrate hydrolyzed/30 min/mg protein) level in nervous tissue of *L. acuminata* after exposure to 40 and 80% of LC_{50} of aqueous and serially purified latex extracts of *E. pulcherima* for 96 h

Parameters	Nature of latex	Control	40% of LC_{50}	80% of LC_{50}
Protein	A	68.42 ± 0.46 (100)	29.24 ± 0.42[a] (43)	20.40 ± 0.43[a] (30)
	B	68.45 ± 0.80 (100)	30.60 ± 0.81[a] (45)	21.08 ± 0.46[a] (31)
Amino acid	A	34.95 ± 0.93 (100)	49.98 ± 0.36[a] (147)	55.76 ± 0.76[a] (164)
	B	35.25 ± 0.94 (100)	49.35 ± 0.28[a] (141)	56.70 ± 0.29[a] (160)
Nucleic acid	DNA			
	A	72.85 ± 0.91 (100)	39.60 ± 0.48[a] (55)	24.48 ± 0.32[a] (34)
	B	72.55 ± 0.48 (100)	41.76 ± 0.72[a] (58)	26.64 ± 0.78[a] (37)
	RNA			
	A	62.24 ± 1.04 (100)	35.34 ± 0.52[a] (57)	24.80 ± 0.68[a] (40)
	B	62.85 ± 1.15 (100)	37.20 ± 0.82[a] (60)	26.04 ± 0.96[a] (42)
Protease	A	0.382 ± 0.060 (100)	0.458 ± 0.076[a] (120)	0.535 ± 0.098[a] (140)
	B	0.390 ± 0.072 (100)	0.464 ± 0.082[a] (119)	0.527 ± 0.072[a] (135)
Acid phosphatase	A	0.198 ± 0.0004 (100)	0.097 ± 0.0004[a] (49)	0.069 ± 0.0005[a] (35)
	B	0.195 ± 0.0005 (100)	0.103 ± 0.0006[a] (53)	0.074 ± 0.0011[a] (38)
Alkaline phosphatase	A	0.412 ± 0.0008 (100)	0.185 ± 0.0012[a] (45)	0.181 ± 0.0007[a] (49)
	B	0.395 ± 0.0014 (100)	0.194 ± 0.0004[a] (49)	0.183 ± 0.0005[a] (46)

Values are mean ± SE of six replicates. Values in parenthesis are percentage change with control taken as 100%. A: Supernatant of aqueous solution of latex. B: Latex serially extracted through chloroform, carbon tetrachloride, acetone, diethyl ether and ethyl alcohol.
[a] Significant ($P < 0.05$) Student's *t*-test was applied between control and treated groups.

Table 4
Changes in total protein, total free amino acids, nucleic acid (DNA and RNA) (μg/mg), protease (μg mol of tyrosine equivalents/mg protein/h), and acid and alkaline phosphatase (μ mol substrate hydrolyzed/30 min/mg protein) level in nervous tissue of *L. acuminata* after exposure to 40 and 80% of LC_{50} of aqueous and serially purified latex extracts of *E. hirta* for 24 h

