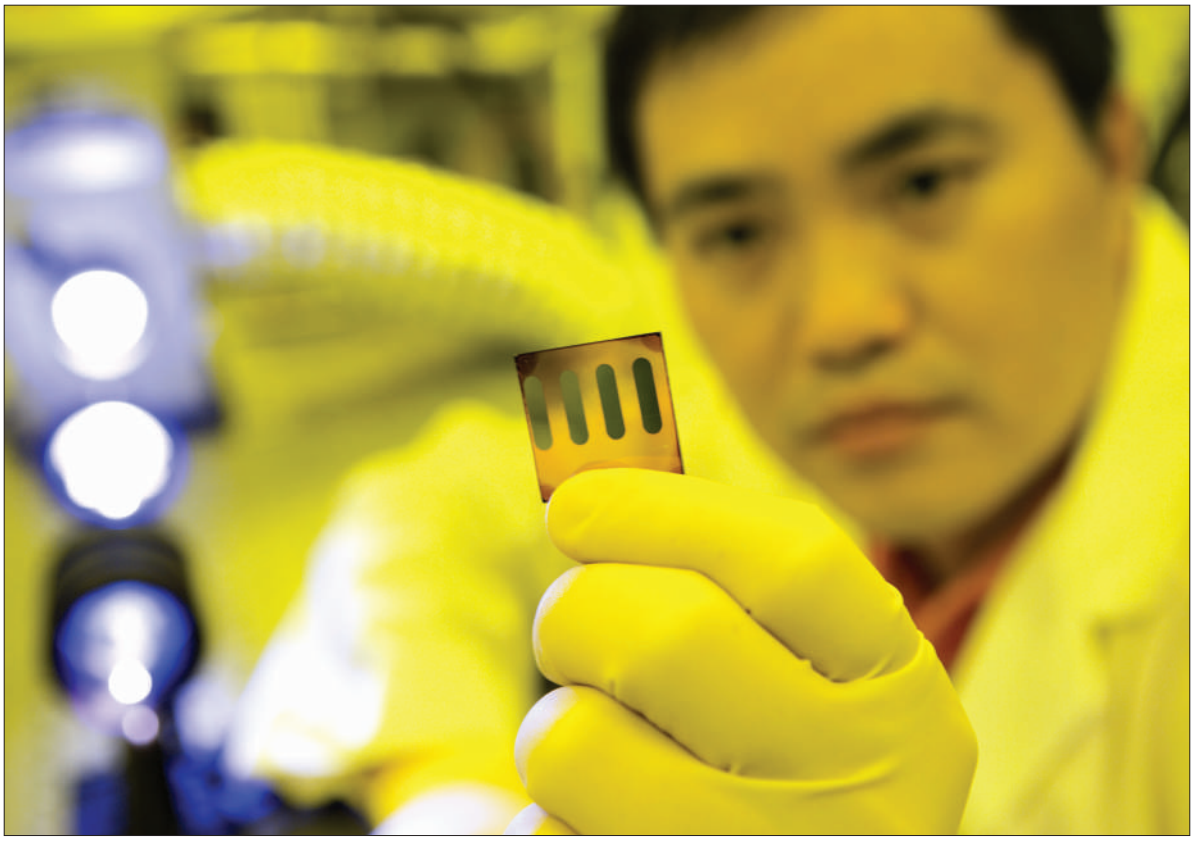

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Feeding of Snail Attractant Pellets (SAP) Containing Papain on Certain Biochemical Parameters in the Gonadal/Nervous Tissue of the Vector Snail (*Lymnaea acuminata*)

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ABSTRACT

Feeding of snail attractant pellets containing papain (40% of 24 h LC_{50}) caused significant reduction in the level of protein, amino acids, DNA, RNA and AChE activity in the gonadal/nervous tissue of *Lymnaea acuminata.* Feeding of papain (40% of 24 h LC_{50}) with serine or starch caused maximum reduction in protein levels in the month of November (64.82% of control) and May (71.23% of control) whereas, amino acids in the month of April (70.54% of control) and September (75.76% of control), respectively. Maximum reduction in DNA levels in the month of September (70.19% of control) and June (61.64% of control) whereas the RNA in the month of May (67.29% of control) and June (66.82% of control), respectively. Bait containing 40% of 24 h LC_{50} of papain with serine or starch caused maximum inhibition in AChE activity in the month of June (68.60 and 50.60% of control).

