European Review for Medical and Pharmacological Sciences 2010; 14: 915-923

Piscicidal activity of leaf and bark extract of *Thevetia peruviana* plant and their biochemical stress response on fish metabolism

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Abstract. – *Objectives:* **The leaf and bark of *Thevetia peruviana* (Family: Apocynaceae) plant was administered for 24 h to the freshwater fish *Catla catla* (Hamilton) to evaluate their piscicidal activity in laboratory and cemented pond condition.**

Materials and Methods and Results: **The LC_{50} values of leaf and bark extracts of different solvents (i.e. acetone, diethyl ether, ethyl alcohol, chloroform and carbon tetrachloride) of this plant to fish *Catla catla* were determined. The LC_{50} values of acetone leaf extract of *Thevetia peruviana* plant is 88.80 mg/L (24h) in laboratory condition and 529.38 mg/L (24h) in cemented pond condition; acetone bark extract of this plant is 99.43 mg/L (24h) in laboratory condition and 591.78 mg/L (24h) in cemented pond condition against freshwater fish *Catla catla*. Similar trend was also observed in case of other solvent (i.e. diethyl ether, ethyl alcohol, chloroform and carbon tetrachloride) of leaf and bark extracts of *Thevetia peruviana* plant against freshwater fish *Catla catla* in laboratory and cemented pond conditions. The acetone leaf and bark extract of this plant was very effective in comparison to other solvent extract in both the conditions. So, the biochemical analysis is taken only acetone leaf and bark extract of *Thevetia peruviana* plant in laboratory condition.**

Conclusions: **Exposure of sub-lethal doses (40% and 80% of LC_{50}) of acetone leaf and bark extract of this plant over 24 h caused significant ($P<0.05$) alterations in total protein, free amino acids, DNA & RNA, protease and acid and alkaline phosphatase activity in muscle, liver and gonadal tissues of fish *Catla catla* in laboratory condition.**

Key Words:

Thevetia peruviana, Catla catla, Leaf, Bark, Solvents, Piscicides.

Introduction

The toxicological actions of *Thevetia peruviana* (Family: Apocynaceae) plant may be due to the presence of apigenin-5-methyl ether (flavonoid) and triterpenoid glycosides[1]. *Thevetia peruviana* plant is also reported in piscicidal activity[2,3]. Plant extracts are referred to as botanicals and when poisonous to fish are called piscicides[4,5]. Such piscicidal plants contain different active ingredients known as alkaloids such as resin, tannins, saponins, nicotine and diosgenin[6]. However, these alkaloids are toxic to fish at high concentrations and wear off within a short time[7,8]. A large number of compounds of various classes have insecticidal, piscicidal and molluscicidal properties[9-18]. The Indian major carps *Catla catla* (Hamilton) was used as the test animal because it is present in almost all freshwater reservoirs in India and is suitable for toxicity monitoring[19,20].

The intensive application of pesticides in modern agriculture and public health operation system has resulted in serious environmental problems[21,23]. The pesticides that have received most attention include pentachlorophenol (PCPs), polychlorinated biphenyls (PCBs), atrasine, organochlorines (OCs), organophosphates (Ops) and carbamates since they are widely used and are continuously being contaminated by the toxic wastes of chemical pesticides[24,25] and pose a potential direct threat to freshwater organism, particularly to sensitive animals, such as fishes and prawns[26,27].

In the present study the piscicidal activity of different solvents of leaf and bark extracts of *Thevetia peruviana* plant to toxic effects on freshwater fish *Catla catla* after 24 hour exposure period in both conditions as well as acetone leaf and bark extract of this plant is alterations in total protein, free amino acids, DNA & RNA, protease and acid and alkaline phosphatase activity in muscle, liver and gonadal tissues of fish *Catla catla* after 24 hour exposure period in laboratory conditions was investigated.

