Afr. J. Trad. CAM (2005) **2 (3):** 326 - 336

Research Paper

Afr. J. Traditional,
Complementary and
Alternative Medicines
www.africanethnomedicines.net

ISSN 0189-6016©2005

THE CONTRIBUTION OF THE ANTICHOLINESTERASE ACTIVITY OF *PEDIALANTHUS TITHYMALOIDE* TO ITS MOLLUSCICIDAL ACTIVITY

Sudhanshu Tiwari*, Sunil K.Singh, Ajay Singh
Natural Product Laboratory, Department of Zoology
D.D.U. Gorakhpur University, Gorakhpur – 273 009 (U.P.), INDIA
Email: sudhansh4@rediffmail.com

Abstract

Experiments were conducted to evaluate the molluscicidal and anticholinesterase activity of aqueous leaf and stem-bark extract of *Pedialanthus tithymaloide* (Family-Euphorbiaceae) *Lymnaea* (Radix) *acuminata* (Lamarack) and *Indoplanorbis exustus* (Deshyas) – intermediate hosts of endemic schistosomiasis and fascioliasis diseases in cattle and livestock. The toxic effect of both extracts was time- as well as dose-dependent. There was a significant negative correlation between LC values and exposure periods observed, thus the LC_{10} values of *P. tithymaloide* leaf decreased from 24.7 mg/L (24 h) to 15.4 mg/L (96 h); LC_{50} decreased from 69.4 mg/L (24 h) to 27.4 mg/L (96 h); LC_{90} decreased from 194.6 mg/L (24 h) to 48.9 mg/L (96 h) against *L. acuminata* and LC_{10} values of *P. tithymaloide* leaf decreased from 5.5 mg/L (24 h) to 0.6 mg/L (96 h); LC_{50} decreased from 35.8 mg/L (24 h) to 4.3 mg/L (96 h); LC_{90} decreased from 233.1 mg/L (24 h) to 32.1 mg/L (96 h) against *Indoplanorbis exustus.* Similar trend was also observed for the aqueous stem-bark extract of *P. tithymaloide*. Sub-lethal exposure at 24h and 96h of the aqueous leaf and stem-bark extract of *P. tithymaloide* caused significant ($P < 0.05$) time- and dose-dependent inhibition of the activity of enzyme acetylcholinesterase (AChE) in the nervous and hepatopancrease tissues of *L. acuminata* after 24 h or 96 h exposure periods. Withdrawal study also shows that there was a significant recovery in the acetylcholinesterase activity in both the tissues of snail after the 7^{th} day of the withdrawal of treatment. Thus, the aqueous extract of *P. tithymaloide* leaf and stem-bark are environmentally safe candidate molluscicides for controlling vector snails of schistosomiasis and fascioliasis.

Key words: Schistosomiasis and Fascioliasis, *Pedialanthus tithymaloide,* Molluscicidal, Anticholinesterase activity

Introduction

Many aquatic snails act as vectors for larvae of parasitic treamatodes, which also spend part of their life in water and thereby causing a number of diseases. Two disease carried by

Afr. J. Trad. CAM (2005) **2 (3):** 326 - 336

aquatic snails, schistosomiasis and fascioliasis cause immense harm to man and his domestic animals (Bali et al., 1986; Singh and Agarwal, 1981).

Fascioliasis, caused by trematode, *Fasciola hepatica* and *Fasciola gigantica*, is a very common disease of sheep, cattle, goat and other herbivorus animals throughout the world (Singh and Agarwal, 1981; Tiwari et al., 2004). Schistosomiasis, caused by *Schistosoma*, is a devastating disease of mankind second only to malaria in its prevalence (Jobin, 1973). Both are transmitted by freshwater intermediate host snails, *Lymnaea acuminata* and *Indoplanorbis exustus* (Hyman, 1970).

A sure way to tackle the problem of schistosomiasis and fascioliasis is to destroy the carrier snails and remove an essential link in the life cycle of both trematodes. This can be achieved by using synthetic or plant origin molluscicides (Singh et al., 1996). A number of synthetic pesticides were used for controlling various species of gastropods pest in aquatic environment (Singh et al., 1993a; 1993b). But heavy application of these pesticides has now become problem of the living organism including human beings (Dua et al., 1996; Walliszeuski et al., 1998). Control of snail vector population through plant origin pesticides is a very effective and new tool of Integrated Vector Managment Programme. Botanical molluscicides can be preferred to synthetic pesticides due to their biodegradability, low cost, safe and effective molluscicidal effect (Marston and Hostettmann, 1985).