Key words: Papain, molluscicides, gonadal tissue, SAP, attractant

INTRODUCTION

Fasciola hepatica (Linnaeus, 1758) and *Fasciola gigantica* (Cobbold, 1856) belong to the family Fasciolidae parasitize the liver and the biliary system of their human and animal hosts, respectively and causes fasciolosis (WHO., 2007). Human fasciolosis has been an underestimated and under-explored disease but is now considered an emerging/reemerging disease (Mas-Coma *et al*., 2009). Recent estimates have cited figures ranging from 2.4-17 million humans infected with fasciolosis, with most infections probably due to *F. hepatica* (Mas-Coma *et al*., 1999). The ecology of the snail intermediate host serves as a major contributing factor in the epidemiology of fasciolosis. The intermediate hosts of liver flukes are hermaphroditic freshwater snail species, belonging to the Family Lymnaeidae (Gastropods: Basommatophora) inhabiting water bodies (Mas-Coma *et al*., 2005). The life cycle of *Fasciola* spp. consists of a wide range of final host, consisting of both, domestic and wild mammals (Boray, 1969). Reproduction rate in *Lymnaea acuminata* is very high throughout the year (Srivastava *et al*., 2014a). The snail control process may be achieved by the use of plant derived molluscicide with attractant (Kumar *et al*., 2012; Srivastava *et al*., 2014b). At present, the snail control is one of the most common methods used all over the world for effective control of trematode infections (Kumar *et al*., 2014; Singh *et al*., 2014).

The aim of the present study is to evaluate the effect of sub-lethal feeding of SAP (Bait formulation) containing papain (40% of 24 h LC_{50}) with attractant (starch/serine) on different biochemical changes (free amino acid, protein, nucleic acid and AChE) in the gonadal/nervous tissue of *Lymnaea acuminata* in each month of the year Nov. 2011 to Oct. 2012.

MATERIALS AND METHODS

Experimental animals: Adult *Lymnaea acuminata* (average length 2.27±0.34 cm) were collected from Ramgarh Lake located almost adjacent to Gorakhpur University Campus, U.P., India. Snails were acclimatized in dechlorinated tap water for 72 h at 25±1°C.

Test material: Papain (E 64; Proteinase Inhibitor E 64; *N*-[*N*-(L-3-trans-carboxyirane-2-carbonyl)-L-leucyl]-agmatine; [1-[*N*-[(L-3-trans-carboxyoxirane-2-carbonyl)-L-leucyl] amino]-4-guanidinobutane)]: was purchased from Sigma-Chemical Co. in the United States.

Preparation of Snail Attractant Pellets (SAP) with molluscicides: SAP (Bait formulation) containing attractant starch or serine and molluscicide papain were prepared in 100 mL of 2% agar solution by the method of Madsen (1992) as modified by Tiwari and Singh (2004a, b). Concentrations of carbohydrate and amino acid were based on the earlier reports of Tiwari and Singh (2004a, b). Papain was added to the attractant food pellets simultaneously with starch or serine in 100 mL of 2% agar solution. These solutions were subsequently spread at a uniform thickness of 5 mm. After cooling, the SAP containing molluscicides were cut out using a corer, measuring 5 mm in diameter. These pellets were used for the toxicity determination against *L. acuminata* in each month of the year Nov. 2011 to Oct. 2012.

Assay apparatus and procedure: To determine the effect of bait containing papain with starch or serine on *Lymnaea acuminata* gonadal/nervous tissue. The experimental snails were fed bait containing (40% of 24 h LC_{50}) of papain. Six aquaria were set up for each experiment. Control aquaria contained only dechlorinated tap water without treatment. After 96 h the treated/control snails were removed from glass aquaria and washed with fresh water. The gonadal and/or nervous tissues were quickly dissected, the adherent tissue was removed and the organs were put on a filter paper for absorption of water and then weigh. Protein, free amino acids, DNA, RNA and enzyme AChE activity were measured.

Biochemical estimations

Estimation of protein and free amino acids: Protein estimations (μg mg^{-1}) were made according to the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. Ten percent trichloroacetic acid (TCA: w/v) was used to prepare homogenates of tissue. Total free amino acid (μg mg^{-1}) estimations were made according to the method of Spices (1957).

