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Eco-Friendly Management of *Lymnaea acuminata*, Snail Vector of Fascioliasis in Livestock in Eastern Uttar Pradesh

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Abstract: The main intention of this study was to evaluate the molluscicidal activity of plant origin pesticides; ethanol extract of leaf and stem bark of common medicinal plant *Lantana indica* (Family: Verbenaceae) against freshwater invertebrate snail *Lymnaea acuminata* in laboratory as well as in pond. The freshwater snail *Lymnaea acuminata* is vector of trematodes; *Fasciola hepatica* and *Fasciola gigantica* which cause fascioliasis in domestic useful animals and very common in the eastern part of Uttar Pradesh, which is major cause of reduction in animal production and mortality in animals. The molluscicidal effect of ethanol extracts of leaf and stem bark of this plant was time as well as dose dependent and there was a significant negative correlation between LC values and exposure periods. LC values decreases as exposure periods increases from 24h to 96h in laboratory and in the pond but the toxicity of plant extracts was reduced in the pond (4.2 times and 3.5 times in the case of stem bark and leaf extracts respectively) than laboratory. The order of toxicity of the plant parts was leaf followed by stem bark. Treatment of snails with sub lethal doses (20% and 40% of 24h LC_{50}) of these extracts affect the reproductive process in negative way i.e. reduction in the fecundity and hatchability. The survival of hatched young snails was observed continuously up to 4 weeks after hatching where hatchability was reduced to 88% and 92% and 83% and 87% after exposure to 20% and 40% of 24h LC_{50} of leaf and stem bark extracts respectively compared with control group 4 weeks post hatching. Sub lethal doses of 24h LC_{50} of these extracts also alter the biochemical parameters significantly in dose dependent manners. It may be stated that plant *L. indica* possess molluscicidal activity against *Lymnaea acuminata* and seemed to be better control agent than the synthetic pesticides due to its biodegradability, easy accessibility and low cost.

Key words: *Lymnaea acuminata* · Ethanol extract · *Lantana indica* · Fascioliasis · Fecundity

INTRODUCTION

Animal diseases are crucial constraints; the animals of poor people are particularly vulnerable to diseases because of the expense, absence or unsuitability of animal health and production inputs. So the loss of individual animals due to diseases has a proportionally greater impact [1]. Fascioliasis is an important live stock heath problem [2, 3]. A large variety of animals, such as sheep, goat, cattle, buffalo, show infection rates that may reach 90% in some areas and consequently leading to significant global economic losses [4, 5]. The snails belonging to the family Lymnaeidae are known to act as intermediate host of animal fascioliasis [6]. *Lymnaea acuminata* act as intermediate host of trematode *Fasciola hepatica* and *Fasciola gigantica*. Hence the control of fascioliasis is very essential to reduce the economic losses as animal production, mortality and morbidity. Snail control with mollucicides has been one of the effective methods used for rapid and effective control of the disease. As the existence of mollusc populations depends on the capacity of adult molluscs to produce high numbers of offspring, any change in either fecundity (number of eggs) or fertility (number of fertile eggs) of the adults could severely endanger the individual populations [7]. Herbal medicine is widely practiced from ancient period throughout the world [8] to replace the synthetic pesticides due to its hazardous effect in the environment. Plants from a variety of families have been shown to possess many classes of products, which have varying degree of molluscicidal activity from past 20 years. A number of medicinal plants belonging to different family's viz *Euphorbia pulcherima, Clitorea ternatea, Guazuma ulmifolia, Madhuca indica* [9, 10].

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The present investigation deals with the comparative evaluation of molluscicidal potentials of ethanolic extract of different parts of common medicinal plant *Lantana indica* (Family: Verbenaceae) commonly known as 'Wild sage' used against snail *Lymnaea acuminata* in laboratory as well as in pond. Its different parts are also used as traditional medicine for the treatment of various human ailments such as ulcers, eczema eruptions and malaria [11]. The effect of the sub lethal dose of these extract on reproduction and biochemical parameters of the snail was also performed.

MATERIALS AND METHODS

Collection of Plant: The leaves and stem bark of the plant *Lantana indica* were collected from the botanical garden of DDU, Gorakhpur University, Gorakhpur, Uttar Pradesh, India. Leaves and stem bark of the plants were washed separately with water and then dried in the shade. Then dried leaves and stem bark of *Lantana indica* were powdered with the help of mechanical device.

