Molluscicidal potential of Lantana indica and Alstonia scholaris plants against freshwater snail Lymnaea acuminata

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Citation

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Abstract

Laboratory evaluation was made to assess the molluscicidal activity of acetone extracts of Alstonia scholaris (family- Apocynaceae) and Lantana indica (family- Verbenaceae) leaf against freshwater snail Lymnaea acuminata. This snail is intermediate hosts of liverfluke, Fasciola hepatica and Fasciola gigantica, which causes endemic fascioliasis in cattle and livestock. The toxic effect of both the plants extracts was time as well as dose dependent. There was a significant negative correlation between LC values and exposure periods, thus the LC_{50} values of Alstonia scholaris leaf acetone extract (ASLAE) was decreased from 25.05 mg/L (24h) to 15.32 mg/L (96h), and from 06.01 mg/L (24h) to 01.31 mg/L (96h) in the case of Lantana indica leaf acetone extract (LILAE). Binary mixture of Lantana indica leaf acetone extract (LILAE) + Alstonia scholaris leaf acetone extract (ASLAE) in 1:5 or 1:1 ratio also shows significant synergistic effect against Lymnaea acuminata. Sub-lethal exposure (20% and 40% of LC_{50} of 24h) of LILAE and ASLAE either individually or in binary combinations, caused significant reduction in the fecundity, hatchability (of eggs) or survival of the hatchlings (hatched young snails) in comparison to control group. On the other hand exposure of sub-lethal doses of this extract also shows significant time and dose dependent alterations in the level of total protein, total free amino acids, glycogen, nucleic acids and the activity of enzyme protease in nervous and hepatopancreas tissue of snail Lymnaea acuminata. Seven days withdrawal experiment shows, there was highly significant ($p<.05$) recovery in all the biochemical parameters in both the tissues of snail. Conclusion: Therefore, acetone extract of leaf of Alstonia scholaris and Lantana indica leaf may eventually be of great value for the control of harmful aquatic snails and other molluscan pests.

INTRODUCTION

Freshwater trematode Lymnaea acuminata is the intermediate host of the Fasciola hepatica and Fasciola gigantica ([1]), which causes endemic fascioliasis in cattle and livestock ([2]). In the Eastern part of Uttar Pradesh, India, the population of this vector snail is pronounced, so the occurance of this disease is very common in this region. Although the snails do not play an active role in transmission of the parasite from one host to other, as do insect vectors; it is an indispensable intermediate host for the development of the parasite ([3]). A large variety of animals, such as sheep, goat, cattle buffalo, horses donkeys, deer, rats, camels and rabbits, show infection rates that may reach 90% in some areas ([4], [5]). According to a World Health Organization ([6]) report the infection was limited in the past to specific and typical geographical areas, but is now widespread throughout the world. One way to tackle the problem of fascioliasis is to destroy the carrier snail and thus remove link in the life cycle of parasite ([7]). This may be achieved with the aid of molluscicides, ([8], [9], [10]). Owing to long term persistence and their toxic effects on non-target aquatic organisms ([11]), the use of synthetic molluscicides is limited. Botanical molluscicides provide an ideal source of low cast, safe and effective molluscicides ([12], [10]).

From ancient time, plants are rich source of effective and safe compounds which are used for different purposes i.e. traditional medicines and control of pests and vectors ([13]). So control of vector snail through plant origin molluscicides is a very effective and new tool of integrated vector management programme.

Plant Lantana indica (Family: Verbinaceae) commonly known as “Indian Lantana” or “Wild Sage”. The leaves of Lantana indica are regarded as a cure for snakebite. Its different parts are used in traditional medicine for the treatment of the various human ailments such as ulcer,

eczema eruption, malaria and rheumatism ([14, 15, 16]). Plant Alstonia scholaris (Family: Apocynaceae) commonly known as "Chatiun". It is large buttressed, evergreen tree. The stem bark of Alstonia scholaris is to be employed in heart diseases, asthama, chronic diarrohea and to stop bleeding of wounds. Latex of this plant is applied to ulcers, sores, tumours, in rheumatic pain and also used for curing tooth ache. In present study, toxicity of LILAE and ASLAE were tested against snail Lymnaea acuminata individuallly or in binary mixtures as well as their sub-lethal effect on its fecundity (egg laying), hatching, and survival of hatchlings and biochemical profiles of the snail were observed.