The plant, *Pedialanthus tithymaloide* (Family-Euphorbiaceae) locally known as Dudhi in India, is a common medicinal plant of India (Satyavati and Gupta, 1987).

In this study, the molluscicidal activity of the leaf and stem bark of *Pedialanthus tithymaloide* was observed against target snails *Lymnaea acuminata* and *Indoplanorbis exustus* and their short and long-term effects on acetylcholinesterase enzyme activity in nervous and hepatopancrease tissues of *Lymnaea acuminata*.

Materials and Methods

Adult *Lymnaea acuminata* (2.5±0.5 cm); *Indoplanorbis exustus* (1.05±0.05 cm) were collected locally from the Ramgarh lake of Gorakhpur district and used as test animal. The collected animals were maintained in glass aquaria containing de-chlorinated tap water for acclimatization to laboratory conditions. The water in aquaria was aerated continuously and changed at every 24 hour. Any dead animal(s) was removed as soon as possible from test container to prevent water fouling.

Pedialanthus tithymaloide was collected locally from botanical garden of D.D.U. Gorakhpur University Gorakhpur and identified by Prof. S.K. Singh, Department of Botany, D.D.U. Gorakhpur University, Gorakhpur, India, where a voucher (voucher number 1031) specimen is deposited.

Preparation of aqueous extract

After collection the leaf and stem bark of *P. tithymaloide* was thoroughly washed with water, dried in incubator at 37°C and powdered. The dried powder was stored in airtight desiccators, for further use. The dried powder was extracted in distilled water for

Afr. J. Trad. CAM (2005) **2 (3):** 326 - 336

one hour, centrifuged at 1000 g for 10 minutes, and only the supernatant was used for treatment.

Toxicity experiment

Toxicity experiment was performed by using method of Singh and Agarwal (1988); ten snails were kept in glass aquaria containing 3 L de-chlorinated tap water. The snails were exposed for 96 h to four different concentrations of *Pedialanthus tithymaloide* leaf and stem bark. Six aquaria were set up for each concentration. Control animals were reared in similar condition without treatment. Behavioural responses of snails were observed for up to 2 h from the beginning of the treatment. Mortality was recorded at every 24 h, 48 h, 72 h and 96 h. LC_{10}, LC_{50} and LC_{90} values, upper and lower confidence limits, slope value, t-ratio, g-factor and heterogeneity were calculated according to probit log analysis method by using POLO computer programme of Russel et al. (1977). Experimental conditions of water was atmospheric temperature 31.0±1.0°C; water temperature 28.5± 0.5°C; pH 7.1 to 7.3; dissolved oxygen 7.5 to 8.0 mg/L; free carbon dioxide 4.0 to 4.5 mg/L; bicarbonate alkalinity 102.0 to 104.0 mg/L (APHA/WEF, 1998).

Treatment protocol for measuring Acetylcholinesterase enzyme activity

Acetylcholinesterase inhibition was measured in the nervous and hepatopancrease tissues of *Lymnaea acuminata*, after exposure to 27.7 mg/L and 55.5 mg/L (40% and 80% of 24 h LC_{50}) and 11.0 mg/L and 21.9 mg/L (40% and 80% of 96 h LC_{50}) of *Pedialanthus tithymaloide* leaf and 13.8 mg/L and 27.6 mg/L (40% and 80% of 24 h LC_{50}) and 2.6 mg/L and 5.2 mg/L (40% and 80% of 96 h LC_{50}) of *Pedialanthus tithymaloide* stem bark. Control animals were kept in similar condition without any treatment. After 24 h or 96 h exposure to 40% and 80% doses of 24 h or 96 h LC_{50} of *P. tithymaloide* leaf and stem bark, acetylcholinesterase (AChE) activity was measured in the nervous and hepatopancrease tissues of snail by the method of Ellman et al. (1961), as modified by Singh and Agarwal (1982). Homogenate (50 mg/ml, W/V) was prepared in 0.1 M-phosphate buffer, pH 8.0 for 5 min in an ice bath and centrifuged at 1000 g for 30 min at 4 ^{0}C. The enzyme containing supernatant (0.05 ml) was pipetted to a cuvette. To this was added 10 µl ($5x10^{-4}$M) of freshly prepared acetylthiocholine iodide solution in distilled water as substrate, 1.45 ml of buffer (pH 8.0) and 0.05 ml of the chromogenic agent, 5:5 dithio-bis-nitrobenzoate (DTNB). The change in optical density at 412 nm, caused by the enzymatic reaction, was monitored for 3 min at 25 ^{0}C. Protein estimation was done by the method of Lowry et al. (1951). Enzyme activity has been expressed as µ mol 'SH' hydrolyzed/min./mg protein.