Extraction of Compounds: Extraction was made by the method of Chauhan and Singh [12]. 50 gram powder of leaves and stem bark of *Lantana indica* were subjected separately to extraction through Soxhlet apparatus in 350 ml Ethanol solvent for about 50 hours and a concentrated solution was obtained. After evaporation of solvent, the extracted compound in dried form was obtained. The extracted compound was stored in air-tight desicator until use for experiments.

Collection of Snails: The fresh water harmful snail *Lymnaea acuminata* (3.65±1.00 cm total shell height and 1.40 ± 0.50 cm total shell width), were collected from the fresh water bodies of Gorakhpur district, U.P. India. Prior to experiment snails were allowed to acclimate to laboratory conditions for 72h.

Toxicity Experiment: Toxicity experiment was performed by the method of Singh and Agarwal [13]. Twenty snails were kept in glass aquaria containing 3L de-chlorinated tap water. Snails were exposed for 24h, 48h, 72h or 96h to four different concentrations of stem bark extract, 7mg/L, 11mg/L, 17mg/L, 21mg/L in laboratory and 28mg/L, 33mg/L, 38mg/L and 43mg/L in pond respectively. The four concentrations of leaf extract was 3.5mg/L, 4.5mg/L, 5.5mg/L, 6.5mg/L in laboratory and 15mg/L, 20mg/L, 25mg/L, 30mg/L in pond respectively. Control, snails were kept in similar conditions without any treatment. Each group of snails was replicated six times. Mortality was recorded after every 24h during the observation period of 96h. Contraction of the snail body within the shell and no response to a needle probe were taken as evidence of death of snails. Dead animals were removed to prevent the decomposition of body in experimental aquarium.

The effective doses (LC values), upper and lower confidence limits, slope value,'t' ratio and heterogeneity were calculated by the probit log method of Robertson *et al.* [14]. Student's' test was applied to determine the significant ($p<0.05$) differences between treated and control animals. Product moment co-relation coefficient was applied in between exposure time and lethal concentrations Sokal and Rohlf [15].

Reproductive Experiment: The experiment was performed according to the method of Presing [16]. In this experiment, fresh water adult *Lymnaea acuminata* were exposed to different sub-lethal [20% & 40% of LC_{50} (24h)] doses of ethanol extract of leaf and stem bark of *Lantana indica* in the laboratory. For fecundity experiments, aquariums were filled with 5L de- chlorinated tap water and required amount of extracts were mixed in each aquarium. Ten adult snails were placed in each aquarium. Six replicates were used for each set of snails; water temperature was kept at 25±1°C during the entire time of experiments. No food was given to the snails during the experimental period. Control groups of snail were kept in similar conditions without any treatment, for smooth spawning fresh lotus leaf was let floated in each aquarium. Lymnaeid snails attached ribbon like egg masses (spawns), containing variable number of eggs to the back surface of lotus leaf and inner wall of the aquarium when reproducing. The egg masses produced by the snail in the experimental aquarium were removed after every 24 hours up to 96 hours and the number of eggs counted under compound microscope. All the spawns of each group were transferred into separate aquarium containing one litter de- chlorinated tap water for hatching under the same exposure conditions as above and kept at 25±1°C for development of embryo in B.O.D. incubator. Hatched snails were counted and their survival rate was recorded for 28 days after hatching. Disintegration of embryos or absence of movement of the embryo was considered for calculating the percent mortality of eggs.

Biochemical Experiment: The biochemical experiments were performed by the method of Tripathi and Singh [17]. The biochemical experiment was conducted in freshwater ponds. Each pond was stocked with 100 snails in 29.28 m^3 in area and 9.19 m^3 in water volume with a size difference not greater than 1.5 times (APHA, 1992). The experimental snails were treated with two different sub-lethal doses; 40% and 80% of LC_{50} (24h) of ethanol extracts of leaf and

stem bark of *Lantana indica* for 96h. Control groups were kept under similar conditions without treatment for same duration. Diet was not given to the snails during the course of experiment. After the completion of 96h of treatment snails were removed from the treated water and the hepatopancreas (HP) and nervous tissue (NT) tissues of both the treated as well as control snails were quickly dissect out and used for biochemical estimations.

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

Protein: Protein levels were estimated according to the method of Lowry *et al.* [19] using bovine serum albumin as standard. Homogenates (5 mg/mL) were prepared in 10% TCA.