Parameters	Nature of latex	Control	40% of LC_{50}	80% of LC_{50}
Protein	A	66.34 ± 0.62 (100)	36.54 ± 0.72[a] (54)	26.40 ± 0.92[a] (40)
	B	66.56 ± 0.86 (100)	36.96 ± 0.51[a] (56)	28.38 ± 0.76[a] (43)
Amino acid	A	35.25 ± 0.62 (100)	47.25 ± 0.72[a] (135)	51.10 ± 0.64[a] (146)
	B	36.15 ± 0.58 (100)	47.16 ± 0.95[a] (131)	51.84 ± 0.66[a] (144)
Nucleic acid	DNA			
	A	73.95 ± 1.12 (100)	60.59 ± 0.56[a] (83)	53.29 ± 0.68[a] (73)
	B	74.25 ± 1.14 (100)	63.64 ± 0.82[a] (86)	55.50 ± 0.78[a] (75)
	RNA			
	A	61.85 ± 1.02 (100)	54.29 ± 0.92[a] (89)	44.53 ± 0.82[a] (73)
	B	60.25 ± 0.62 (100)	54.62 ± 0.98[a] (91)	45.60 ± 0.96[a] (76)
Protease	A	0.385 ± 0.082 (100)	0.431 ± 0.078[a] (112)	0.454 ± 0.068[a] (122)
	B	0.372 ± 0.064 (100)	0.409 ± 0.062[a] (110)	0.454 ± 0.068[a] (120)
Acid phosphatase	A	0.193 ± 0.0004 (100)	0.168 ± 0.0005[a] (87)	0.145 ± 0.0006[a] (75)
	B	0.190 ± 0.0003 (100)	0.171 ± 0.0006[a] (90)	0.146 ± 0.0005 [a] (77)
Alkaline phosphatase	A	0.415 ± 0.0012 (100)	0.374 ± 0.0012[a] (90)	0.311 ± 0.0006[a] (75)
	B	0.414 ± 0.0006 (100)	0.377 ± 0.0014[a] (91)	0.319 ± 0.0004[a] (77)

Values are mean ± SE of six replicates. Values in parenthesis are percentage change with control taken as 100%. A: Supernatant of aqueous solution of latex. B: Latex serially extracted through chloroform, carbon tetrachloride, acetone, diethyl ether and ethyl alcohol.
[a] Significant ($P < 0.05$) Student's *t*-test was applied between control and treated groups.

after treatment with 40 and 80% of LC_{50} (24 h) of aqueous latex extracts respectively in nervous tissue of *L. acuminata*. The maximum decrease in RNA (40% of control) was observed in snails treated with 80% of LC_{50} (96 h) of aqueous latex extract. Total free amino acid levels were increased up to 147 and 164% of controls after treatment with 40 and 80% of LC_{50} (96 h) of aqueous latex extracts respectively in nervous tissue of the snail *L. acuminata* (Tables 2 and 3).

Activity of acid phosphatase was inhibited to 88 and 76% of controls after treatment with 40 and 80% of LC_{50} (24 h)

Table 5
Changes in total protein, total free amino acids, nucleic acid (DNA and RNA) (μg/mg), protease (μg mol of tyrosine equivalents/mg protein/h), and acid and alkaline phosphatase (μ mol substrate hydrolyzed/30 min/mg protein) level in nervous tissue of *L. acuminata* after exposure to 40 and 80% of LC_{50} of aqueous and serially purified latex extracts of *E. hirta* for 96 h

Parameters	Nature of latex	Control	40% of LC_{50}	80% of LC_{50}
Protein	A	65.60 ± 0.48 (100)	29.25 ± 0.68[a] (45)	22.10 ± 0.72[a] (34)
	B	66.22 ± 0.56 (100)	31.02 ± 0.76[a] (47)	23.12 ± 0.48[a] (35)
Amino acid	A	35.26 ± 0.74 (100)	50.05 ± 0.88[a] (143)	55.65 ± 0.72[a] (159)
	B	36.48 ± 0.56 (100)	50.76 ± 0.86[a] (141)	56.16 ± 0.62[a] (156)
Nucleic acid	DNA			
	A	73.95 ± 0.96 (100)	44.53 ± 0.76[a] (61)	27.74 ± 0.82[a] (38)
	B	75.60 ± 1.02 (100)	46.50 ± 0.82[a] (62)	30.75 ± 0.95[a] (41)
	RNA			
	A	60.12 ± 0.72 (100)	35.40 ± 0.76[a] (59)	25.80 ± 0.68[a] (43)
	B	61.25 ± 0.72 (100)	37.21 ± 0.82[a] (61)	28.06 ± 0.56[a] (46)
Protease	A	0.348 ± 0.078 (100)	0.411 ± 0.072[a] (118)	0.480 ± 0.074[a] (138)
	B	0.332 ± 0.066 (100)	0.382 ± 0.086[a] (115)	0.448 ± 0.062[a] (135)
Acid phosphatase	A	0.194 ± 0.0005 (100)	0.093 ± 0.0003[a] (48)	0.074 ± 0.0012[a] (38)
	B	0.193 ± 0.0007 (100)	0.098 ± 0.0006[a] (51)	0.072 ± 0.0006 [a] (37)
Alkaline phosphatase	A	0.382 ± 0.0006 (100)	0.187 ± 0.0010[a] (49)	0.141 ± 0.0038[a] (37)
	B	0.392 ± 0.0007 (100)	0.199 ± 0.0008[a] (51)	0.157 ± 0.0005[a] (40)