MATERIALS AND METHODS

Plant: The leaves of the plant Lantana indica and Alstonia scholaris were collected from the botanical garden of DDU, Gorakhpur University, Gorakhpur, Uttar Pradesh, India. First of all, leaves of both the plants were washed separately with water and then dried in an incubator at about 37 C. Then dried leaves of Lantana indica and Alstonia scholaris were powdered with the help of mechanical device.

Extraction of compounds: 50 gram powder of leaf of Lantana indica or Alstonia scholaris were subjected to extraction through Soxhlet apparatus in 350 ml acetone 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 and further used for experiments.

Animal: 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 ([17]). Twenty animals were kept in glass aquaria containing 3L de-chlorinated tap water. Snails were exposed for 24h, 48h, 72h or 96h at four different concentrations of single or binary mixtures of both the plants extracts, control animals were kept in similar condition without any treatment. Each set of experiments, were 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., ([18]). 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 ([19]).

FECUNDITY HATCHABILITY AND SURVIVABILITY EXPERIMENT

These experiments were performed according to the method of Presing, ([20]). In this experiment, fresh water adult Lymnaea acuminata were exposed to different sub-lethal [20% & 40 % of LC_{50} (24h)] doses of Lantana indica and Alstonia scholaris leaf acetone extract. 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 experiment, water temperature were kept at 25±1ºC 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 experiment 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 petri-dishes containing one litter de- chlorinated tap water for hatching under the same exposure condition as above and kept at 25 ±1ºC 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 experimental animals were treated with sub-lethal doses, i.e. 40% (2.33 mg/L) and 80% (4.67 mg/L) of LC_{50} of 24h of binary mixture of LILAE+ASLAE (in 1:1 ratio) for 96h exposure periods. Control groups were kept under similar conditions without any treatment, after completion of 96 hrs.

the hepatopancreas and nervous tissue (brain tissue) of the treated as well as control group animal were quickly dissect out and used for bio-chemical estimations. In order to see the effect of 7th day of withdrawal, animals were exposed to sub lethal doses i.e. 80% of LC_{50} of 24h in binary mixture of LILAE+ASLAE in 1:1 ratio for 96h exposure periods. After 96h animals were transferred to freshwater free from any treatment, water was changed every 24h for the next six day. After completion of 7th day the nervous and hepatopancreas tissue was quickly dissect out and used for bio-chemical parameters.

Total free amino acid: Estimation of to total free amino acid was made according to the method of Spies 1957 ([21]). Homogenates (10 mg/mL, w/v) were prepared in 95% ethanol, centrifuged at 6000 xg and used for amino acid estimation.

Protein: Protein levels were estimated according to the method of Lowry et al., 1951 ([22]) using bovine serum albumin as standard. Homogenate (5 mg/mL, w/v) were prepared in 10% TCA.

Glycogen: Glycogen was estimated by the Anthrone method of Vander Vies 1954 ([23]) as modified by Mahendru and Agarwal 1982 ([24]) for snail L. acuminata. 50 mg of tissue were homogenised with 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) performed by the method of Schneider 1957 ([25]) using diphenylamine and orcinol reagents, respectively. Homogenates (1 mg/mL, w/v) were prepared in 5% TCA and centrifuged at 5000 xg for 20 minute and supernatant was prepared and used for estimation.

Activity of enzyme protease: Protease activity was measured according to the method of Moore and Stein 1954 ([26]); homogenate (50 mg/L, w/v) was prepared in cold distilled water (0^{o} C) and optical density was measured at 570 nm. Protease activity is expressed in as micromoles of tyrosine equivalents per milligram of protein /hour.

RESULT

TOXICOLOGICAL OBSERVATIONS (TABLE1 AND FIG. 1)

Behavioral change was seen after few minutes of exposure to the extracts. In initial 30-45 minutes snails were started to aggregating, and they started crawling on each others. As poison enters in the body of snails, there was a muscular twitching and snails become spirally twisted, which resulted ataxia, convulsion, paralysis and finally death of snails. In control groups, there were no such behavioural response and symptoms occur and there was no death also. The contraction of the body within the shell and no response to a niddle probe were taken as evidence of snail death.