Glycogen: Glycogen was estimated by the Anthrone method of Vander Vies [20] as modified by Mahendru and Agarwal [21] for snail *L. acuminata.* 50 mg of tissue were homogenized in 5 mL of cold 5% TCA. The homogenate was filtered and 1.0 mL of filtrate was used for assay.

Nucleic Acid: Estimation of nucleic acid (DNA and RNA) was performed by the method of Schneider [22] using diphenylamine and orcinol reagents, respectively. Homogenates (1 mg/mL) were prepared in 5% TCA and centrifuged at 5000 xg for 20 minute and the supernatant was used for the estimation.

Activity of Enzyme Protease: Protease activity was measured according to the method of Moore and Stein [23] homogenate (50 mg/L) was prepared in cold distilled water and optical density was measured at 570 nm. Protease activity is expressed in as micromoles of tyrosine equivalents per milligram of protein /hour.

RESULTS

Molluscicidal Potentials of Plant Extracts: The toxicity of extracts of both parts of *L. indica* plant was time and dose-dependent. There was a significant negative correlation between LC values and exposure periods. Thus with increase in exposure periods the LC_{50} values of ethanol extract of *L. indica* stem bark decreased from 20.06 mg/L (24h) to 8.21 mg/L (96h) and 6.59 mg/L (24h) to 3.82 mg/L (96h) in the case of leaf in the laboratory (Table1). Similarly LC_{50} values decreased from 86.19 mg/L (24h) to 31.19 mg/L (96h) and 23.72 mg/L (24h) to 15.66 mg/L (96h) in case of stem bark and leaf extracts in pond respectively (Table2).

Snails Fecundity and Hatchability: Treatment of snails with sub lethal doses (20% and 40% of LC_{50} of 24h) of ethanol extract of *L. indica* stem bark resulted in reduction in snails fecundity to 87% and 81% of control after 96h of exposure respectively and the number of hatched eggs was reduced to 78% and 71% of control respectively (Table 3). The survival rate of the hatched snails was reduced to 71% and 68% of control after one week of hatching and it was further reduced to 63% and 58% after

Table 1: Toxicity (LC_{50} values) of different concentrations of ethanol extracts of stem bark and leaf of *Lantana indica* against freshwater snail *Lymnaea acuminata* at different time intervals in laboratory conditions

Ethanol extract of stem bark of *Lantana indica*						
	Effective dose	Limits (mg/L)				
Exposure Periods	(mg/L) LC_{50}	LCL	UCL	Slope value	't' ratio	Heterogeneity
24h	20.06	17.17	25.79	2.02±0.33	7.96	0.70
48h	14.55	13.06	16.44	2.48±0.33	7.42	0.03
72h	10.91	09.04	12.62	1.82±0.32	5.66	0.51
96h	08.21	06.65	09.45	2.30±0.33	6.85	0.69
Ethanol extract of leaf of *Lantana indica*						
24h	06.59	05.97	07.83	3.54±0.62	5.66	0.45
48h	05.84	05.39	06.60	3.56±0.60	5.87	0.30
72h	04.75	04.21	05.30	2.54±0.58	4.37	0.64
96h	03.82	02.83	04.34	5.28±0.64	8.14	0.28

Twenty snails were exposed to four different concentrations of the extract. Concentrations given are the final concentration (w/v) in the de-chlorinated water. Mortality was determined at every 24h. Regression coefficient showed that there was significant ($P<0.05$) negative Regression between exposure time and different LC values. LCL-Lower confidence limit; UCL-Upper confidence limit. There was no mortality in control group.

Table 2: Toxicity (LC_{50} values) of different concentrations of ethanol extracts of stem bark and leaf of *Lantana indica* against freshwater snail *Lymnaea acuminata* at different time intervals in pond

Exposure Periods	Effective dose (mg/L) LC_{50}	Limits (mg/L) LCL	UCL	Slope value	't' ratio	Heterogeneity
Ethanol extract of stem bark of *Lantana indica*						
24h	86.19	75.24	98.30	2.35±0.48	7.12	0.72
48h	56.74	43.66	68.36	3.78±0.53	6.58	0.32
72h	46.91	35.25	55.65	2.10±0.33	5.54	0.64
96h	31.19	19.47	48.05	2.34±0.34	7.11	0.33
Ethanol extract of leaf of *Lantana indica*						
24h	23.72	18.23	26.78	3.39±0.41	6.03	0.09
48h	21.78	13.63	24.36	2.87±0.32	5.78	0.02
72h	16.62	10.57	22.62	2.46±0.35	7.25	0.25
96h	15.66	9.81	18.08	1.96±0.35	6.54	0.30

Hundred snails were exposed to four different concentrations of the extract. Concentrations given are the final concentration (w/v) in the de-chlorinated water. Mortality was determined at every 24h.Regression coefficient showed that there was significant ($P<0.05$) negative Regression between exposure time and different LC values. LCL-Lower confidence limit; UCL-Upper confidence limit. There was no mortality in control group.