Withdrawal experiment

In order to see the effect of 7th day of withdrawal of treatment, *Lymnaea acuminata*, were exposed at sub-lethal doses of 55.5 mg/L (80% of the 24 h LC_{50}) for 24 h exposure period and 21.9 mg/L (80% of the 96 h LC_{50}) for 96 hour exposure period of *P. tithymaloide* leaf

Afr. J. Trad. CAM (2005) **2 (3):** 326 - 336

and at sub-lethal doses of 27.6 mg/L (80% of the 24 h LC_{50}) for 24 hour exposure period and 5.2 mg/L (80% of the 96 h LC_{50}) for 96 hour exposure period of *P. tithymaloide* stem bark.

After termination of experiments, 50% of the test animals were sacrificed and the activity of acetylcholinesterase enzyme was measured in nervous and hepatopancrease tissues. The other half was transferred to fresh water free from any treatment, which was changed every 24 h for the next six days. Following this, the activity of acetylcholinesterase enzyme was measured in both nervous and hepatopancrease tissues. Control animals were kept in similar condition without any treatment.

Each experiment was replicated at least six times and data have been expressed as mean± SE. Test of correlation, two-way analysis of variance and Student's t-test were applied for locating significant differences (Sokal and Rohlf, 1973).

Results

Effect on behavioral Changes

Exposure to leaf and stem bark extracts of *Pedialanthus tithymaloide* caused significant behavioural changes in the freshwater snails *L. acuminata* and *I. exustus.* Behavioural changes appear within 5 to 15 min of exposure. The initial 30-40 min. was a period of hyperactivity.

Toxicity

Table 1: Toxicity (LC_{10}, LC_{50} and LC_{90}) of aqueous leaf extract of *Pedialanthus tithymaloide* against *Lymnaea acuminata* at different time intervals

Exposure periods	Effective dose (mg/L)	Limits (mg/L)		Slope value	'g' factor	't' ratio	Heterogeneity
		LCL	UCL				
24h	LC_{10}=24.7	9.1	35.7	2.9±0.7	0.2	4.1	0.1
	LC_{50}=69.4	58.6	79.2				
	LC_{90}=194.6	140.0	475.0				
48h	LC_{10}=19.8	7.1	29.8	3.4±0.8	0.2	4.4	0.1
	LC_{50}=47.2	32.8	55.6				
	LC_{90}=112.3	94.5	166.2				
72h	LC_{10}=12.9	1.2	23.9	3.8±0.2	0.4	3.3	0.4
	LC_{50}=28.1	8.1	39.3				
	LC_{90}=61.2	48.6	72.4				
96h	LC_{10}=15.4	1.7	25.9	5.1±0.9	0.06	2.6	0.4
	LC_{50}=27.4	7.8	37.3				
	LC_{90}=48.9	34.1	56.1				

After some time they started crawling on each other. As the poison took over, there was a muscular twitching and the snails become spirally twisted. Prior to death, there was complete withdrawal of the body inside the shell. At the highest doses mortality occurred

Afr. J. Trad. CAM (2005) **2 (3):** 326 - 336

in about 100-150 min. of exposure period. Even with the weaker doses the sequences of events leading to death were the same.

The LC values of aqueous extract of *P. tithymaloide* leaf and stem bark on snails for periods ranging from 24 hour to 96 h are given in Tables 1 - 4.

The toxicity was both time as well as dose dependent. There was a significant negative correlation between LC values and exposure periods. Thus with an increase in exposure time, the LC_{50} values of leaf extract decreased from 69.4 mg/L (24 h) > 47.2 mg/L (48 h) > 28.1 mg/L (72 h) > to 27.4 mg/L (96 h) in case of *L. acuminata* (Table 1). LC_{50} values were also decreased from 35.8 mg/L (24 h) > 23.8 mg/L (48 h) > 13.4 mg/L (72 h) > to 4.3 mg/L (96 h) in case of *I. exustus* (Table 3).

