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Metabolic Changes in Freshwater Harmful Snail *Lymnaea acuminata* Due to Aqueous Extract of Bark and Leaf of *Euphorbia pulcherima* Plant

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Abstract: The aqueous extract of bark and leaf of *Euphorbia pulcherima* plant (Family: Euphorbiaceae) have potent molluscicidal activity. Sub-lethal doses (40% and 80% of 24h or 96h LC_{50}) of aqueous extract of bark and leaf of this plant also significantly alter the levels of total protein, total free amino acid, nucleic acids (DNA and RNA) and the activity of enzyme protease and acid and alkaline phosphatase in several tissues (i.e. nervous, hepatopancreas and ovotestis) of the harmful snail *Lymnaea acuminata* in time and dose dependent manner. This snail is the intermediate host of *Fasciola hepatica* which causes endemic fascioliasis in cattle and livestock in Northern part of India. *Euphorbia pulcherima* is a common medicinal plant of India, which is useful for a variety of conditions, such as rheumatism, snakebite, asthma, obstipation and skin-diseases.

Key words: *Lymnaea acuminata* · *Euphorbia pulcherima* · DNA and RNA and Metabolism

INTRODUCTION

Fascioliasis is caused by *Fasciola hepatica*, the large liver-fluke and common in sheep, cattle, goat and other herbivorous animals throughout the World. Froyed, [1] reported that about 21% cattle and 7% sheep were infected with liver fluke in Great Britain. In India, the freshwater harmful snail *Lymnaea acuminata* is the intermediate host of *Fasciola hepatica* [2-4] which causes immense harm to domestic animals of this country. One of the effective methods of controlling fascioliasis is to destroy the intermediate snail hosts with molluscicides [5-7]. Widespread and heavy use of synthetic pesticides, have been found to affect water bodies due to their high toxicity, bioaccumulation and long term persistence [8, 9]. The hazardous nature of synthetic pesticides has prompted the scientists to find out the least disruptive newer options in the field of pest control technologies. Molluscicides of plant origin are being widely used, because of the fact that the toxicity of these products is very high, easily biodegradable in nature and are safe to the users along with their low cast.

Earlier studies indicate that the euphorbiales have potent molluscicidal activity against freshwater harmful snail *Lymnaea acuminata* [10-13]. *Euphorbia pulcherima* (Family: Euphorbiaceae) plant is a common medicinal plant of India, which has a variety of use, such as in the treatment of rheumatism, snakebite, asthma, obstipation and skin-diseases [14].

The objective of present investigation is to measure the effects of sub-lethal doses (40 and 80% of 24h or 96h LC_{50}) of aqueous extract of bark and leaf of *Euphorbia pulcherima*, plant on different biochemical parameters of several tissues of freshwater harmful snail *Lymnaea acuminata*.

MATERIALS AND METHODS

Snail: The freshwater harmful snail *Lymnaea acuminata* (2.6±0.3 cm in total shell height) were collected from the local freshwater bodies of Gorakhpur district, in India and stored in glass aquaria containing de-chlorinated tap water for acclimatization to laboratory conditions. Experimental conditions of water were determined in the beginning of the experiments by the method of [15]. Dead animals were immediately removed from the aquaria to avoid any contamination. Average sized adult animals were used for the biochemical experiments.

Atmospheric and water temperature were ranging from 30.6 to 31.6°C and 26.0 to 27.0°C, respectively, pH of water was 7.3 to 7.5, while dissolved oxygen, free carbon dioxide and bicarbonate alkalinity ranged from 6.6 to 7.8 mg/L, 4.5 to 6.6 mg/L and 106.0 to 109.0 mg/L, respectively, during the experiments.