Table 3: Numbers of laid eggs, egg masses duration of hatching, hatched eggs and survival of hatched young snails (hatchlings) after treatment with 20% and 40% of LC_{50} (24h) of *Lantana indica* stem bark and leaf ethanol extracts to the freshwater snails, *Lymnaea acuminata*

	Ethanol extract of *L. indica* Stem bark			**Ethanol extract of *L. indica* leaf**		
	Control	20% of LC_{50} (24h) (4.01mg/L)	40% of LC_{50} (24h) (8.02mg/L)	Control	20% of LC_{50} (24h) (1.31mg/L)	40% of LC_{50} (24h) (2.63mg/L)
No. of laid eggs (after 96h of treatment)	407.6±0.96 (100)	342.5±0.13* (87)	314.3±0.73* (81)	537.16±1.03 (100)	437.50±0.83* (81)	409.16±0.65* (76)
No. of eggs masses	10.50±0.83	9.0±1.05	8.50±0.83	12.6±0.73	11.3±0.96	9.3±0.96
No. of hatched eggs	407.5±0.83 (100)	229.8±0.66* (78)	223.5±0.83* (71)	534.50±0.83 (100)	330.50±0.83* (76)	284.16±0.65* (72)
Survival of hatched young snails (hatchlings)						
After 1st week of hatching	404.5±0.83 (99)	162.2±0.66** (71)	152.5±0.83** (68)	531.50±0.83 (99)	286.50±0.83** (70)	175.16±0.65** (62)
After 2nd week of hatching	401.6±0.96 (98)	142. 3±0.33** (63)	130.4±0.83** (58)	522.66±0.73 (97)	195.50±0.83** (59)	151.50±0.83** (53)
After 3rd week of hatching	392.6±0.83 (96)	105.30±0.83**c (46)	91.6±0.96** (41)	518.10±0.83 (96)	133.66±0.96** (40)	91.50±0.83** (32)
After 4thweek of hatching	384.5±0.83 (94)	38.80±0.66** (17)	28.8±0.83** (13)	511.01±0.63 (95)	39.20±0.96** (12)	21.50±1.03** (8)

All experiments were replicated six times. Values are means ± SE of six replicates.Values in parentheses are percentages of the corresponding value with control taken as 100%.*, Significant ($P<0.05$), when Student's 't' test was applied between control and treated groups. **, Significant ($P<0.05$), when Student's 't' test applied between number of hatched eggs and survival rate of hatchlings in corresponding treated groups.

two weeks, 46% and 41% after three weeks and 17% and 13% of control after four weeks of hatching, respectively. Similarly treatment of snails with sub-lethal doses; 20% and 40% of LC_{50} (24h) of ethanol extract of *L. indica* leaf caused reduction in fecundity to 81% and 76% of control and r eduction in number of hatched eggs estimated 76% and 72% of control respectively (Table 3). The survival rate of hatched snails was reduced to 70% and 62% of control after one week of hatching and it was only 12% and 8% of control after four weeks of hatching respectively.

Snail Biochemical Alterations: Sub-lethal doses (40% and 80% of LC_{50} of 24h) of plant extracts caused significant alterations in the level of total protein, total free amino acids, glycogen and nucleic acids and activity of enzyme protease in nervous and HP tissue of the snail *Lymnaea acuminata* (Table 4). When snails were exposed to ethanol extract of stem bark of the plant, total protein level was significantly reduced to 86% and 64% of control in nervous tissue and 81% and 61% of control in HP tissue respectively. Glycogen level was reduced to 79% and 69% of control in nervous tissue and 72% and 65%

Table 4: Changes in total free amino acids (FAA) (µg/mg), total protein (TP) (µg/mg), glycogen level (G) (mg/g), nucleic acid level [DNA and RNA (µg/mg)] and enzyme protease (µmol tyrosine/mg protein/h) in nervous and hepatopancreas (HP) tissues of freshwater snail *Lymnaea acuminata* after exposure to sub-lethal doses (40% and 80% of LC_{50} of 24h) of ethanol extract of stem bark and leaf of *Lantana indica* after 96h of exposure in pond.