Values are mean ± SE of six replicates. Values in parenthesis are percentage change with control taken as 100%. A: Supernatant of aqueous solution of latex. B: Latex serially extracted through chloroform, carbon tetrachloride, acetone, diethyl ether and ethyl alcohol.
[a] Significant ($P < 0.05$) Student's *t*-test was applied between control and treated groups.

of aqueous latex extracts respectively in nervous tissue of snail. Activity of alkaline phosphatase was reduced to 88 and 65% of control after treatment with 40 and 80% of LC_{50} (24 h) of aqueous latex extracts respectively in nervous tissue of snail. The maximum decrease in acid and alkaline phosphatase 35 and 44% of control, respectively, was observed in snails treated with 80% of LC_{50} (96 h) of aqueous latex extract. Protease activity was increased to 114 and 129% of controls after treatment with 40 and 80% of LC_{50} (24 h) of aqueous latex extracts respectively in the nervous tissue of snail *L. acuminata*. The maximum increase in protease activity (140% of control) was observed in snails treated with 80% of LC_{50} (96 h) of aqueous latex extract (Tables 2 and 3).

Latex was serially extracted with different organic solvents also caused a similar alteration in the nitrogenous metabolism of snail. Alterations caused by serially extracted latex, though statistically insignificant, appeared lower than the water extracted latex at all the doses and exposure periods (Tables 2 and 3).

3.2. *Euphorbia hirta*

Exposure of snails to 40 and 80% of LC_{50} of aqueous latex extracts of *E. hirta* for 24 or 96 h caused significant alterations in nitrogenous metabolism in nervous tissue of the freshwater snail *L. acuminata* (Tables 4 and 5). Total protein levels were reduced to 54 and 40% of controls, respectively after exposure to 40 and 80% of LC_{50} (24 h) of aqueous latex extract. The maximum decrease in protein level (34% of control) was observed in snails treated with 80% of LC_{50} (96 h) of aqueous latex extract. DNA level was reduced to 83 and 73% of controls after treatment with 40 and 80% of LC_{50} (24 h), respectively. The maximum decrease in DNA (38% of control) was observed in snails treated with 80% of LC_{50} (96 h) of aqueous latex extract. RNA level was reduced to 89 and 73% of controls after treatment with 40 and 80% of LC_{50} (24 h) of aqueous latex extracts respectively in nervous tissue of *L. acuminata*. The maximum decrease in RNA (43% of control) was observed in snails treated with 80% of LC_{50} (96 h) of aqueous latex extract. Total free amino acid levels were induced to 143 and 159% of controls after treatment with 40 and 80% of LC_{50} (96 h) of aqueous latex extracts respectively in nervous tissue of snail (Tables 4 and 5).

Activity of acid phosphatase was inhibited to 87 and 75% of controls after treatment with 40 and 80% of LC_{50} (24 h) of aqueous latex extracts respectively in nervous tissue of snail. The activity of alkaline phosphatase was reduced to 90 and 75% of controls after treatment with 40 and 80% of LC_{50} (24 h) of aqueous latex extracts respectively in nervous tissue. Protease activity was increased to 112 and 122% of controls after treatment with 40 and 80% of LC_{50} (24 h) of aqueous latex extracts respectively in the nervous tissue of snail *L. acuminata*. The maximum increase in protease activity (138% of control) was observed in snails treated with 80% of LC_{50} (96 h) of aqueous latex extract, respectively (Tables 4 and 5).