The toxicity of both plant extracts were 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 decreased from 25.05 mg/L (24h) to 15.32 mg/L (96h) and 06.01 mg/L (24h) to 01.31 mg/L (96h) in case of ASLAE and LILAE, respectively (Table1). In binary mixtures of the extracts, LC values decreased from 07.07 mg/L (24h) 03.43 mg/L (96h) and 05.84 mg/L (24h) to 03.30 mg/L (96h) in case of LILAE + ASLAE in (1:5) ratio and in (1:1) ratio, respectively (Fig. 1).

The steep slope value given in the toxicity table was steep and the heterogeneity factor was less than 1.0, which indicates that the results found fall within 95% confidence limits of LC values. The regression test ('t' ratio) was greater than 1.96 and the potency estimation test ('g' value) was greater than 0.5 at all probability levels

Figure 1

Table 1. Toxicity (LC values) of different concentrations of ASLAE and LILAE against freshwater snail at different time intervals.

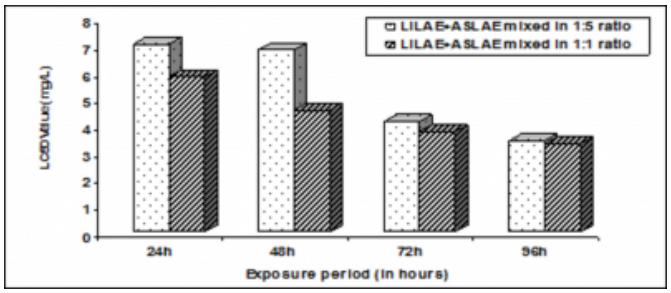
Exposure periods	Effective dose (mg/L)	Limits (mg/L)		Slope value	't' ratio	Hetero-geneity
		LCL	UCL			
Alstonia scholaris						
24h	LC_{50} = 25.05	22.98	28.71	4.08±0.61	6.64	0.74
48h	LC_{50} = 23.05	21.30	25.93	3.83±0.59	6.50	0.26
72h	LC_{50}= 17.85	16.53	19.15	3.81±0.56	6.74	0.86
96h	LC_{50}= 15.32	12.43	17.21	5.35±0.60	8.80	0.65
Lantana indica						
24h	LC_{50} = 06.01	4.06	14.97	1.56±0.21	7.36	0.30
48h	LC_{50} =04.04	2.31	9.78	1.37±0.19	7.08	0.22
72h	LC_{50}= 02.07	1.23	2.87	1.90±0.19	9.59	0.45
96h	LC_{50}= 01.31	1.04	1.56	2.09±0.21	9.88	0.85

Batches of twenty snails were exposed to four different concentrations of the extract. Concentrations given are the final concentration (w/v) in the aquarium water containing de-chlorinated tap water. Each set of experiment was replicated six times. Mortality was recorded after every 24h. 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. There was no mortality in the control group.

Figure 2

Fig.1. Bar diagram showing the toxicity of binary mixtures (LC value in mg/L) of LILAE + ASLAE mixed in 1:5 or 1:1 ratio against freshwater snail at different time intervals.



FECUNDITY, HATCHABILITY AND SURVIVABILITY EXPERIMENT (TABLE 2, 3 AND 4)

The results of the fecundity, hatchability and survivability experiment on the freshwater snail Lymnaea acuminata are given in Table 2, 3 and 4. There was significant reduction in the cumulative number of laid eggs (fecundity), number of hatched eggs and survival of hatchlings (i.e. hatched young snails) after exposure to sub-lethal doses (i. e. 20% and 40% of LC_{50} of 24h) of acetone extracts of both the plants individually or in binary mixtures of 1:1 ratio.

Treatment of snail with sub lethal doses (20% and 40% of LC_{50} of 24h) of LILAE (Table 2), fecundity was reduced to 88.77% to 83.30% of control after 96h exposure periods, and the number of hatched eggs was reduced to 80.33% to 69.63% of control. The survival rate of the hatched snails was greatly reduced to 73.48% to 62.91% of control after 7 days of hatching and it was further reduced to 17.99% to 5.86% of control after 28 days of hatching, respectively.

Treatment of snail with sub-lethal doses 20% and 40% of LC_{50} (24h) of ASLAE (Table 3), caused reduction in fecundity to 88.17% to 80.17% of control, and reduction in number of hatched egg to 78.41% to 72.68% of control. The survival rate of hatched snails was reduced to 71.49% to 67.22% of control after 7 days of hatching and it was again reduced to 23.50% and 10.85% of control after 28 days of hatching, respectively.