		Ethanol extract of *L. indica* stem bark			**Ethanol extract of *L. indica* leaf**		
Parameter	Tissue	Control	40% of 24h LC_{50} (17.23mg/L)	80% 24h LC_{50} (68.95mg/L)	Control	40%24h LC_{50} (4.74mg/L)	80% 24h LC_{50} (18.97mg/L)
FAA	NT	21.54±0.12	26.0±0.21* (120)	31.42±0.83* (145)	42.30±0.21	54.71±0.34* (129)	65.31±0.09* (154)
	HP	18.65±0.30	21.52±0.33* (115)	24.51±0.02* (131)	36.45±0.54	45.43±0.11* (125)	49.52±0.06* (136)
TP	NT	33.65±0.21	29.01±0.95* (86)	21.62±0.69* (64)	57.31±0.54	46.02±0.36* (80)	31.31±1.01* (55)
	HP	30.10±0.12	24.32±0.08* (81)	18.47±0.62* (61)	50.21±0.12	39.69±0.45* (79)	26.57±0.02* (53)
G	NT	10.32±0.04	8.13±0.22* (79)	07.13±0.32* (69)	8.32±0.65	5.89±0.32* (71)	4.66±0.12* (56)
	HP	8.36±0.23	6.06±0.19* (72)	5.45±1.12* (65)	6.35±0.52	4.14±0.05* (65)	3.27±0.82* (51)
DNA	NT	55.36±0.55	45.10±0.31* (81)	43.20±0.30* (78)	41.82±0.01	33.57±0.12* (80)	30.87±0.01* (74)
	HP	43.80±0.23	35.24±0.02* (80)	33.86±0.05* (77)	39.33±0.24	30.87±0.03* (78)	27.66±0.40* (70)
RNA	NT	53.65±0.83	47.80±0.40* (89)	44.53±0.22* (83)	61.22±0.31	51.86±0.62* (85)	45.09±0.24* (74)
	HP	49.36±0.81	38.83±0.03* (79)	34.36±0.31* (70)	55.87±0.09	40.18±0.22* (72)	37.84±0.54* (68)
Protease	NT	0.439±0.66	0.538±0.01* (123)	0.624±0.13* (142)	0.254±0.12	0.341±0.01* (134)	0.375±0.12* (148)
	HP	0.467±0.24	0.552±0.03* (118)	0.641±0.02* (137)	0.233±0.04	0.294±1.02* (126)	0.321±0.83* (138)

All these values are ± SE of six replicates. Values in parenthesis are percent change with control taken as 100%. Data were analyzed through student's't' test. *, shows significant ($P<0.05$) changes, when treated groups were compared with controls. Levels of total free amino acids, total protein, nucleic acid (DNA and RNA) are expressed in µg/mg and glycogen level are expressed in mg/g and activity of protease in micromoles of tyrosine equivalents/mg protein /h.

of control in HP tissue respectively. DNA level was reduced to 81% and 78% and 80% to 77% of control in nervous and HP tissues respectively. RNA level was reduced to 89% and 83% and 79% and 70% of control in nervous and hepatopancreas tissues respectively. Control group was taken as 100%. While total free amino acids level was significantly increased and it was 120% and 145% of control in nervous tissues and 115% and 131% of control in hepatopancreas tissues respectively. Protease activity was significantly increased to 123% and 142% of control in nervous tissue and 118% and 137% of control in hepatopancreas tissue of snails respectively. Similar results were observed in the case of ethanol extract of *L. indica* leaf (Table 4).

DISCUSSION

From results it was proved that the ethanolic extract of leaf and stem bark of *L. indica* was a potential source of herbal molluscicides. There was a positive correlation between dose and mortality of snails. The increases in mortality with increase in exposure period could be due to several factors, which may be act separately or conjointly. The uptake of the active moiety of ethanolic extract of leaf and stem bark of *Lantana indica* could be time dependent leading to a progressive increase in the titre of the active ingredients and its effects in the snails [24, 3]. In the pond, the toxicity of plant extracts was reduced to 3.5 to 4.2 times (24h toxicity) in the case of leaf and stem bark respectively. It may be due to, under natural conditions many factors such as temperature, sunlight, adsorption by soil particles etc. influence toxicity (i.e. lowered) and toxicant degradation. Dawson *et al.* [25] found that rotenone disappeared two to three times faster in earthen ponds than in concrete ponds. Perchbacher and Sarkar, [26] reported that the detoxification of Phostoxin (Phosphine) occurred in 4 days in laboratory tank at 23°C and in 1 day in earthen ponds at 37°C. In natural

conditions of the toxicity of *Euphorbia pulcherima* latex powder with other plant derived molluscicides were reduced against freshwater vector snails *L. acuminata* [9].