Table 2: Toxicity (LC_{10}, LC_{50} and LC_{90}) of aqueous stem bark extract of *Pedialanthus tithymaloide* against *Lymnaea acuminata* at different time intervals.

Exposure periods	Effective dose (mg/L)	Limits (mg/L)		Slope value	'g' factor	't' ratio	Heterogeneity
		LCL	UCL				
24h	LC_{10}=2.7	0.4	6.2	1.2±0.3	0.2	4.4	0.3
	LC_{50}=34.5	24.3	50.1				
	LC_{90}=435.8	189.3	3713.1				
48h	LC_{10}=2.2	0.4	4.2	1.8±0.3	0.2	6.1	0.3
	LC_{50}=11.1	5.9	15.6				
	LC_{90}=56.5	40.3	105.6				
72h	LC_{10}=1.9	0.6	3.7	2.0±0.3	0.1	6.0	0.6
	LC_{50}=8.6	5.0	11.9				
	LC_{90}=38.6	29.6	56.7				
96h	LC_{10}=1.3	0.2	3.0	1.9±0.4	0.2	5.3	0.2
	LC_{50}=6.4	2.7	9.9				
	LC_{90}=31.7	23.7	49.2				

Similar trend was also observed in stem bark extract. In this treatment LC_{50} decreased from 34.5 mg/L (24 h) > 11.1 mg/L (48 h) > 8.6 mg/L (72 h) > to 6.4 mg/L (96 h) against *L. acuminata* (Table 2). In case of *I. exustus* LC_{50} were decreased from 28.2 mg/L (24 h) > 20.5 mg/L (48 h) > 17.2 mg/L (72 h) > to 12.7 mg/L (96 h) (Table 4).

The slope values given in (Tables 1 - 4) were steep and the separate estimation of LC based on each of the six replicates was found to be within the 95% confidence limits of LC doses. The't' ratio was greater than 1.96 and heterogeneity factor was less than 1.0. The 'g' value was less than 0.5 at all the probability levels.

Effect on Acetylcholinesterase activity

Table 5, shows that treatment of snail *L. acuminata* with sub lethal doses of aqueous extract of leaf and stem bark of *P. tithymaloide* for the exposure period of 24 h or 96 h, which caused significant ($P<0.05$) inhibition in acetylcholinesterase activity in the nervous and hepatopancrease tissues.

Afr. J. Trad. CAM (2005) **2 (3):** 326 - 335

Table 3: Toxicity (LC_{10}, LC_{50} and LC_{90}) of aqueous leaf extract of *Pedialanthus tithymaloide* against *Indoplanorbis exustus* at different time intervals.

Exposure periods	Effective dose (mg/L)	Limits (mg/L)		Slope value	'g' factor	't' ratio	Heterogeneity
		LCL	UCL				
24h	LC_{10}=5.5	1.1	10.3	1.6±0.3	0.2	5.7	0.6
	LC_{50}=35.8	25.4	52.5				
	LC_{90}=233.1	119.6	1273.5				
48h	LC_{10}=4.0	0.8	7.8	1.6±0.3	0.2	6.0	0.6
	LC_{50}=23.8	15.4	32.7				
	LC_{90}=143.4	83.5	514.8				
72h	LC_{10}=3.5	1.6	5.5	2.2±0.3	0.07	7.1	0.8
	LC_{50}=13.4	9.7	16.8				
	LC_{90}=51.1	40.2	73.2				
96h	LC_{10}=0.6	0.0	1.9	1.5±0.3	0.2	4.3	0.5
	LC_{50}=4.3	1.0	7.2				
	LC_{90}=32.1	22.8	53.4				

Thus 24 h exposure to 27.7 mg/L and 55.5 mg/L of *P. tithymaloide* leaf reduced the AChE activity up to 81 and 70% of controls in nervous tissue; up to 90 and 85% of controls in hepatopancrease tissue of *L. acuminata*. It was observed that 96 h exposure reduced the AChE activity up to 72 and 56% of controls in nervous; ; up to 80 and 70% of controls in hepatopancrease tissue of *L. acuminata* while 24 h exposures to 13.8 mg/L and 27.6 mg/L of *P. tithymaloide* stem bark inhibited the AChE activity up to 66 and 48% of controls in nervous tissues ; up to 76 and 60% of controls in hepatopancrease tissue of *L. acuminata* (Table 5). Similarly, 96 h exposure inhibited the AChE activity up to 52 and 34% of controls in nervous tissue; up to 60 and 50% of controls in hepatopancrease tissue of *L. acuminata* (Table 5). Analysis of variance demonstrated that the inhibition of AChE activity was both time and dose dependent ($P < 0.05$).