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Materials and Methods

Animal

The freshwater fish *Catla catla* (6.50±0.6 cm in total length and 469 mg wet weight) was collected from the Government Hatcheries Centre Chappia, district Gorakhpur, Uttar Pradesh, India. The fishes were stocked in pond containing 1000 L de-chlorinated tap water for acclimatization. Care was taken to remove the dead animals as soon as possible in order to prevent the decomposition of the body in the pond. The stocking cemented ponds are large (5' × 10' × 6' feet) while, the experimental cemented ponds are 5' × 5' × 6' feet in size.

Plant

Plant *Thevetia peruviana* (Family: Apocynaceae) 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, Uttar Pradesh, India, where a voucher specimen is deposited.

Extraction of Active Compounds

The fresh leaves and bark were dried 40°C over night and pulverized in a mortar and pestle. The dried powder of leaf and bark was extracted in Soxhlet apparatus, using 200 mL of five different solvents, i.e. acetone, diethyl ether, ethyl alcohol, chloroform and carbon tetrachloride by the method of Jain[28]. The extracted material was dried through vacuum pump. The extracted dried powder was stored in airtight desiccators and used for further toxicity and biochemical studies.

Toxicity Experiments

Toxicity experiments were performed by the method of Singh and Agarwal[29]. Ten experimental animals like *Catla catla* were kept in cemented pond condition containing 50 L de-chlorinated tap water for 24 hour exposure periods and ten experimental animals were kept in laboratory condition containing 10 L de-chlorinated tap water for 24 hour exposure periods. These were exposed to four different concentrations of different solvent leaf extracts of *Thevetia peruviana* plant (70, 80, 90, 100 mg/L in laboratory condition and 350, 450, 550, 650 mg/L in cemented pond condition), respectively and four different concentrations of different solvent bark extracts of *Thevetia peruviana* plant (80, 90, 100, 110 mg/L in laboratory condition and 400, 500, 600, 700 mg/L in cemented pond condition). Control groups were kept in de-chlorinated tap water without any treatment. Each set of experiments was replicated six times. The LC values, upper and lower confidence limits, slope value, "t" ratio and heterogeneity were calculated by the probit log method (POLO computer programme) of Robertson et al[30].

Biochemical Experiments

The acclimatized animals were treated with 40% and 80% of 24 h LC_{50} of acetone leaf and bark extract for 24h exposure periods. Six aquariums were set up for each dose and each aquarium contained 10 fishes in 10L de-chlorinated tap water in laboratory condition. After termination of treatment, the test animals were removed from aquaria as well as pond and washed with water and killed. Dissect the treated animals, liver, muscle and gonadal tissues of fish were collect in ice tray and used for biochemical analysis. Control animals were held in similar condition without any treatment. Each experiment was replicated at least six times and the values have been expressed as means ± SE of six replicates. Student'*t* test was applied to locate significant changes with controls[31].

Total Protein: Total Protein levels were estimated according to the method of Lowry et al[32] using bovine serum albumin (BSA) as standard. Homogenates (5 mg/mL, w/v) were prepared in 10% TCA.

Total free amino acids: Estimation of total free amino acid was made according to the method of Spices[33]. Homogenates (10 mg/mL, w/v) were prepared in 95% ethanol, centrifuged at 6000 × g and used for amino acid estimation.

Nucleic acids (DNA & RNA): Nucleic acids were estimated by the methods of Schneider[34]. Homogenates (1 mg/mL, w/v) were prepared in 5% tri-chloroacetic acid (TCA) at 90°C, centrifuged at 5000 × g for 20 min and the supernatant was used for the estimation.

Protease: Protease activity was estimated by the method of Moore and Stein[35]. Homogenate (50 mg/mL, w/v) was prepared in cold distilled water.

Acid and alkaline phosphatase: Acid and alkaline phosphatase activity was determined by

the method of Anderson and Szcypinski[36]. Homogenates (2% w/v) were prepared in ice-cold 0.9% NaCl solution and centrifuged at 5000 xg at 0°C for 15 min.