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Table 1: Sub-lethal doses of bark and leaf extracts, of *Euphorbia pulcherima* plant used for biochemical studies in the freshwater harmful snail *Lymnaea acuminata*

Plant		Doses (mg/L)			
Euphorbia pulcherima	Plant parts	40% of LC_{50} (24h)	80% of LC_{50} (24h)	40% of LC_{50} (96h)	80% of LC_{50} (96h)
	Bark	36.54	73.08	9.21	18.42
	Leaf	55.29	110.58	15.55	31.10

Plant: The plant *Euphorbia pulcherima* (Family: Euphorbiaceae) was collected from Botanical Garden of D.D.U. Gorakhpur University, Gorakhpur, Uttar Pradesh, India and identified by Plant taxonomist Department of Botany, D.D.U. Gorakhpur University, Gorakhpur, (U.P.) India, where a voucher specimen is deposited.

Extraction of Active Moiety: The fresh bark and leaf were mined in 5.0 mL of distilled water, homogenised for 5 min and centrifuged at 1000 g for 10 min. The supernatant was used as a water extract for the biochemical studies. The desired concentrations, (40% and 80% of 24h or 96h LC_{50}) has been given in Table 1.

Treatment Protocol for Dose-Response Relationship: *Lymnaea acuminata* was kept in glass aquaria containing 3L de-chlorinated tap water. Each aquarium contains ten experimental animals. Snails were exposed for 40% and 80% of LC_{50} doses of 24h or 96h aqueous extracts of *Euphorbia pulcherima* bark and leaf (Table 1) separately. Control animals were held under similar condition but without any plant extract of treatment. After completion of the treatment, the test animals were removed from the aquaria and washed with tap water. Several tissues (i.e. nervous, hepatopancreas and ovotestis) of snail *Lymnaea acuminata* were removed for biochemical analysis. Each experiment had 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 [16].

Methods Used for Biochemical Estimation

Total Protein: Total protein levels were estimated according to the method of [17], using bovine serum albumin as standard. Homogenates (5 mg/mL, w/v) were prepared in 10% Tri Chloro Acetic acid (TCA).

Total Free Amino Acids: Estimation of total free amino acids was made according to the method of [18]. Homogenates (10 mg/mL, w/v) were prepared in 95% ethanol, centrifuged at 6000 xg and used for amino acid estimation.

Nucleic Acids: Estimation of nucleic acid (DNA and RNA) was performed, by the methods of [19] using diphenylamine and orcinol reagents, respectively. Homogenates (1 mg/mL, w/v) were prepared in 5% TCA at 90°C, centrifuged at 5000 xg for 20 min and the supernatant was used for the estimation of nucleic acids.

Protease: Protease activity was estimated by the method of [20]. Homogenate (50 mg/mL, w/v) was prepared in cold distilled water. Optical density was measured at 570 nm and the enzyme activity was expressed in µ mole of tyrosine equivalent/mg protein/h.

Acid and Alkaline Phosphatase: The activity of acid and alkaline phosphatase in the various tissues was determined, according to the method of [21] and modified by [22, 23]. Tissue homogenates (2% w/v) were prepared in ice-cold 0.9% sodium chloride solution and centrifuged at 5000 xg 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 ñ-nitrophenol formed/30min/mg protein in the supernatant.

RESULTS

Exposure to the aqueous extracts of bark and leaf of *Euphorbia pulcherima* caused significant behavioural changes in the freshwater harmful snail *Lymnaea acuminata*. Behavioural changes appeared with in 5 to 10 min of exposure. The initial 30-40 min was a period of hyperactivity during which, sluggish snails moved rapidly in the aquarium water. After some time they started crawling on each other. With the entering of the toxicant in the snails body muscular twitching developed and the snails became spirally twisted, resulting in ataxia, convulsion, paralysis and death. Complete withdrawal of the body inside prior to death is perhaps due to the shell of nerve poisoning. Such behavioural symptoms and death did not occurred in control groups comprising the specific role played by the plant extract for the altered behaviour leading to the death of the snail.