Statistical analysis of the data on toxicity brings out several important points. The χ^2 test for goodness of fit 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 graph exhibits steep slope values. The steepness of slope line indicates that there is a large increase in the mortality of vectors population with relatively small increase in the toxicant.

Sub lethal dose of ethanol extract of the *L. indica* treated animal's shows reproductive toxicology also i.e. significant reduction in the fecundity, hatchability and survival. The reduction in fecundity associated to the intermediate and final phases of the *L. acuminata* larval development, coinciding with the depletion of the energy stores, as the glycogen deposits and protein content in this study in different tissues, verifying that the eggs production decreases after 96h of treatment. Similar response was also obtained by [27-30]. It can also be inferred from the results of Adewunmi *et al.* (30) that the significant reduction in the glycogen and protein content could be held responsible for the reduction in egg production of *Biomphalaria glabrata* and *Lymnaea columella*. Because gastropods in general, use glycogen as a reserve [31-34].

Hatchability of hatched young snails was significantly reduced in treated animals. Plants extracts perhaps induce embryonic malformations, delays in embryogenesis and, for most toxic effects, death of embryos [35] and hence reduction in hatchability. Bakry [36] found that exposure of methanol extract of *E. splendens*, *A. stylosa* and *G. officinalis* also reduce their growth rate of young snail.

The survival of hatched young snail was also significantly reduced. A similar result was observed by Khangarot and Das [37], they found that percentage survival of embryos treated with Cu reduced sharply after 240 h of exposure. Survival of hatched snails was significantly reduced. It may be due to the plant products affect the respiratory function of the snails by acting as uncoupler of oxidative phosphorylation at the mitochondrial level [38, 39]. So sub lethal dose of this plant disrupt the reproductive function of snails and according to Woin and Bronmark [40] disruption in the reproduction will ultimately affect the abundance and distribution of snails and act as alternate method to manage the size of the snail populations.

Sub lethal doses of ethanol extract of the *L. indica* were adequate to alter the different biochemical parameters of the snail. After exposure to sub lethal doses, total protein level, glycogen and nucleic acid level were significantly reduced while total free amino acids and protease level was significantly increased. Carbohydrates are the primary and immediate source of energy [41] in living creatures. The fall in glycogen content in the body tissues of *L. acuminata* indicates its rapid utilization by the respective tissues as a consequence of pesticide toxic stress. Under hypoxic conditions, animals derive their energy from anaerobic breakdown of glucose, which is available to the cells by increased glycogenolysis [42]. Glycogen levels appear to be related, at least to some extent, to the detoxification mechanisms essential for metabolism or degradation and elimination of pesticides from the body [43]. The reduction of protein fraction in both nervous and hepatopancreas tissues of snails may be due to their degradation and possible utilization of degraded products for metabolic purposes. Inhibition in DNA synthesis might affect both protein as well as amino acid levels by decreasing the level of RNA in protein synthesis machinery [44]. The increase in total free amino acids level was the result of breakdown of protein for energy requirements and impaired incorporation of amino acids in protein synthesis. It is also attributed to lesser use of amino acids [45] and their involvement in the maintenance of an acid-base balance [46]. The protease enzyme functions in hydrolyzing proteins to free amino acids and small peptides. Increased protease activity in the body tissues of treated snails was evidence that proteins had undergone degradation processes such as proteolysis and used the degrade products for increased energy metabolism in stress conditions. Similar results were also reported by several researchers in different animals as *Tilapia mossambica*, *Pila globosa* and various mammals [47-50].

CONCLUSION

It concluded that the plant *L. indica* has molluscicidal effect against *L. acuminata* snails and is considered a potential source of herbal and safer molluscicides which are the need today. This study would also be helpful in promoting to develop a new agent for snail control based on bioactive chemical compounds from indigenous plant sources. Furthermore the developmental study should be done in the pond to check its efficacy in natural condition.

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