Experimental Conditions of Experimental Water

Experimental conditions of water were determined in the beginning of the experiments by the methods of APHA[37]. In case of laboratory condition the atmospheric and water temperature were ranging from 30.5-31.5°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 6.8-7.6 mg/L, 4.4-6.5 mg/L and 105.0-109.0 mg/L, respectively, during the experiments in laboratory condition.

In case of cemented pond condition the atmospheric and water temperature were ranging from 31.6-32.8°C and 28.0-29.0°C, respectively, pH of water was 7.5-7.6, while dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were ranging from 7.2-8.3 mg/L, 5.4-7.5 mg/L and 108.0-113.0 mg/L, respectively, during the experiments.

Results

In the present study different solvent of leaf and bark extracts of *Thevetia peruviana* plant was tested against freshwater fish *Catla catla*. Fish mortality was used as a bioassay for monitoring the piscicidal activity. The toxicity (lethal concentration: LC) values of the different solvent leaf and bark extracts of this plant for periods ranging from 24h of *Catla catla* is shown in Table I to II. Thus, the LC_{50} of acetone leaf extracts of *Thevetia peruviana* plant against the fish *Catla catla* decreased from 88.80 mg/L (24h) in laboratory conditions, respectively (Table I) and 529.38 mg/L (24h) in cemented pond conditions, respectively (Table II). Similar trend of toxicity was also observed in case of other different solvent (i.e. diethyl ether, ethyl alcohol, chloroform and carbon tetrachloride) leaf extracts of *Thevetia peruviana* plant against fish *Catla catla* in laboratory and cemented pond condition, respectively (Table I and II). While the LC_{50} of acetone bark extracts of *Thevetia peruviana* plant against the fish *Catla catla* decreased from 99.43 mg/L (24h) in laboratory conditions, respectively (Table I) and 591.78 mg/L (24h) in cemented pond conditions, respectively (Table II). Similar trend of toxicity was also observed in case of other different solvents (i.e. diethyl ether, ethyl alcohol, chloroform and carbon tetrachloride) of bark extracts of *Thevetia peruviana* plant against fish *Catla catla* in laboratory and cemented pond conditions, respectively (Table I and II).

Both the plant parts were effective in killing the freshwater fish *Catla catla* at different concentrations. Bark extracts, of *Thevetia peruviana* plant is the least effective plant part against the fish *Catla catla* in laboratory and cemented pond conditions, respectively (Table I to II). While the leaf extracts of *Thevetia peruviana* plant are the most effective plant parts against the fish *Catla catla* in both the conditions, respectively (Table I to II).

Statistical analysis of the data on toxicity brings out several important points. The X^2 test for goodness of fit (heterogeneity) demonstrated that the mortality counts were not found to be significantly heterogeneous and other variables, e.g. resistance etc. do not significantly affect the LC_{50} values, as these were found to lie within the 95% confidence limits. The dose mortality graphs exhibit steep values. The steepness of the slope line indicates that there is a large increase in the mortality of freshwater fish *Catla catla* with relatively small increase in the concentration of the toxicant. The slope is, thus an index of the susceptibility of the animal to the plant origin pesticides used.

Exposure of sub-lethal doses of (40% and 80% of LC_{50} 24 h) of leaf and bark extract of *Thevetia peruviana* plant against freshwater fish *Catla catla* for 24 h caused significant ($P<0.05$) alterations in total protein, free amino acids, DNA & RNA, protease and acid and alkaline phosphatase activity in various tissues (Table III to IV) in laboratory condition.