Table 2: Changes in total protein, total free amino acids, nucleic acid (DNA and RNA) (µg/mg), protease (µg mole of tyrosine equivalents/mg protein/h) and acid and alkaline phosphatase (µ mole substrate hydrolysed/30 min/mg protein) level in various tissues (i.e. nervous, hepatopancreas and ovotestis) of freshwater harmful snail *Lymnaea acuminata* after exposure to 40% and 80% of LC_{50} of aqueous bark extracts of *Euphorbia pulcherima* for 24h

Parameter	Tissues	Control	40% of LC_{50} (36.54 mg/L)	80% of LC_{50} (73.08 mg/L)
Total Protein	NT	67.18±0.82 (100)	36.94±0.24$^+$ (55)	26.20±0.98$^+$ (39)
	HP	66.08±0.24 (100)	34.32±0.56$^+$ (52)	25.74±0.67$^+$ (39)
	OT	72.34±0.80 (100)	36.17±0.89$^+$ (50)	26.76±0.97$^+$ (37)
Total free Amino acid	NT	36.22±0.71 (100)	51.07±0.45$^+$ (141)	55.05±0.48$^+$ (152)
	HP	28.73±0.38 (100)	36.48±0.98$^+$ (127)	39.64±0.78$^+$ (138)
	OT	34.28±0.43(100)	44.90±0.23$^+$ (131)	48.67±0.45$^+$ (142)
DNA	NT	74.38±1.54 (100)	60.99±1.23$^+$ (82)	52.06±0.98$^+$ (70)
	HP	67.18±0.56 (100)	55.08±0.58$^+$ (82)	51.72±0.78$^+$ (77)
	OT	74.66±0.80 (100)	53.00±0.89$^+$ (71)	49.27±0.56$^+$ (66)
RNA	NT	61.28±0.69 (100)	54.53±0.92$^+$ (89)	44.12±0.78$^+$ (72)
	HP	57.09±0.56(100)	46.81±0.45$^+$ (82)	38.25±0.92$^+$ (67)
	OT	63.34±0.89 (100)	50.03±0.34$^+$ (79)	45.60±0.43$^+$ (72)
Protease	NT	0.350±0.064 (100)	0.402±0.067$^+$ (115)	0.455±0.056$^+$ (130)
	HP	0.367±0.066 (100)	0.447±0.067$^+$ (122)	0.469±0.076$^+$ (128)
	OT	0.389±0.058 (100)	0.451±0.056$^+$ (116)	0.505±0.067$^+$ (130)
Acid phosphatase	NT	0.185±0.004 (100)	0.162±0.003$^+$ (88)	0.144±0.004$^+$ (78)
	HP	0.178±0.006 (100)	0.145±0.005$^+$ (82)	0.122±0.006$^+$ (69)
	OT	0.192±0.008 (100)	0.163±0.009$^+$ (85)	0.124±0.012$^+$ (65)
Alkaline phosphatase	NT	0.389±0.015 (100)	0.338±0.013$^+$ (87)	0.260±0.012$^+$ (67)
	HP	0.356±0.008 (100)	0.320±0.009$^+$ (90)	0.252±0.008$^+$ (71)
	OT	0.388±0.004 (100)	0.306±0.005$^+$ (79)	0.244±0.006$^+$ (63)

- Values are mean ±SE of six replicates.
- Values in parenthesis are % change with control taken as 100%.
- $^+$, Significant ($P<0.05$) student's't' test was applied between control and treated groups.
- NT = Nervous tissue; HP = Hepatopancreas tissue and OT = ovotestis tissue.

Table 3: Changes in total protein, total free amino acids, nucleic acid (DNA and RNA) (µg/mg), protease (µg mole of tyrosine equivalents/mg protein/h) and acid and alkaline phosphatase (µ mole substrate hydrolysed/30 min/mg protein) level in various tissues (i.e. nervous, hepatopancreas and ovotestis) of freshwater harmful snail *Lymnaea acuminata* after exposure to 40% and 80% of LC_{50} of aqueous bark extracts of *Euphorbia pulcherima* for 96h