DNA and RNA: DNA and RNA (μg mg^{-1}) in the gonadal tissue of *L. acuminata* were estimated according to the method of Schneider (1957) using diphenylamine and orcinol reagent, respectively. Homogenates (1 mg mL^{-1}, w/v) of gonadal tissue were prepared in 10% TCA at 90°C and centrifuged at 5000 g for 20 min and used for the estimations.

Enzyme acetylcholinesterase (AChE): Acetylcholinesterase activity was measured according to the method of Ellman *et al.* (1961) as modified by Singh and Agarwal (1982). Fifty milligram of nervous tissue of *L. acuminata* was taken around the buccal mass and homogenized in 1.0 mL of 0.1 M phosphate buffer pH 8.0 for 5 min in an ice bath then centrifuged at 1000 g for 30 min at 4°C. Enzyme activity was measured in a 10 mm path length cuvette using an incubation mixture consisting of 0.1 mL of enzyme source, 2.9 mL of 0.1 M buffer pH 8.0, 0.1 mL of chromogenic agent

DTNB (5, 5-dithiobis-2-nitrobenzoic acid) and 0.02 mL of freshly prepared ATChI (acetylthiocholine iodide) solution in distilled water. The change in optical density at 412 nm was recorded for 3 min after every 30 sec interval at 25°C. Enzyme activity has been expressed as μmol 'SH' hydrolyzed/min/mg protein.

Statistical analysis: Each result was six times replicate estimation (measurement in six different pools of gonadal/nervous tissue). LC_{50} value was calculated by using the POLO computer programme software of Russell *et al*. (1977). The values were expressed as Mean±SE. Two way analysis of variance and Student's t-test was applied to determine the significant ($p<0.05$) difference between treated and control group of snails (Sokal and Rohlf, 1995).

RESULTS

Table 1 shows that there was a significant ($p<0.05$) decrease in protein, amino acids, nucleic acids and AChE activity in the gonadal/nervous tissue of snails exposed to 40% of 24 h LC_{50} of papain with attractant starch/serine in each month of the year Nov. 2011 to Oct. 2012.

Feeding of SAP containing plant molluscicides Papain+Serine+Agar (40% of 24 h LC_{50}) caused maximum reduction in protein (64.82% of control) in month of November, whereas snail fed Papain+Starch+Agar (40% of 24 h LC_{50}) caused maximum reduction (71.23% of control) in month of May (Table 1, 2).

Feeding of bait containing plant molluscicides Papain+Serine+Agar (40% of 24 h LC_{50}) caused maximum reduction in amino acids (70.54% of control) in month of April, whereas snail fed Papain+Starch+Agar (40% of 24 h LC_{50}) caused maximum reduction in amino acids levels (75.76% of control) in month of September (Table 1, 2).

Snail exposed to bait containing plant molluscicides Papain+Serine+Agar (40% of 24 h LC_{50}) caused maximum reduction in DNA (70.19% of control) in month of September, whereas snail fed Papain+Starch+Agar (40% of 24 h LC_{50}) caused maximum reduction (61.64% of control) in month of June in the gonadal tissue of *L. acuminata* (Table 1, 2).

Feeding of SAP Papain+Serine+Agar (40% of 24 h LC_{50}) caused maximum reduction in RNA (67.29% of control) in month of May whereas Papain+Starch+Agar (40% of 24 h LC_{50}) caused maximum reduction in RNA (66.82% of control) in month of June (Table 1, 2).

Feeding of Papain+Serine+Agar (40% of 24 h LC_{50}) caused maximum inhibition in the nervous tissue of *L. acuminata* in AChE activity (68.67% of control) in month of June, whereas snail fed Papain+Starch+Agar (40% of 24 h LC_{50}) caused maximum inhibition (50.60% of control) in month of June in the nervous tissue of *L. acuminata* (Table 1, 2).

DISCUSSION

Present study reveals that feeding of 40% of 24 h LC_{50} of papain resulted in a significant decrease in the different biochemical parameters, namely protein, amino acids, DNA, RNA and AChE in the gonadal/nervous tissue of snail *L. acuminata.* It seems that the biochemical changes in the gonadal/nervous tissue are the causes of mortality of these snails is due to inactivation of ribosomal unit as well as interference of papain with the biosynthetic process amino acids, DNA, RNA and AChE. Jaiswal and Singh (2008) reported that reduction in protein levels may be due to the direct interference of the active component of *Carica papaya* fruit with the protein biosynthesis. It is evident from the results section that active molluscicidal components of *Carica papya* (papain) in bait formulations were more effective in killing the *L. acuminata*. It was reported that function