Total protein levels were reduced to 69%, 78% and 77% of controls in muscle, liver and gonad tissues, respectively after exposure to 40% of LC_{50} (24 h) of acetone leaf extract. The maximum decrease in protein level (34% of control) was observed in fish treated with 80% of LC_{50} (24 h) of acetone leaf extract. DNA level was reduced to 76%, 80% and 78% of controls after treatment with 40% of LC_{50} (24 h), respectively. The maximum decrease in DNA (57% of control) was observed in fish treated with 80% of LC_{50} (24 h) of acetone leaf extract. RNA level was reduced to 80%, 90% and 78% of controls

Table I. Toxicity ($LC_{10,50,90}$) of different solvent bark and leaf extracts of *Thevetia peruviana* plant against fish *Catla catla* in laboratory conditions after 24 hour exposure period.

Solvents	Plant parts	Effective dose LC_{50} (mg/L)	Limits (mg/L)		Slope value	"t" ratio	Hetero-
			LCL	UCL			
Acetone	Bark	LC_{10} = 72.79 **LC_{50} = 99.43** LC_{90} = 135.81	88.70	108.85	9.46 ± 5.01	3.80	0.06
	Leaf	LC_{10} = 64.65 **LC_{50} = 88.80** LC_{90} = 121.98	78.97	97.27	9.29 ± 4.73	3.86	0.05
Diethyl ether	Bark	LC_{10} = 72.59 **LC_{50} = 103.41** LC_{90} = 147.32	91.90	115.35	8.34 ± 4.73	3.56	0.37
	Leaf	LC_{10} = 65.64 **LC_{50} = 94.95** LC_{90} = 137.34	84.62	107.38	7.99 ± 4.55	3.47	0.15
Ethyl alcohol	Bark	LC_{10} = 73.49 **LC_{50} = 106.33** LC_{90} = 153.84	94.86	120.57	7.99 ± 4.69	3.45	0.17
	Leaf	LC_{10} = 68.20 **LC_{50} = 99.46** LC_{90} =145.06	89.25	115.59	7.82 ± 4.65	3.33	0.15
Chloroform	Bark	LC_{10} = 72.43 **LC_{50} = 109.97** LC_{90} = 166.95	97.39	130.21	7.07 ± 4.55	3.15	0.42
	Leaf	LC_{10} = 66.92 **LC_{50} = 103.42** LC_{90} = 159.83	91.88	131.36	6.78 ± 4.57	2.94	0.14
Carbon tetrachloride	Bark	LC_{10} = 78.34 **LC_{50} = 114.75** LC_{90} = 168.09	103.35	137.09	7.73 ± 4.76	3.30	0.09
	Leaf	LC_{10} = 68.28 **LC_{50} = 107.32** LC_{90} = 168.69	95.16	148.78	6.53 ± 4.61	2.81	0.08

Batches of 10 fishes were exposed to four different concentrations of bark and leaf of *Thevetia peruviana* plant. Concentrations given are the final concentrations (w/v) in laboratory conditions. Regression coefficient showed that there was significant (P<0.05) negative correlation between exposure time and different LC values. LCL = Lower confidence limit; UCL = Upper confidence limit. Hetero. = Heterogeneity.

after treatment with 40% of LC_{50} (24 h) of acetone leaf extract, respectively in various tissues of fish *Catla catla*. The maximum decrease in RNA (60% of control) was observed in fish treated with 80% of LC_{50} (24 h) of acetone leaf extract. Activity of acid phosphatase was inhibited to 30%, 35% and 32% with respect to controls after treatment with 40% of LC_{50} (24 h) of acetone leaf extract respectively in various tissues of fish. The activity of alkaline phosphatase was reduced to 41%, 37% and 47% than the controls after treatment with 40% of LC_{50} (24 h) of acetone leaf extract respectively in various tissues of *Catla catla*, respectively (Table III).