Parameter	Tissues	Control	40% of LC_{50} (9.21 mg/L)	80% of LC_{50} (18.42 mg/L)
Total Protein	NT	68.26±0.45 (100)	30.71±0.23$^+$ (45)	21.82±0.89$^+$ (32)
	HP	66.2±0.54 (100)	26.48±0.56$^+$ (40)	23.17±0.67$^+$ (35)
	OT	62.5±0.89 (100)	20.62±0.98$^+$ (33)	18.75±0.56$^+$ (39)
Total free Amino acid	NT	37.16±0.88 (100)	54.98±0.87$^+$ (148)	60.94±0.34$^+$ (164)
	HP	26.8±0.34 (100)	34.84±0.78$^+$ (130)	37.52±0.67$^+$ (140)
	OT	31.4±0.82 (100)	44.90±0.2$^+$ (142)	47.72±0.78$^+$ (152)
DNA	NT	71.10±0.85 (100)	39.81±0.98$^+$ (56)	24.88±0.56$^+$ (35)
	HP	61.2±0.35 (100)	42.84±0.78$^+$ (70)	40.39±0.46$^+$ (66)
	OT	71.7±0.82 (100)	48.73±0.45$^+$ (68)	43.79±0.34$^+$ (61)
RNA	NT	63.16±1.22 (100)	35.36±0.56$^+$ (56)	25.83±0.98$^+$ (41)
	HP	61.2±0.55 (100)	50.18±0.45$^+$ (82)	40.39±0.45$^+$ (66)
	OT	65.4±0.90 (100)	54.23±0.34$^+$ (83)	45.12±0.45$^+$ (69)
Protease	NT	0.334±0.065 (100)	0.407±0.078$^+$ (122)	0.467±0.078$^+$ (140)
	HP	0.296±0.066 (100)	0.331±0.024$^+$ (112)	0.378±0.067$^+$ (128)
	OT	0.334±0.078 (100)	0.397±0.067$^+$ (119)	0.467±0.045$^+$ (140)
Acid phosphatase	NT	0.189±0.004 (100)	0.094±0.005$^+$ (50)	0.073±0.007$^+$ (39)
	HP	0.180±0.007 (100)	0.124±0.007$^+$ (69)	0.113±0.005$^+$ (63)
	OT	0.186±0.009 (100)	0.156±0.008$^+$ (84)	0.123±0.004$^+$ (66)
Alkaline phosphatase	NT	0.425±0.009 (100)	0.195±0.008$^+$ (46)	0.212±0.006$^+$ (50)
	HP	0.318±0.008 (100)	0.263±0.004$^+$ (83)	0.193±0.008$^+$ (61)
	OT	0.267±0.006 (100)	0.226±0.008$^+$ (85)	0.165±0.009$^+$ (62)

- Details are as given in Table 2.

Table 4: Changes in total protein, total free amino acids, nucleic acid (DNA and RNA) (µg/mg), protease (µg mole of tyrosine equivalents/mg protein/h) and acid and alkaline phosphatase (µ mole substrate hydrolysed/30 min/mg protein) level in various tissues (i.e. nervous, hepatopancreas and ovotestis) of freshwater harmful snail *Lymnaea acuminata* after exposure to 40% and 80% of LC_{50} of aqueous leaf extracts of *Euphorbia pulcherima* for 24h