After seven days withdrawal experiment of after exposure to sub-lethal doses of 55.5 mg/L of leaf and 27.6 mg/L of stem bark for 24 h exposure period and for 96 h exposure period at sub-lethal doses of 21.9 mg/L of leaf and 5.2 mg/L of stem bark (Table 5) shows, there was highly significant ($P < 0.05$) recovery observed in the acetylcholinesterase enzyme activity in both nervous and hepatopancrease tissues of snail *L. acuminata* (Figure 1).

Discussion

The nature and rapid onset of these behavioral responses of snails indicates that both leaf and stem bark extracts perhaps contain some neurotoxins, which might be active at neuromuscular system of the exposed animals. Similar behavioral response was also observed in case of *Euphorbia royleana*, *Euphorbia antisyphliatica* and *Jatropha*

Afr. J. Trad. CAM (2005) **2 (3):** 326 - 336

gossypifolia on snail *L. acuminata* (Singh and Agarwal, 1990; Tiwari et al., 2004). The behavioral changes are indeed reminiscent to the response of snails to organophosphorus and carbamate pesticides (Singh and Agarwal, 1981).

Table 4: Toxicity (LC_{10}, LC_{50} and LC_{90}) of aqueous stem bark extract of *Pedialanthus tithymaloide* against *Indoplanorbis exustus* at different time intervals.

Exposure periods	Effective dose (mg/L)	Limits (mg/L)		Slope value	'g' factor	't' ratio	Heterogeneity
		LCL	UCL				
24h	LC_{10}=11.5	8.3	14.0	3.3±0.5	0.07	7.2	0.6
	LC_{50}=28.2	24.9	32.5				
	LC_{90}=69.1	54.1	104.1				
48h	LC_{10}=10.2	8.0	12.1	4.2±0.5	0.04	9.1	0.8
	LC_{50}=20.5	18.4	22.7				
	LC_{90}=41.2	35.7	50.6				
72h	LC_{10}=9.2	7.3	10.8	4.7±0.5	0.04	9.4	0.9
	LC_{50}=17.2	15.5	19.0				
	LC_{90}=32.3	28.5	38.2				
96h	LC_{10}=7.0	5.3	8.4	5.0±0.6	0.05	8.5	0.5
	LC_{50}=12.7	11.1	14.2				
	LC_{90}=23.1	20.4	27.2				

No such behavioral symptoms and death occurred in control groups indicating that no factor other than plant moieties was responsible for altered behavior and mortality in the experimental animals.

Mortality caused by stem bark and latex showed a clear significant positive correlation with dose of the extract. The positive correlation between dose and mortality in leaf and stem bark may be explained by assuming that increase in the concentration of toxicant in aquarium water resulted in more intake or entry of toxicant in the snail body. Stability (life span) of active moiety of pesticides in environment and the rate of their detoxification in animal body also alter the mortality and exposure period relationship (Matsumura, 1985). Increased mortality with increase in exposure periods could be affected by several factors. For example, uptake of active moiety is time dependent, which leads to progressive increase in the uptake of drug and its effect on snail body (Goodmann et al., 1985; Singh and Agarwal, 1988; 1993a; 1993b). Statistical analysis of the data on toxicity brings out several important points. The χ^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 values as these were found to lie within 95% confidence limits. The steepness of the slope line indicates that there is a large increase in the mortality of snail with relatively small increase in the concentration of the toxicant. A steep slope is also indicative of rapid absorption and onset of effects. Since the LC values lay within 95% confidence limits, it is obvious that in replicate test of random samples, the concentration response lines would fall in the same range (Rand and Petrocelli, 1988).