Total free amino acid levels were increased to 105%, 117% and 119% with respect to controls after treatment with 40% of LC_{50} (24 h) of acetone leaf extract in various tissues of fish *Catla catla*, respectively. The maximum increase in total free amino acid levels (146% of controls) was observed in fish treated with 80% of LC_{50} (24 h) of acetone leaf extract, respectively. Protease activity was increased to 135%, 123% and 130% than the controls after treatment with 40% of LC_{50} (24 h) of acetone leaf extract, respectively in the various tissue of fish *Catla catla*. The maximum increase in protease activity (151% of controls) was observed in fish treated with 80% of LC_{50} (24 h) of acetone leaf extract of *Thevetia peruviana* plant, respectively (Table III).

Similar trend of biochemical alterations was observed in sub-lethal doses of (40% and 80% of

Table II. Toxicity ($LC_{10,50,90}$) of different solvent bark and leaf extracts of *Thevetia peruviana* plant against fish *Catla catla* in cemented pond conditions after 24 hour exposure period.

Solvents	Plant parts	Effective dose LC_{50} (mg/L)	Limits (mg/L)		Slope value	"t" ratio	Hetero-
			LCL	UCL			
Acetone	Bark	LC_{10} = 359.78 **LC_{50} = 591.78** LC_{90} = 973.35	494.43	683.51	5.93 ± 4.43	3.75	0.14
	Leaf	LC_{10} = 313.04 **LC_{50} = 529.38** LC_{90} = 895.24	435.03	615.06	5.62 ± 4.03	3.84	0.09
Diethyl ether	Bark	LC_{10} = 344.49 **LC_{50} = 628.64** LC_{90} = 1147.15	514.21	764.56	4.91 ± 4.23	3.25	0.07
	Leaf	LC_{10} = 321.13 **LC_{50} = 590.35** LC_{90} = 1086.82	487.78	723.58	4.84 ± 3.90	3.44	0.20
Ethyl alcohol	Bark	LC_{10} = 352.54 **LC_{50} = 659.49** LC_{90} = 1233.68	544.21	832.86	4.71 ± 4.22	3.14	0.21
	Leaf	LC_{10} = 311.36 **LC_{50} = 642.12** LC_{90} = 1324.22	522.59	897.21	4.08 ± 3.81	2.97	0.29
Chloroform	Bark	LC_{10} = 358.06 **LC_{50} = 693.60** LC_{90} = 1343.58	574.72	940.79	4.46 ± 4.21	2.98	0.29
	Leaf	LC_{10} = 313.62 **LC_{50} = 661.50** LC_{90} = 1395.27	538.46	983.62	3.95 ± 3.81	2.88	0.20
Carbon tetrachloride	Bark	LC_{10} = 411.71 **LC_{50} = 718.48** LC_{90} = 1253.83	615.84	925.72	5.30 ± 4.53	3.30	0.03
	Leaf	LC_{10} = 363.32 **LC_{50} = 667.26** LC_{90} = 1225.44	563.84	880.11	4.85 ± 4.10	3.29	0.03

Batches of 50 fishes were exposed to four different concentrations of bark and leaf of *Thevetia peruviana* plant. Concentrations given are the final concentrations (w/v) in pond conditions. Regression coefficient showed that there was significant ($P<0.05$) negative correlation between exposure time and different LC values. LCL = Lower confidence limit; UCL = Upper confidence limit. Hetero. = Heterogeneity.

LC_{50} 24h) acetone bark extract of *Thevetia peruviana* plant against freshwater fish *Catla catla* in various tissues, respectively (Table IV) in laboratory condition.

Discussion

The present study shows that the different solvent of leaf and bark extracts of *Thevetia peruviana* plant have a piscicidal activity in laboratory and cemented pond conditions. The results of this study are similar to that of other Authors[38-39] who reported different tolerance limits of various aquatic organisms to various piscicides. Same results were also observed in case of karanj, *Pongamia pennata* seed on different fishes i.e. *Gudsia giuris*, *Chanda nama, Oreochromis mossambicus*[40]. *Maesa ramentacea* and *Sapindus emarginatus* are the most effective plants against the *Moina* sp. *Oreochromis niloticus* and *Anabas testudineus*[39]. *Euphorbia heterophylla* plant is the most effective against the fingerlings of *Barbus occidentalis*[41]. Same results were also found in case of *Euphorbia pulcherima* against fingerlings of *Labeo rohita* (Hamilton) in different culturing conditions[5]. The toxicological actions of *Thevetia peruviana*, may be due to the presence of apigenin-5-methyl ether (flavonoid) and triterpenoid glycosides[1].