Parameter	Tissues	Control	40% of LC_{50} (55.29 mg/L)	80% of LC_{50} (110.58 mg/L)
Total Protein	NT	67.19±0.54 (100)	37.62±0.34^{+} (56)	26.87±0.35^{+} (40)
	HP	67.12±0.55 (100)	39.58±0.56^{+} (59)	31.53±0.98^{+} (47)
	OT	82.08±0.69 (100)	63.14±0.98^{+} (77)	41.82±0.76^{+} (51)
Total free Amino acid	NT	36.65±0.42 (100)	52.04±0.57^{+} (142)	56.44±0.56^{+} (154)
	HP	37.8±0.37 (100)	53.29±0.45^{+} (141)	67.28±0.67^{+} (178)
	OT	30.2±0.84 (100)	20.83±0.67^{+} (145)	57.38±0.56^{+} (190)
DNA	NT	75.24±1.02(100)	62.44±0.34^{+} (83)	54.92±0.45^{+} (73)
	HP	77.27±1.12(100)	47.13±0.24^{+} (61)	20.86±0.97^{+} (27)
	OT	67.60±0.82 (100)	38.53±0.56^{+} (57)	25.68±0.57^{+} (38)
RNA	NT	58.14±0.51 (100)	52.31±0.34^{+} (90)	41.86±0.96^{+} (72)
	HP	79.03±0.84 (100)	52.15±0.24^{+} (66)	39.51±0.78^{+} (50)
	OT	72.30±0.89 (100)	39.04±0.26^{+} (54)	23.13±0.54^{+} (32)
Protease	NT	0.318±0.062(100)	0.368±0.056^{+} (116)	0.416±0.067^{+} (131)
	HP	0.382±0.034 (100)	0.488±0.078^{+} (128)	0.530±0.078^{+} (139)
	OT	0.312±0.052(100)	0.411±0.078^{+} (132)	0.446±0.098^{+} (143)
Acid phosphatase	NT	0.198±0.004 (100)	0.180±0.006^{+} (91)	0.156±0.007^{+} (79)
	HP	0.257±0.006 (100)	0.129±0.004^{+} (50)	0.146±0.004^{+} (57)
	OT	0.248±0.003 (100)	0.136±0.005^{+} (55)	0.158±0.008^{+} (64)
Alkaline phosphatase	NT	0.380±0.013 (100)	0.345±0.012^{+} (91)	0.258±0.009^{+} (68)
	HP	0.370±0.004 (100)	0.173±0.007^{+} (47)	0.162±0.008^{+} (44)
	OT	0.452±0.003 (100)	0.207±0.008^{+} (46)	0.163±0.007^{+} (36)

• Details are as given in Table 2.

Table 5: Changes in total protein, total free amino acids, nucleic acid (DNA and RNA) (µg/mg), protease (µg mole of tyrosine equivalents/mg protein/h) and acid and alkaline phosphatase (µ mole substrate hydrolysed/30 min/mg protein) level in various tissues (i.e. nervous, hepatopancreas and ovotestis) of freshwater harmful snail *Lymnaea acuminata* after exposure to 40% and 80% of LC_{50} of aqueous leaf extracts of *Euphorbia pulcherima* for 96h

Parameter	Tissues	Control	40% of LC_{50} (15.55 mg/L)	80% of LC_{50} (31.10 mg/L)
Total Protein	NT	69.26±0.51 (100)	31.85±0.97^{+} (46)	22.85±0.67^{+} (33)
	HP	66.12±0.52 (100)	34.38±0.78^{+} (52)	26.44±0.56^{+} (40)
	OT	72.32±0.82 (100)	38.33±0.56^{+} (53)	26.03±0.45^{+} (36)
Total free Amino acid	NT	36.25±0.95 (100)	54.37±0.45^{+} (150)	60.90±0.78^{+} (168)
	HP	28.61±0.33 (100)	45.61±0.46^{+} (162)	48.43±0.57^{+} (172)
	OT	37.32±0.82 (100)	57.09±0.67^{+} (153)	63.81±0.45^{+} (171)
DNA	NT	74.22±0.92 (100)	42.30±0.78^{+} (57)	27.46±0.78^{+} (37)
	HP	71.11±1.12 (100)	44.08±0.57^{+} (62)	31.99±0.56^{+} (45)
	OT	74.60±0.78 (100)	37.30±0.98 (50)	22.38±0.45^{+} (30)
RNA	NT	64.24±1.10 (100)	37.25±0.78^{+} (58)	28.26±0.96^{+} (44)
	HP	58.30±0.81 (100)	38.47±0.77^{+} (66)	27.40±0.67^{+} (47)
	OT	65.33±0.78 (100)	34.62±0.56^{+} (53)	24.17±0.57^{+} (37)
Protease	NT	0.358±0.063 (100)	0.443±0.078^{+} (124)	0.511±0.067^{+} (143)
	HP	0.368±0.035 (100)	0.434±0.098^{+} (118)	0.500±0.069^{+} (136)
	OT	0.378±0.046 (100)	0.438±0.067^{+} (116)	0.506±0.078^{+} (134)
Acid phosphatase	NT	0.196±0.005 (100)	0.099±0.008^{+} (51)	0.078±0.007^{+} (40)
	HP	0.185±0.003 (100)	0.096±0.007^{+} (52)	0.129±0.005^{+} (70)
	OT	0.184±0.004 (100)	0.080±0.005^{+} (44)	0.099±0.008^{+} (54)
Alkaline phosphatase	NT	0.418±0.006 (100)	0.204±0.009^{+} (49)	0.197±0.007^{+} (47)
	HP	0.368±0.003 (100)	0.139±0.007^{+} (38)	0.128±0.009^{+} (35)
	OT	0.390±0.004 (100)	0.149±0.006^{+} (38)	0.125±0.005^{+} (32)