Afr. J. Trad. CAM (2005) **2 (3):** 326 - 336

Table 5: Inhibition of AChE activity in nervous and hepatopancrease tissues of freshwater snail *Lymnaea acuminata* exposed to 24 h or 96 h to 40% and 80% LC_{50} of aqueous extract of *Pedialanthus tithymaloide* leaf, stem bark, and recovery of AChE activity after 7th day withdrawal experiment of treatment.

Aqueous Leaf Extract				
Tissue	**Control**	**24 hour Exposure period**		
		40% of 24h LC_{50} (27.7 mg/L)	**80% of 24h LC_{50}** (55.5 mg/L)	**7th day after withdrawal** (55.5 mg/L)
Nervous	0.081±0.001(100)	0.066±0.002*(81)	0.057±0.005*(70)	0.077±0.007**(95)
Hepatopancrease	0.066±0.004(100)	0.059±0.003*(90)	0.056±0.001*(85)	0.065±0.005**(98)
Tissue	**Control**	**96 hour Exposure period**		
		40% of 96h LC_{50} (11.0 mg/L)	**80% of 96h LC_{50}** (21.9 mg/L)	**7th day after withdrawal** (21.9 mg/L)
Nervous	0.078±0.003(100)	0.056±0.009*(72)	0.044±0.001*(56)	0.070±0.003**(90)
Hepatopancrease	0.062±0.002(100)	0.050±0.005*(80)	0.043±0.007*(70)	0.060±0.001**(96)
Aqueous Stem Bark Extract				
Tissue	**Control**	**24 hour Exposure period**		
		40% of 24h LC_{50} (13.8 mg/L)	**80% of 24h LC_{50}** (27.6 mg/L)	**7th day after withdrawal** (27.6 mg/L)
Nervous	0.081±0.001(100)	0.053±0.003*(66)	0.039±0.004*(48)	0.075±0.002**(92)
Hepatopancrease	0.066±0.004(100)	0.050±0.006*(76)	0.040±0.005*(60)	0.063±0.008**(95)
Tissue	**Control**	**96 hour Exposure period**		
		40% of 96h LC_{50} (2.6 mg/L)	**80% of 96h LC_{50}** (5.2 mg/L)	**7th day after withdrawal** (5.2 mg/L)
Nervous	0.078±0.003(100)	0.041±0.006*(52)	0.027±0.008*(34)	0.069±0.005**(89)
Hepatopancrease	0.062±0.002(100)	0.037±0.001*(60)	0.031±0.005*(50)	0.056±0.009**(91)

- Values are mean ± SE of six replicates.
- Values in parentheses are % change with control taken as 100%.
- Data were analyzed through student's test.
- *, Significant (P< 0.05), when treated groups were compared with controls.
- **, Significant (P< 0.05), when withdrawal groups were compared with treated groups.