Table III. Changes in total protein, total free amino acids, nucleic acid (DNA & RNA) (g/mg) level and activity of protease (mol of tyrosine equivalents/mg protein/h) and acid and alkaline phosphatase (mol substrate hydrolysed/30 min/mg protein) in muscle, liver and gonad tissues of freshwater fish *Catla catla* after exposure to sub-lethal doses of 40% and 80% of LC_{50} (35.20 mg/L and 70.40 mg/L) of acetone leaf extract of *Thevetia peruviana* plant after 24h in laboratory conditions.

Parameter	Tissue	Control	40% of LC_{50} (24h); 35.20 mg/L	80% of LC_{50} (24h); 70.40 mg/L-
Protein	M	161.01 ± 0.61 (100)	111.16 ± 0.23 (69)	54.74 ± 0.23 (34)
	L	144.01 ± 0.58 (100)	112.32 ± 0.10 (78)	109.51 ± 0.18 (76)
	G	146.02 ± 0.33 (100)	112.58 ± 0.13 (77)	58.48 ± 0.05 (40)
Amino acid	M	34.18 ± 0.20 (100)	35.88 ± 0.27 (105)	42.72 ± 0.31 (125)
	L	21.28 ± 0.23 (100)	24.89 ± 0.41 (117)	30.00 ± 0.37 (141)
	G	36.00 ± 0.78 (100)	42.84 ± 0.01 (119)	52.56 ± 0.02(146)
DNA	M	148.40 ± 0.66 (100)	112.78 ± 0.13 (76)	86.07 ± 0.13 (58)
	L	146.02 ± 0.66 (100)	116.81 ± 0.06 (80)	99.29 ± 0.11 (68)
	G	143.01 ± 0.77 (100)	111.54 ± 0.41 (78)	81.51 ± 0.25 (57)
RNA	M	100.00 ± 0.28 (100)	80.00 ± 0.01 (80)	65.00 ± 0.03 (65)
	L	105.00 ± 0.58 (100)	94.50 ± 0.15 (90)	73.50 ± 0.15 (70)
	G	0.563 ± 0.011 (100)	0.439 ± 0.38 (78)	0.337 ± 0.26 (60)
Protease	M	0.544 ± 0.011 (100)	0.734 ± 0.041 (135)	0.821 ± 0.013 (151)
	L	0.644 ± 0.016 (100)	0.792 ± 0.013 (123)	0.888 ± 0.012 (138)
	G	0.600 ± 0.010 (100)	0.780 ± 0.011 (130)	0.900 ± 0.011 (150)
Acid phosphatase	M	0.211 ± 0.011 (100)	0.063 ± 0.09 (30)	0.569 ± 0.014 (27)
	L	0.291 ± 0.010 (100)	0.101 ± 0.10 (35)	0.072 ± 0.008 (25)
	G	0.265 ± 0.015 (100)	0.084 ± 0.05 (32)	0.060 ± 0.015 (23)
Alkaline phosphatase	M	0.444 ± 0.003 (100)	0.182 ± 0.003 (41)	0.115 ± 0.002 (26)
	L	0.400 ± 0.021 (100)	0.148 ± 0.006 (37)	0.092 ± 0.007 (22)
	G	0.395 ± 0.010 (100)	0.185 ± 0.005 (47)	0.122 ± 0.003 (31)

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. M = Muscle; L = Liver; G = Gonad.