• Details are as given in Table 2.

Exposure to 40% and 80% of LC_{50} of aqueous bark and leaf extracts of *Euphorbia pulcherima* for 24h or 96h caused significant alterations in nitrogenous metabolism in various tissues of the snail *Lymnaea acuminata* (Table 2 to 5).

Treatment of total protein and nucleic acids (DNA and RNA) were significantly reduced, while free amino acid level was significantly enhanced following exposure to the sub-lethal doses in all the body tissues of freshwater harmful snail *Lymnaea acuminata*. While activity of enzyme acid and alkaline phosphatases was reduced, protease activity was increased after the exposure.

Bark Extract of *Euphorbia pulcherima:* Total protein levels in nervous, hepatopancreas and ovotestis tissues, were reduced to 55%, 52% and 50% respectively levels following treatment with aqueous 40% of concentration of (24h) LC_{50} value bark extracts. The maximum decrease in protein level (32% of control) was observed in the batches snails treated with 80% of (96h) LC_{50} concentration of aqueous bark extract. DNA levels the three tissues were reduced to 82%, 82% and 71% of controls after treatment with 40% of LC_{50} (24h), respectively. The maximum decrease in DNA (35% of control) was observed in groups treated with 80% of LC_{50} (96h) of aqueous bark extract. RNA levels in there three tissues were reduced to 89%, 82% and 79% respectively after treatment with 40% of (24h) LC_{50} of aqueous bark extracts. The maximum decrease in RNA (41% of control) was observed in snails treated with 80% of LC_{50} (96h) of aqueous bark extract. Total free amino acid levels were induced to 141%, 127% and 131% of controls after treatment with 40% of (24h) LC_{50} of aqueous bark extracts respectively in various tissues of harmful snail *Lymnaea acuminata*, respectively. The maximum increase in total free amino acid levels (164% of control) was observed in snails treated with 80% of (96h) LC_{50} of aqueous bark extract, respectively (Table 2 and 3).

Activity of acid phosphatase was inhibited to 88%, 82% and 85% of controls after treatment with 40% of (24h) LC_{50} of aqueous bark extracts respectively in all the tissues. The activity of alkaline phosphatase was reduced to 87%, 90% and 79% of controls after treatment with 40% of (24h) LC_{50} concentration respectively in all the tissues of harmful snail *Lymnaea acuminata*. Protease activity was increased to 115%, 122% and 116% of controls after treatment with 40% of (24h) LC_{50} of aqueous bark extracts, in the three different tissues, respectively. The maximum increase in protease activity (140% of control) was observed in snails treated with 80% of (96h) LC_{50} of aqueous bark extract, respectively (Table 2 and 3).