Table 1: Feeding of snail attractant pellets containing papain 40% of 24 h LC_{50}) in bait formulation on the biochemical changes in gonadal and AChE activity in nervous tissue of snail *Lymnaea acuminata*

				Protein		Amino acid		DNA		RNA		AchE	
Months	Treatments	24 h LC_{50}	Sub-lethal dose (mg L^{-1})	Mean±SD	Percentage	Mean±SD	Percentage	Mean±SD	Percentage	Mean±SD	Percentage	Mean±SD	Percentage
November 2011	Control	-	-	84.12±0.89	100	62.45±0.84	100	89.54±0.85	100	96.21±0.96	100	1.65±0.06	100
	Pa+Se+Ag	11.14	4.45	54.53±0.27*	64.82	58.29±0.75	93.33	85.37±0.44	95.34	83.57±0.45*	86.86	1.33±0.04*	80.60
December 2011	Control	-	-	82.91±0.81	100	62.47±0.88	100	90.84±0.85	100	95.31±0.98	100	1.63±0.06	100
	Pa+Se+Ag	12.58	5.03	71.41±0.64*	86.12	58.76±0.65	94.06	81.31±0.34	89.50	84.23±0.44*	88.37	1.23±0.03*	75.46
January 2012	Control	-	-	83.96±0.80	100	65.45±0.64	100	81.84±0.95	100	95.58±0.86	100	1.62±0.06	100
	Pa+Se+Ag	16.54	6.61	75.53±0.44	89.95	54.83±0.81*	83.77	76.26±0.63*	93.18	82.67±0.36*	86.49	1.18±0.02*	72.83
February 2012	Control	-	-	82.90±0.82	100	66.45±0.74	100	88.34±0.89	100	94.61±0.86	100	1.64±0.06	100
	Pa+Se+Ag	13.43	5.37	62.24±0.54*	75.07	52.97±0.45*	79.71	74.63±0.76*	84.48	79.43±0.64*	83.95	1.35±0.04*	82.31
March 2012	Control	-	-	85.97±0.81	100	67.15±0.74	100	88.58±0.95	100	96.78±0.76	100	1.63±0.04	100
	Pa+Se+Ag	14.52	5.80	58.62±0.68*	68.18	59.18±0.54	88.13	76.93±0.73*	86.84	82.62±0.43*	85.36	1.37±0.02*	84.04
April 2012	Control	-	-	85.38±0.83	100	65.35±0.94	100	87.72±0.95	100	95.21±0.96	100	1.64±0.03	100
	Pa+Se+Ag	13.74	5.49	72.67±0.54*	85.11	46.10±0.45*	70.54	71.23±0.36*	81.20	82.44±0.44*	86.58	1.19±0.04*	72.56
May 2012	Control	-	-	86.91±0.84	100	64.65±0.84	100	87.98±0.75	100	98.68±0.98	100	1.66±0.06	100
	Pa+Se+Ag	12.73	5.09	66.36±0.85*	76.35	58.41±0.93	90.34	65.65±0.65*	74.61	66.41±0.61*	67.29	1.34±0.03*	80.72
June 2012	Control	-	-	87.95±0.83	100	67.65±0.74	100	86.81±0.95	100	96.76±0.88	100	1.66±0.03	100
	Pa+Se+Ag	8.58	3.43	77.69±0.85*	88.33	52.51±0.69*	77.62	68.62±0.43*	79.04	68.83±0.48*	71.13	1.14±0.04*	68.67
July 2012	Control	-	-	81.91±0.82	100	66.55±0.54	100	86.34±0.75	100	93.41±0.86	100	1.67±0.06	100
	Pa+Se+Ag	8.76	3.50	71.54±0.64*	87.33	46.45±0.47*	69.79	66.46±0.36*	76.97	78.20±0.17*	83.71	1.56±0.01*	93.41
August 2012	Control	-	-	82.98±0.85	100	68.46±0.89	100	87.56±0.85	100	96.25±0.99	100	1.67±0.06	100
	Pa+Se+Ag	9.15	3.66	65.30±0.81*	78.69	52.34±0.93*	76.45	62.83±0.64*	71.75	77.51±0.82*	80.52	1.64±0.02*	98.20
September 2012	Control	-	-	81.47±0.89	100	68.42±0.88	100	87.54±0.85	100	96.21±0.96	100	1.68±0.06	100
	Pa+Se+Ag	10.32	4.12	68.62±0.86*	84.22	56.31±0.24*	82.30	61.45±0.64*	70.19	84.43±0.84*	87.75	1.28±0.03*	76.19
October 2012	Control	-	-	83.93±0.81	100	67.15±0.84	100	86.73±0.97	100	97.64±0.86	100	1.68±0.04	100
	Pa+Se+Ag	10.16	4.06	72.48±0.64*	86.35	61.65±0.54*	91.80	74.61±0.50*	86.02	84.24±0.57*	86.27	1.55±0.03*	92.26