Figure 3

Table 2. Numbers of laid eggs, egg masses duration of hatching, hatched eggs, and survivability of hatched young snails (hatchlings) after treatment with 20% and 40% of LC (24h) of leaf acetone extract to the freshwater snails, .

	Control	20% of LC_{50}(24h) (1.20mg/L)	40% of LC_{50}(24h) (2.40 mg/L)
No. of laid eggs (after 96 h treatment)	454.5±0.83 (100)	403.50±0.83* (88.77)	378.66±0.73* (83.30)
No. of eggs masses	15.50±0.83 (100)	12.16±0.65 (76)	11.16±0.65 (69.75)
Duration of hatching (in days)	10-13	12-13	12-13
No. of hatched eggs	435.16±0.86 (100)	364.00±0.63* (80.33)	315.50±0.83* (69.63)
Survivability of hatchlings			
After 7 days	447.16±0.77 (98.67)	267.50±0.83* (73.48)	198.50±0.83* (62.91)
After 14 days	445.83±0.65 (98.38)	233.50±0.83* (63.87)	146.50±0.83* (46.43)
After 21days	443.83±0.65 (97.94)	166.16±1.03* (45.63)	65.50±0.83* (20.76)
After 28 days	442.33±0.78 (97.61)	65.50±0.83* (17.99)	18.50±0.83* (5.86)

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.

Figure 4

Table 3. Numbers of laid egg, egg masses, and hatched eggs, duration of hatching, and survivability of hatched young snails (hatchlings) after treatment with 20% and 40% of LC(24h) of leaf acetone extract to the freshwater snails, .

	Control	20% of LC_{50} (24h) (5.01 mg/L)	40% of LC_{50} (24h) (10.02 mg/L)
No. of laid eggs (after 96h treatment)	524.50±0.83 (100)	462.50±0.83* (88.17)	420.50±0.83* (80.17)
No. of eggs masses	17.5±0.83 (100)	15.50±0.46 (88.57)	14.83±0.65 (85.71)
Duration of hatching (in days)	10-13	10-13	10-13
No. of hatched eggs	523.50±0.83 (100)	410.50±0.83* (78.41)	380.50±0.83* (72.68)
Survivability of hatchlings			
After 7 days	518.50±0.83 (99.04)	293.50±0.83* (71.49)	255.83±0.365* (67.22)
After 14 days	516.50±0.83 (98.66)	262.50±0.83* (63.94)	185.50±0.83* (48.75)
After 21days	512.50±0.83 (97.89)	178.83±0.65* (43.55)	120.50±0.83* (31.66)
After 28 days	511.50±0.83 (97.70)	96.50±0.83* (23.50)	41.33±0.96* (10.85)

Other details are as given in Table 2.

Similarly the binary mixture of extract i.e. 20% and 40% of LC_{50} (24h) LILAE + ASLAE in 1:1 ratio (Table 4) also caused reduction in the fecundity of snail Lymnaea acuminata, here the fecundity was reduced to 71.16% to 57.96% of control after 96h exposure period, and the number of hatched eggs was reduced to 66.48% to 55.38% of control

respectively (Table 4). The survival rate of hatched eggs after 20% of LC_{50} treatment was only 64.85% of control after 7 days of hatching, and 29.52% after 21 days of hatching, and there was no survival of hatched egg was observed after 28 days of hatching in 20% of LC_{50} treatment. In the case of 40% of LC_{50} treatment the number of hatched was reduced to 52.50% of control after 7 days of hatching, and it was again reduced to 38.40% only after 14 days of hatching, there was no survival was observed after 21 days after hatching.

Figure 5

Table 4. Numbers of laid egg, egg masses, and hatched eggs, duration of hatching, and survivability of hatched young snails (hatchlings) after treatment with 20% and 40% of LC(24h) of leaf acetone extract+ leaf acetone extract in 1:1 ratio to the freshwater snails, .