Afr. J. Trad. CAM (2005) **2 (3):** 326 - 336

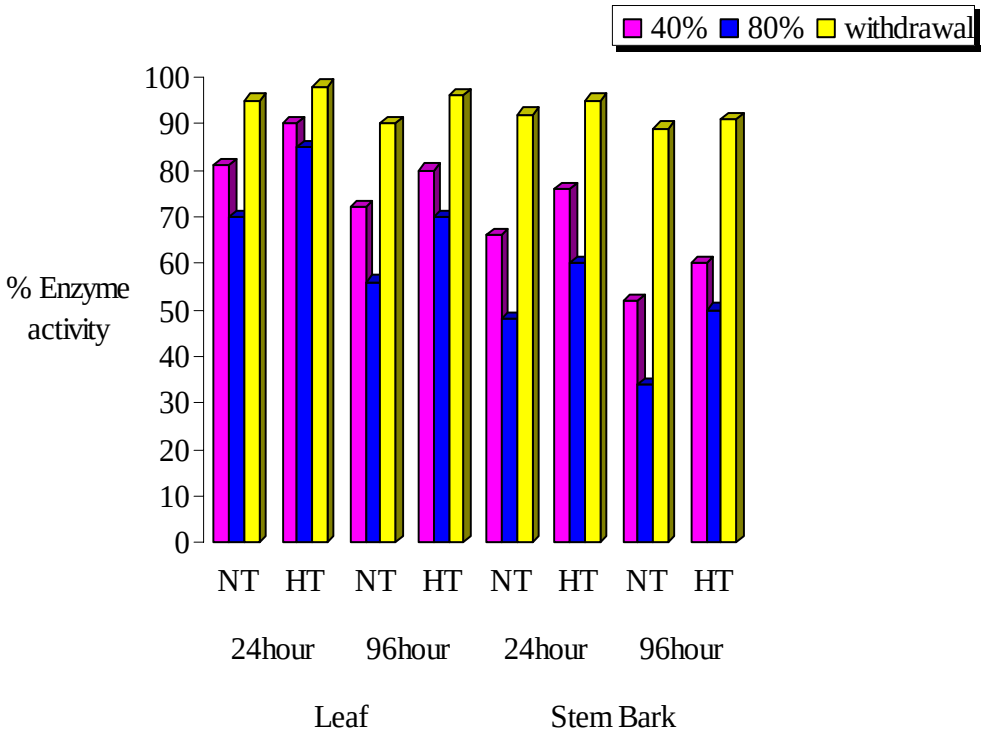

Figure 1: Inhibition of % AChE activity in nervous (NT) and hepatopancrease (HT) tissues of snail *Lymnaea acuminata* exposed to 24 h or 96 h to 40% and 80% LC_{50} of aqueous leaf, stem bark extract of *Pedialanthus tithymaloide* and their recovery after 7^{th} day withdrawal experiment of treatments.

Animal behaviour is a neurotropically regulated phenomenon, which is mediated by neurotransmitter substances such as AChE (Bullock et al., 1977). The present study shows that aqueous extract of leaf and stem bark of *P. tithymaloide* caused significant time and dose dependent inhibition in the activity of enzyme acetylcholinesterase in the snails. This enzyme present in synaptic regions and mediates transmission of impulses by breaking acetylcholine into acetic acid and choline (O'Brien, 1976). The acetylcholine at neural and neuromotar regions upon accumulation causes 'hyper-excitability' (Kabeer Ahammad Sahib and Ramana Rao, 1980), which in turn might also influence behaviour pattern of animals. Plants belonging to family Euphorbiaceae have significant anti-AChE activity (Tiwari et al., 2004). Kinghorn and Evans (1975) reported that the pharmacological action of Euphorbious plants is due to presence of a group of diterpen phorbal esters, which are reported to promote activity of the enzyme protein kinase C (Aitken, 1987; Evans and Edwards, 1987), which specifically phosphorylates serine and threonine residues in proteins (Aitken, 1987). Since the active site of enzyme contains a serine residue (Koelle, 1975), it is possible that the inhibition of this enzyme is due to the phosphorylation of the active site. So altered behaviors and snail's mortality in

Afr. J. Trad. CAM (2005) **2 (3):** 326 - 336

present study is due to the inhibition of enzyme acetylcholinesterase activity in both tissues of the snails.

Thus we can conclude that *P. tithymaloide* leaf and stem-bark extracts are highly toxic to *L. acuminata* and *I. exustus* snails and can be developed as molluscicides for controlling snail's populations. The reversibility of the action of both extracts despite the high toxicity would be an added advantage in their use.

References

1. APHA/AWWA/WEF, (1998). Standard methods for the examination of water and wastewater. 20th edition, American public health association, New York, USA.
2. Aitken, A., (1987). The activation of protein kinase C by dephnane, ingenane and tigliane diterpenoid esters. Botanical J. Linnaean Soci., **94**: 247-263.
3. Bali, H.S., Singh, S., Sharma, S., (1986).The distribution and ecology of vectors snails of Punjab. Indian J. Ecol., **13**:31-37.
4. Bullock T.H., Orkand, R.., Grinnella, A., (1977). In: Introduction to nervous system, (D. Kennedy Ed.) W.H. Freeman and Company, San Francisco.
5. Dua, V.K., Pant, C.S., Sharma, V.P., Pathak, G.K., (1998). Determination of HCH and DDT in finger-prick whole blood dried on filter paper and its field application for monitoring concentrations in blood. Bull. Environ. Contam. Toxicol., **56:** 50-57.
6. Ellman, G.L., Courtney, K.D., Andres, Jr. V., Featherstone, R.M., (1961). A new and rapid Colorimetric determination of acetylcholinesterase activity. Biol. Pharmacol., **7:** 88.
7. Evans, F.J., Edwards, M.C. (1987). Activity correlation in the phorbol ester series. Botanical J. Linnaean Soci., **94:** 231-246.
8. Goodmann L.S., Gillman, A.G., Rall, T.W., Murad, F., (1985). The pharmacological basis of therapeutics. Macmillan Publishing Company, New York.
9. Hyman, L.H., (1970). The invertebrate. Mollusca I. Vol. VI., Mc Graw Hill, New York.
10. Jobin, W.R., (1973). Environmental control of bilharizia snail in small reservoir. J. Irrig. Drain. Div., **99:**365-373.
11. Kabeer Ahammad Sahib, I., Ramana Rao, K.V., (1980). Toxicity of malathion to the freshwater fish, *Tilapia mossambica*. Bull. Environ. Contam. Toxicol.**, 24:**870-874.
12. Kinghorn, A.D., Evans, F.J., (1975). A biological screen of selected species of the genus Euphorbia for skin irritant effect. Planta Medica, **28:** 325-335.
13. Koelle, G.B., (1975). Anticholinesterase agents. In: The Pharamacological basis of therapeutics (L.S. Goodman and A. Gillman Eds.) Macmillan Publishing Co., New York, pp. 404-466.
14. Lowry, O.H., Rosenbrough, N.J., Farr A.L., Randell, R.J., (1951). Protein measurement with foline phenol reagent. J. Biolo. Chem., **193:** 265 – 275.
15. Marston, A., Hostettmann, K., (1985). Plant molluscicides. Phytochemistry, **24:** 639-652.