Each experiment was replicated six times and the value of protein, amino acid, DNA, RNA and AChE is the mean of six replicate. Two way analysis of variance and student 't'-test were applied between control and treated group indicate (*) significant ($p<0.05$) test. Protein, amino acid, DNA and RNA levels measured in µg mg^{-1}. Acetylcholinesterase activity, µmol 'SH' hydrolyzed/min/mg protein. Abbreviation: Pa: Papain, Se: Serine, Ag: Agar

Table 2: Feeding of snail attractant pellets containing papain (40% of 24 h LC_{50}) in bait formulation on the biochemical changes in gonadal and AChE activity in nervous tissue of snail *Lymnaea acuminata*

				Protein		Amino acid		DNA		RNA		AchE	
Months	Treatments	24 h LC_{50}	Sub-lethal dose (mg L^{-1})	Mean±SD	Percentage	Mean±SD	Percentage	Mean±SD	Percentage	Mean±SD	Percentage	Mean±SD	Percentage
November 2011	Control	-	-	84.12±0.89	100	62.45±0.84	100	89.54±0.85	100	96.21±0.96	100	1.65±0.06	100
	Pa+St+Ag	11.84	4.737	4.34±0.75*	88.37	59.36±0.51	95.05	82.36±0.49	91.98	88.51±0.55	91.99	1.37±0.01*	83.03
December 2011	Control	-	-	82.91±0.81	100	62.47±0.88	100	90.84±0.85	100	95.31±0.98	100	1.63±0.06	100
	Pa+St+Ag	12.85	5.14	72.62±0.55*	87.58	58.28±0.46	93.29	84.60±0.81	93.13	87.62±0.54	91.93	1.14±0.04*	69.93
January 2012	Control	-	-	83.96±0.80	100	65.45±0.64	100	81.84±0.95	100	95.58±0.86	100	1.62±0.06	100
	Pa+St+Ag	16.75	6.70	76.62±0.69	91.25	55.62±0.69*	84.98	73.52±0.55*	89.83	82.52±054*	86.33	1.25±0.03*	77.16
February 2012	Control	-	-	82.90±0.82	100	66.45±0.74	100	88.34±0.89	100	94.61±0.86	100	1.64±0.06	100
	Pa+St+Ag	14.70	5.88	76.26±0.81	91.99	56.16±0.55	84.51	76.03±0.65*	86.06	83.56±0.45*	88.32	1.16±0.03*	70.73
March 2012	Control	-	-	85.97±0.81	100	67.15±0.74	100	88.58±0.95	100	96.78±0.76	100	1.63±0.04	100
	Pa+St+Ag	14.25	5.70	64.26±0.73*	74.74	51.91±0.82*	77.30	73.36±0.45*	82.81	76.56±0.45*	79.10	1.38±0.04*	80.98
April 2012	Control	-	-	85.38±0.83	100	65.35±0.94	100	87.72±0.95	100	95.21±0.96	100	1.64±0.03	100
	Pa+St+Ag	13.57	5.42	68.83±0.64*	80.61	56.62±0.88	86.64	68.63±0.56*	78.23	72.18±0.36*	75.81	0.94±0.04*	57.31
May 2012	Control	-	-	86.91±0.84	100	64.65±0.84	100	87.98±0.75	100	98.68±0.98	100	1.66±0.06	100
	Pa+St+Ag	12.30	5.92	61.91±0.81*	71.23	60.84±0.68	94.10	67.52±0.62*	76.74	68.43±0.54*	69.34	0.96±0.02*	57.83
June 2012	Control	-	-	87.95±0.83	100	67.65±0.74	100	86.81±0.95	100	96.76±0.88	100	1.66±0.03	100
	Pa+St+Ag	9.85	3.94	64.12±0.44*	72.90	53.63±0.55*	79.27	53.51±0.64*	61.64	64.66±0.30*	66.82	0.84±0.01*	50.60
July 2012	Control	-	-	81.91±0.82	100	66.55±0.54	100	86.34±0.75	100	93.41±0.86	100	1.67±0.06	100
	Pa+St+Ag	9.16	3.66	65.62±0.56*	80.11	58.68±0.66*	88.17	56.33±0.71*	65.24	66.48±0.54*	71.17	1.65±0.04*	98.80
August 2012	Control	-	-	82.98±0.85	100	68.46±0.89	100	87.56±0.85	100	96.25±0.99	100	1.67±0.06	100
	Pa+St+Ag	9.25	3.70	66.60±0.46*	80.26	56.38±0.46*	82.35	58.76±0.66*	67.10	64.71±0.68*	67.23	1.32±0.03*	79.04
September 2012	Control	-	-	81.47±0.89	100	68.42±0.88	100	87.54±0.85	100	96.21±0.96	100	1.68±0.06	100
	Pa+St+Ag	10.20	4.08	78.47±0.96	96.31	51.84±0.66*	75.76	65.69±0.49*	75.03	76.36±0.36*	79.36	1.47±0.03*	87.50
October 2012	Control	-	-	83.93±0.81	100	67.15±0.84	100	86.73±0.97	100	97.64±0.86	100	1.68±0.04	100
	Pa+St+Ag	10.76	4.30	74.38±0.74	88.62	61.25±0.45	91.21	78.68±0.55	90.71	86.39±0.36*	88.47	1.56±0.02*	92.85