	Control	20% of LC_{50} (24h) (1.16 mg/L)	40% of LC_{50} (24h) (2.33 mg/L)
No. of laid eggs (after 96 hrs. of exposure)	492.50±0.83 (100%)	350.50±0.83* (71.16%)	285.50±0.83* (57.96%)
No. of eggs masses	14.50±0.83	10.50±0.83	9.50±0.83
Duration of hatching (in days)	10-12	11-13	11-13
No. of hatched eggs	486.66±0.96 (100%)	326.50±0.83* (66.48%)	269.50±0.83* (55.38%)
Survivability of hatchlings			
After 7 days	485.33±0.83 (99.73%)	209.83±0.66* (64.85%)	141.50±0.83* (52.50%)
After 14 days	481.66±0.96 (98.97%)	141.16±0.95* (43.61%)	103.50±0.83* (38.40%)
After 21days	478.83±0.66 (98.39%)	95.50±0.83* (29.52%)	-
After 28 days	474.50±0.83 (97.51%)	-	-

-, No survivability was recorded. Other details are as given in Table 2.

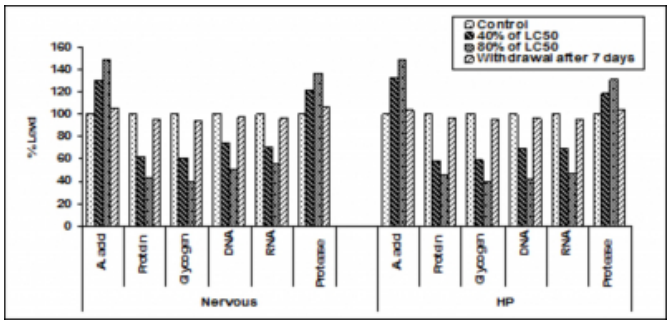
BIOCHEMICAL ESTIMATIONS: (FIG. 2)

Sub-lethal doses (40% and 80% of LC_{50} of 24h) of binary mixture of LILAE + ASLAE in 1:1 ratio, 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 hepatopancreas tissue of the snail Lymnaea acuminata (Fig. 2). Total protein level was significantly reduced to 61.63% to 43.96% of control in nervous and 57.83% to 44.86% of control in hepatopancreas tissue respectively. Glycogen level was reduced to 61.33% to 40% of control in nervous tissue and 58.655 to 39.90% of control in hepatopancreas tissue. DNA level was reduced to 75.22% to 50.44% and 69.35% to 41.93% of control and RNA level was reduced to 70.83% to 55.20% and 69.44% to 46.29% of control in nervous and hepatopancreas tissue respectively. Control group was taken as 100%. While total free amino acid level was significantly increased and it was 130.41% to 148.21% of control in nervous tissue and 132.35% to 149.15% of control in hepatopancreas tissue respectively. Protease activity was significantly increased to 121% to 136% of control in nervous tissue and 118% to 131% of control in hepatopancreas tissue of snail.

Students 't' test showed that these biochemical changes were significantly ($p<0.05$) time and dose dependent. Seven days withdrawal experiment shows, there was highly significant ($p<0.05$) recovery in all the above biochemical parameters in both the tissues of snail Lymnaea acuminata.

Figure 6

Fig. 2. Bar diagram showing the percent level of total free amino acid (Âµg/mg), total protein (Âµg/mg), glycogen (mg/g), DNA and RNA (Âµg/mg), protease level (Âµmol tyrosine/mg protein/h) in nervous and hepatopancreas (HP) tissue of the freshwater snail after 96h exposure to 40% and 80% of LC (24h) of binary combination of acetone extract of leaf in 1:1 ratio, and 7 days after withdrawal.



DISCUSSION

It is evident from results section that Alstonia scholaris and Lantana indica leaf are toxic against snail Lymnaea acuminata. These plant extract bring out significant behavioural changes in the snail Lymnaea acuminata. In initial 30-45 minutes snails were started to aggregating and started crawling on each other. As poison (acetone extract) enters in the body of snails, there was hyperactivity, muscular twitching, ataxia, convulsion, paralysis and finally complete withdrawal of body from the shell prior to death. In the control group, no such behavioural symptoms and death occurred, that means no other factor other than the plant moieties were responsible for alterations in behaviour and mortality of snail. Similar behavioral responses were also observed by Singh and Agarwal ([27]) in the case of Euphorbia royleana, Jatropha gossypifolia plant extracts against snail Lymnaea acuminata.

From result section it is also clear that there was a positive correlation between exposure period and mortality. The increases in mortality with increase in exposure period could be due to several factors, which may be acting separately are conjointly. The uptake of the active moiety of acetone extract of both plant Lantana indica and Alstonia scholaris could be time dependent leading to a progressive increase in the titre of the active ingredients and it effects in the snails ([28, 17]).