In laboratory conditions, the LC_{50} values of the tested plant against the freshwater fish *Catla catla* was 88.80 mg/L (24 h) in acetone leaf extracts of *Thevetia peruviana* plant and 99.43 mg/L (24 h) in acetone bark extracts of *Thevetia peruviana* plant. In a laboratory conditions, the toxicity of *Thevetia peruviana* plant acetone leaf extracts was 529.38 mg/L (24 h) and the toxicity of *Thevetia peruviana* plant acetone bark extracts was 591.78 mg/L (24 h) against the fish *Catla catla* in cemented pond condition.

Obviously under cemented pond conditions the toxicity of tested plants was reduced. The reason for this reduced toxicity could be a sand particle adsorption or acceleration of the toxicant degradation process by temperature[42]. A similar trend was reported by Perchbacher and Sarkar[43] in which the toxicity persistence of *Masea ramentacea* and tea seed cake was short and fish could be stocked into ponds 4 days after applying the pesticides. The potential for using *Masea ramentacea* as a substitute for tea seed cake for killing predatory fish in freshwater has been shown. However, the effective concentration must be determined against the predatory air-breathing fish, such as *Clarias* sp. *Ophicephalus striatus* and *Anabas testudineus* that are generally more tolerant of toxicants than other fishes[43].

Statistical analysis of the data on toxicity brings out several important points. The X^2 test for goodness of fit (heterogeneity) demonstrated that the mortality counts were not found to be significantly heterogeneous and other variables, e.g. resistance etc. do not significantly affect the LC_{50} values, as these were found to lie within the 95% confidence limits. The dose mortality graphs exhibit steep values[44]. The steepness of the slope line indicates that there is a large increase in the mortality of freshwater fish *Catla catla* with relatively small increase in the concentration of the toxicant. The slope is, thus an index of the susceptibility of the non-target animal to the plant origin pesticides used.

The depletion of protein fraction in various tissues may have been due to their degradation and possible utilization of degraded products for metabolic purposes. Mommensen and Walsh[45] reported that proteins are mainly involved in the

Table IV. Changes in total protein, total free amino acids, nucleic acid (DNA & RNA) (g/mg) level and activity of protease (mol of tyrosine equivalents/mg protein/h) and acid and alkaline phosphatase (mol substrate hydrolysed/30 min/mg protein) in muscle, liver and gonad tissues of freshwater fish *Catla catla* after exposure to sub-lethal doses of 40% and 80% of LC_{50} (39.77 mg/L and 79.54 mg/L) of acetone bark extract of *Thevetia peruviana* plant after 24h in laboratory conditions.