Leaf Extract of *Euphorbia pulcherima*: Total protein levels were reduced to 56, 59 and 77% of controls in nervous, hepatopancreas and ovotestis tissues, respectively after exposure to 40% of (24h) LC_{50} of aqueous leaf extract. The maximum decrease in protein level (33% of control) was observed in snails treated with 80% of LC_{50} (96h) of aqueous leaf extract. DNA level was reduced to 83%, 61% and 57% of controls after treatment with 40% of (24h), LC_{50} respectively. The maximum decrease in DNA (30% of control) was observed in snails treated with 80% of LC_{50} (96h) of aqueous leaf extract. RNA level was reduced to 90%, 66% and 54% of controls after treatment with 40% of (24h) LC_{50} of aqueous leaf extracts respectively in various tissues of harmful snail *Lymnaea acuminata*. The maximum decrease in RNA (37% of control) was observed in snails treated with 80% of LC_{50} (96h) of aqueous leaf extract. Total free amino acid levels were induced to 142%, 141% and 145% of controls after treatment with 40% of (24h) LC_{50} of aqueous leaf extracts respectively in various tissues of harmful snail *Lymnaea acuminata*, respectively. The maximum increase in total free amino acid levels (172% of control) was observed in snails treated with 80% of LC_{50} (96h) of aqueous leaf extract, respectively (Table 4 and 5).

Activity of acid phosphatase was inhibited to 91%, 50% and 55% of controls after treatment with 40% of (24h) LC_{50} of aqueous leaf extracts respectively in all the tissues of harmful snail. The activity of alkaline phosphatase was reduced to 91%, 47% and 46% of controls after treatment with 40% of (24h) LC_{50} of aqueous leaf extracts, respectively in all the tissues of harmful snail *Lymnaea acuminata*. Protease activity was increased to 116%, 128% and 132% of controls after treatment with 40% of (24h) LC_{50} of aqueous leaf extracts, respectively in the all the tissues of harmful snail *Lymnaea acuminata*. The maximum increase in protease activity (143% of control) was observed in snails treated with 80% of LC_{50} (96h) of aqueous leaf extract, respectively (Table 4 and 5).

DISCUSSION

It is clear from the result section, that exposure to sub-lethal doses of aqueous bark and leaf extracts of *Euphorbia pulcherima* against harmful snail *Lymnaea acuminata* significantly altered the level of total protein,

total free amino acid and nucleic acids (DNA and RNA) and activity of enzyme protease, acid and alkaline phosphatase.

The depletion of protein fraction in all the three tissues may have been due to their degradations and possible utilization of degraded products for metabolic purposes. Mommensen and Walsh, [24] reported that proteins are mainly involved in the architecture of the cell, which is the chief source of nitrogenous metabolism and during chronic period of stress they are also a source of energy.

The quantity of protein depends on the rate of protein synthesis or its degradation. It also affected due to impaired incorporation of amino acids into polypeptide chains [25]. 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 to freshwater harmful snail *Lymnaea acuminata* 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 [26] and their involvement in the maintenance of an acid-base balance [27].

Aqueous bark and leaf extract of this plant was also significantly decreased the level of nucleic acids in the various tissues of the freshwater harmful snail *Lymnaea acuminata*. Several reports are available on the reduction in DNA and RNA level on exposure to different pesticides [28, 29]. 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, [30] suggested that the anti-AChE compounds attack many enzymes responsible for normal metabolism pathway.

The increase in free amino acid level suggests tissues damage probably due to the increased proteolytic activity under extracted extracts toxic stress. However, the elevated levels of free amino acids (FAA) can be utilised for energy production by feeding them in to the TCA cycle through aminotransferase reaction. The increase in the levels of FAA can also be attributed to the synthesis of amino acids in addition to their elevation by protein hydrolysis. A third possibility for increased FAA level might be their increase due to transamination and amination of keto acids [31, 32]. 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 [33] and other secretary activities [34].

CONCLUSIONS

Thus we can conclude that aqueous extracts of *Euphorbia pulcherima* plant caused significant time and dose dependent alteration in nitrogenous metabolism of freshwater harmful snail *Lymnaea acuminata*. We therefore believe that the plant extracts may eventually be of great value for the control of aquatic target organisms.

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