Statistical analysis of the data on toxicity brings out several important points. The X^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. The slope is thus an index of the susceptibility of the target animal to the pesticides used. A steep slope is also indicative of rapid absorption and onset of effects. Even though the slope alone is not a very reliable indicator of toxicological mechanism, yet it is a useful parameter ([29]) for such a study. Since the LC_{50} of the plant extracts lay within the 95% confidence limits, it is obvious that in replicate test of random samples, the concentration response lines would fall in the same range ([29]).

It is also clear from the result section that acetone extract of both the plants in binary combination are more effective than individual extract towards snail. It seems that action of binary mixture is non-interactive as the components do not affect the transport and final concentration of each other at the site of action. Moreover they also do not influence the changes induced by each other at the site of action ([30,31]).

In the developmental study of snail it was found that number of laid egg (fecundity), number of hatched eggs, survival rate and development of the hatchlings reduced after exposure to all the sub-lethal doses of the acetone extracts of both the plants (Table 2, 3 and 4). There was a significant reduction in the fecundity (egg laying) was observed in comparison to control groups, it may be due to the significant reduction in the level of glycogen and protein content in the different body tissues of snail Lymnaea acuminata after the exposures of the acetone extracts of leaf of Lantana indica and Alstonia scholaris in different concentrations using as individually or in binary combinations. It can also be inferred from the results of Adewunmi et al., 1987 ([32]) 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, an amount that depends on seasonal, nutritional or reproductive factors ([33,34,35,36]).

The hatchability of eggs were also significantly decreased (Table 2, 3, and 4) after exposure to the acetone extract than control group. A similar trend of observations was reported by Mostafa and Tantawy, 2000 ([37]), they observed that Calendula micrantha and Anagallis arvensis plant induced significant reduction in hatchability of egg masses of snail Biomphalaria alexandrina than the control group.

The survivability of the hatched young snails were significantly decreased than the control group after exposure to sub lethal doses of acetone extracts of Lantana indica and Alstonia scholaris either singly or in binary combinations, and survival rate was reduced to zero in both 20% treatment after 28 days and after 21days in 40% treatment. (Table 2, 3, and 4). Mostafa and Tantawy, 2000 ([37]) also reported similar result, that the survival rate of snail B. alexandrina, maintained in aqueous solutions of the two plants (Calendula micrantha and Anagallis arvensis) decreased gradually with time until the 9th and 10th week where the survival rate was zero in the higher concentration of Anagallis arvensis and Calendula micrantha, respectively. Deformations in developing eggs were also observed during developmental period. This data is supported by Zhang and Guo ([38]), that the eggs of Oncomelania snails exposed to bromoacetamide showed the deformation in the earlier stages but not in the later stages.

From biochemical results it is clear that these extract alters the different biochemical parameters. Total protein level, glycogen and nucleic acid level were significantly reduced while total free amino acid and protease level was significantly increased. The depletion of protein fraction in nervous and hepatopanceas tissue of snail may be due to their degradation and possible utilization of degraded products for metabolic purposes. The enzyme protease functions in hydrolyzing proteins to free amino acids and small peptides. Increased protease activity in the body tissue of both groups was evidence that proteins had undergone degradation processes such as proteolysis and used the degrades products for increased energy metabolism. Similar trend in protease has been reported by several workers in

different animals as Tilapia mossambica, Pila globosa and various mammals, ([39,40,41,42]). The increase in free amino acid 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 ([43]) and their involvement in the maintenance of an acid-base balance ([44]). The decrease in the glycogen content in tissues indicates its rapid utilization by the perspective tissues as a consequence of toxic stress felt by the snails during the experiment. Inhibition in DNA synthesis might affect both protein as well as amino acid levels by decreasing the level of RNA in protein synthesis machinery ([45]). Similar results were also observed by Yadav et al., 2005 ([46]).

Withdrawal experiment indicates that the toxicity of leaf extract of both the plant against snail was significantly reversible at 7 days of withdrawal from treatment. The reversibility of the action of plant extract despite of the high toxicity would be an added advantage in their use.

It is our belief that the use of Lantana indica and Alstonia scholaris plant moieties either singly or in combinations for management of harmful snail population would be less expensive and may be less hazardous to environment than synthetic molluscicides.

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