Parameter	Tissue	Control	40% of LC_{50} (24h); 39.77 mg/L	80% of LC_{50} (24h); 79.54 mg/L-
Protein	M	151.20 ± 0.65 (100)	46.87 ± 0.27 (31)	45.36 ± 0.43 (90)
	L	138.30 ± 0.57 (100)	76.06 ± 0.25 (55)	89.89 ± 0.16 (65)
	G	153.00 ± 0.47 (100)	76.30 ± 0.11 (50)	76.58 ± 0.03 (50)
Amino acid	M	35.20 ± 0.21 (100)	34.49 ± 0.23 (103)	39.42 ± 0.28 (112)
	L	28.23 ± 0.28 (100)	29.65 ± 0.33 (105)	32.18 ± 0.31 (114)
	G	36.09 ± 0.80 (100)	38.89 ± 0.01 (108)	45.73 ± 0.01(127)
DNA	M	148.32 ± 0.65 (100)	97.89 ± 0.11 (66)	86.02 ± 0.11 (58)
	L	140.01 ± 0.55 (100)	105.00 ± 0.03 (75)	91.00 ± 0.14 (65)
	G	131.02 ± 0.71 (100)	104.81 ± 0.37 (80)	68.13 ± 0.23 (52)
RNA	M	100.00 ± 0.20 (100)	73.00 ± 0.03 (73)	60.00 ± 0.01 (60)
	L	105.03 ± 0.27 (100)	89.28 ± 0.14 (85)	73.52 ± 0.01 (70)
	G	58.20 ± 0.78 (100)	68.74 ± 0.33 (70)	56.85 ± 0.25 (58)
Protease	M	0.431 ± 0.009 (100)	0.425 ± 0.038 (142)	0.603 ± 0.012 (140)
	L	0.548 ± 0.003 (100)	0.548 ± 0.013 (100)	0.739 ± 0.011 (135)
	G	0.590 ± 0.010 (100)	0.619 ± 0.010 (105)	0.808 ± 0.010(137)
Acid phosphatase	M	0.300 ± 0.011 (100)	0.084 ± 0.008 (28)	0.078 ± 0.014 (26)
	L	0.273 ± 0.015 (100)	0.101 ± 0.010 (37)	0.062 ± 0.007 (23)
	G	0.195 ± 0.011 (100)	0.058 ± 0.013 (30)	0.040 ± 0.013 (21)
Alkaline phosphatase	M	0.300 ± 0.003 (100)	0.120 ± 0.004 (40)	0.054 ± 0.001 (18)
	L	0.395 ± 0.002 (100)	0.150 ± 0.007 (38)	0.063 ± 0.005 (16)
	G	0.402 ± 0.012 (100)	0.168 ± 0.005 (42)	0.084 ± 0.003 (21)

Details are same as given in Table III.

architecture of the cells, which is the chief source of nitrogenous metabolism and during chronic period of stress they are also a source of energy.

The quantity of proteins depends on the rate of protein synthesis or its degradation. It also affected due to impaired incorporation of amino acids into polypeptide chains[46]. 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.

The increase in free amino acid level suggests tissues damage probably due to the increased proteolytic activity under acetone leaf and bark extract toxic stress. However, the elevated levels of free amino acids can be utilised for energy production by feeding them in to the TCA cycle (tri chloro acetic acid) through aminotransferase reaction. The increase in the levels of free amino acid can also be attributed to the synthesis of amino acids in addition to their elevation by protein hydrolysis. A third possibility for increased free amino acid level might be their increase due to transamination and amination of keto acids[47].

Vorbrodt[48] has reported that phosphatase is an important enzyme of animal metabolism, which plays an important role in the transport of metabolites to cross the membranes. Since, acetone leaf and bark extracts used in the present study may also have anti-phosphatase activity. So, the reduction in proteins level may be due to the inhibition of acid and alkaline phosphatase activity, as it plays an important role in protein synthesis[49].

Fish under stress mobilizes triglycerides and protein to meet an increased demand for energy resulting from increased physical activity, biotransformation and excretion of xenobiotics[50]. This suggests there was effective utilization of amino acids for metabolic processes in exposed fish. But stress generally is known to elevate amino transferase activity[51]. Under stress conditions, fish need more energy resulting in higher demand for carbohydrate and their precursors to

keep the glycolytic pathway and TCA cycles at sustained levels[17]. Depressed acid and alkaline phosphatase activity suggest a decrease in energy demand, metabolic pathway and amino acids. Decrease in acid and alkaline phosphatase activity in the organs and muscle tissue could be due to a fall in the synthesis of glycogen caused by lowered metabolic demands and also to electrolytic imbalance caused by tissue over hydration[52].

In conclusion, the different solvents of leaf and bark extracts of *Thevetia peruviana* plant may be used as potent source of piscicidal activity. Because plant products are less expensive, easily available and easily soluble in water, they may be preferred over commercial pesticides.

Acknowledgements

One of the Authors (Sunil Kumar Singh) is thankful to Ministry of Science and Technology, Department of Science and Technology, New Delhi, Govt. of India, for award of the SERC Fast Track Young Scientist Fellowship (No. SR/FT/L-44/2006 dated 18.06.2007) for financial assistance.

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