16. Matsumura, F., (1985). Toxicity of Insecticides. 2nd edition, Plenum Press, New York. pp. 47, 74, 78-80, 163-165, 446.
17. O'Brien, R.D., (1976). Acetylcholinesterase and its inhibition. In; Insecticide Biochemistry and Physiology (C.F. Wilkinson Ed.) Plenum Press, New York. pp. 271-293.
18. Rand, G.M., Petrocelli, S.R., (1988). Fundamentals of aquatic toxicology. Hemisphere Publishing Corporation, New York, pp. 415.
19. Russel, R.M., Robertson, J.L., Savin, N.E., (1977). POLO a new computer programme for probit analysis. Bull, Entomol. Soci. America, **23:** 209-213.
20. Satyavati, G.V., Gupta, D.K. (1987). Medicinal plants of India. I.C.M.R. New Delhi.
21. Singh, A., Agarwal, R.A., (1988). Possibility of using latex of Euphorbiales for snail control. The Sci. Total Environment, **77:** 231-236.
22. Singh, A., Agarwal, R.A., (1990). Molluscicidal properties of synthetic pyrethroids. J. Medi. Appli. Malaco., **2:**141-144.
23. Singh, A., Agarwal, R.A., (1993a). Toxicity of the synthetic pyrethroids fenvalerate, on enzymes of the target snail *Lymnaea acuminata* and the non-target fish *Channa striatus*. J. Medi. Appli. Malaco., **5:** 87-91.
24. Singh, A., Agarwal, R.A., (1993b). Effect of cypermethrin on lactate succinic dehydrogenase and cytochrome oxides of snail and fish. Bull. Environ. Cont. Toxico., **51:** 445-452.
25. Singh, D.K., Agarwal, R.A., (1982). Comparative study of cholinesterase in two snails *Pila globosa* and *Lymnaea acuminata*, J. Physiol., **78:** 467.
26. Singh, K., Singh, A., Singh, D.K., (1996). Molluscidal activity of Neem (*Azadirachta indica*). J. Ethnopharmacol., **52:**35-40.
27. Singh, O., Agarwal, R.A., (1981). Toxicity of certain pesticide to two economic species of snails in northern India. J. Econo. Entomol., **74:**568-571.
28. Sokal, R.R., Rohlf, F.J. (1973). Introduction of Biostatistics. W.H. Freeman and company, San Francisco, pp. 368.
29. Tiwari, S., Singh, S.K., Singh, A., (2004). Toxicological and biochemical alterations induced by different fractions of *E. royleana* latex in freshwater harmful vector snail *L. acuminata*. Indian J. Expi. Biol., **42:** 1220-1225.
30. Waliszewski, S.M., Aguirre, A.A., Benitez, A., Infanzon, R.M., Infanzon, R., Rivera, J., (1999). Organochlorine pesticide residue in human blood serum of inhabitants of Vera Cruz, Mexico. Bull. Environ. Contam. Toxicol., **62:** 397-402.