Each experiment was replicated six times and the value of protein, amino acid, DNA, RNA and AChE is the mean of six replicate. Two way of analysis of variance and student 't'-test were applied in between control and treated group (*) significant ($p<0.05$) test. Protein, amino acid, DNA and RNA levels measured in µg mg^{-1}. Acetylcholinesterase activity, µmol 'SH' hydrolyzed/min/mg protein. Pa: Papain, St: Starch, Ag: Agar

of papain is possible is through the cysteine-25 portion of the triads in the active site that attack the carbonyl carbon in the backbone of the peptide chain freeing the amino terminal portion. As this occurs throughout the peptides chain of the protein, the protein breaks apart. The mechanism by which it breaks peptides bonds involves deprotonation of Cys-25 by His-159. It was also reported that it preferentially cleaves peptide bonds involving basic amino acids, particularly arginine, lysine and residues following phenylalanine. The synthesis of protein in any of a tissue can be affect in two ways by a chemical, (i) it either affect the RNA synthesis at the transcription stage or (ii) it somehow affects the uptake of amino acid in the polypeptide chain. In this case the RNA synthesis would be inhibited resulting in reduced RNA as well as protein content and only the protein content would be affected (Tariq *et al*., 1977; Singh *et al*., 2010). Amino acid levels in the gonadal tissue of the snail exposed to different preparation was significantly lower than control. It indicates that they also interfere with the biosynthesis of amino acid in the cell (Singh *et al*., 1998).

It may be possible that papain affect the gonadal tissue of the treated snails directly or indirectly through the neurohormones. The upsetting of glycemic homeostasis is an indication of intoxication (Mello-Silva *et al*., 2010). The detoxification processes consume high amounts of energy, resulting in exhaustion of the carbohydrate deposits, thus leading to the reduction of fecundity that would otherwise be used for reproductive activity (Silva *et al*., 2012). Papain is a cysteine hydrolase that is stable and active under a wide range of conditions. It is very stable even at elevated temperatures (Dubey *et al*., 2007).

It is clear from the result section that the sub-lethal treatment of papain in bait formulation (24 h LC_{50}) caused a significant inhibition of AChE activity in the nervous tissue of *L. acuminata.* AChE inhibition results in accumulation of acetylcholinesterase at the nerve synapses so that the post synaptic membrane is in a state of permanent stimulation producing paralysis, ataxia and general lack of coordination in neuromuscular system and eventual death (Jaiswal *et al*., 2008).

CONCLUSION

It can be concluded from the above study that the seasonal variation and bait containing plant molluscicides (papain) with attractant (starch/serine) can significantly alter the biochemical parameters in gonadal/nervous tissue of snail *Lymnaea acuminata* in each month of the year Nov. 2011 to Oct. 2012. The result show clearly from this experiment that May-August is most suitable period for the control of the snail at threshold level which ultimately reduces the incidence of fasciolosis in eastern part of India.

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