Latex of *E. hirta* was serially purified extracts with organic solvents also caused a similar alteration in nitrogenous metabolism of snail. Alterations caused by serially purified latex, though statistically insignificant, appeared lower than the water extracted latex at all the doses and exposure periods (Tables 4 and 5).

4. Discussion

It is clear from the results that the treatment with sub-lethal doses of aqueous and serially purified latex extracts of *E. pulcherima* and *E. hirta*, significantly alter the level of total protein, total free amino acid, nucleic acid, activity of enzyme protease, acid and alkaline phosphatase. The rate of alteration in all the cases was significant ($P < 0.05$) time and dose dependent.

Proteins are mainly involved in cell architecture. During chronic periods of stress they are also a source of energy (Umminger, 1977). The decrease in protein levels observed in this study may be due to their degradation and also to their possible utilization for metabolic purposes. Bradbury et al. (1987) reported that the decrease in protein content might also be attributed to the destruction or necrosis of cells and consequent impairment in protein synthesis machinery.

The quantity of protein depends on the rate of protein synthesis or its degradation. It is affected due to impaired incorporation of amino acids into polypeptide chains (Singh et al., 1996). The synthesis of RNA plays an important role in protein synthesis. The inhibition of RNA synthesis at transcription level, thus may affect the protein level. In this study, a significant decline in RNA level in exposed snail was observed. The decrease in the RNA concentration may also have been a cause of protein depletion. Alternatively, the increase in protease activity may be the cause of increased protein degradation.

Enhanced protease activity and decreased protein level have resulted in a marked elevation of free amino acids in the snail tissue. The accumulation of free amino acids can also be attributed to the less use of amino acids (Seshagiri Rao et al., 1987) and their involvement in the maintenance of an acid–base balance (Moorthy et al., 1984). Extracts of both the plants also significantly decreased the level of nucleic acids in the nervous tissue of the snail. Several reports are available on the reduction in DNA and RNA level on exposure to different pesticides Tarig et al. (1977); Nordenskjold et al. (1979). Data attained in this study make it clear that these plant extracts are potential inhibition of DNA synthesis, resulting in reduction of the RNA level. Mahendru (1981) suggested that the anti-AChE compounds attack many enzymes responsible for normal metabolism pathway.

In present study, the aqueous and serially extracted latices of *Euphorbia pulcherima* and *E. hirta* reduced the alkaline

phosphatase activity in nerve tissue of *L. acuminata*. The inhibition of alkaline phosphatase activity was found to be both dose and time dependent. Alkaline phosphatase which contains a serine residue at its active site also get inhibited by the latices of both the plants, like-wise enzyme acetylcholinesterase (Singh, 2000).

Another side enzyme acid phosphatase also play an important role in catabolism, pathological necrosis, autolysis, and phagocytosis (Abou-Donia, 1978). Aqueous and serially extracted latices of all these plants significantly ($P < 0.05$) decreased the activity of this enzyme in time and dose dependent manner. Singh et al. (1998) have reported significant reduction in acid and alkaline phosphatase activity in nerve tissue of *L. acuminata*, on exposure to sub-lethal doses of aqueous extracts of *Nerium indicum* and *Argemone maxicana*. In another study Singh et al. (1997) found sub-lethal doses of aqueous extracts of spices also caused a significant reduction in acid and alkaline phosphatase activity in nerve tissue of snail. Vorbrodt (1959) reported that alkaline phosphatase is an important enzyme of animal metabolism, which plays an important role in the transport of metabolites across the membranes. Since, both the plants used in the present study may also have anti-phosphatases activity. So the reduction in protein level may be due to the inhibition of alkaline phosphatase activity, as it plays an important role in protein synthesis Pilo et al. (1972) and also involved in the synthesis of certain enzymes Sumner (1965).

Thus, aqueous and serially purified latex extracts contains biochemical alterations which are more potent than synthetic molluscicides. We believe that these extracts may eventually be of great value of the control of snails and safe for other non-target organism.

Acknowledgements

S.K. Singh thanks the Department of Environment, New Delhi (Sanction No. F-14/35/96 MAB-RE dated: 09/11/1999), for financial support.

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