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Molluscicidal Effect of Medicinal Plant *Euphorbia tirucalli* on the Harmful Snails in Experimental Ponds

Saroj Chauhan and Ajay Singh

Department of Zoology, Natural Product Laboratory,
D.D.U. Gorakhpur University, Gorakhpur-273009 (U.P.) India

Abstract: Snail borne infections are of significant importance in livestock production in India especially in eastern Uttar Pradesh. Snails *Lymnaea acuminata* and *Indoplanorbis exustus* live in muddy vicinity and standing water and act as the intermediate hosts of the parasitic disease fascioliasis in the livestock. This disease is responsible for significant economic losses due to negative effects to animal health and productivity. A better approach to tackle the problem is to destroy the carrier snails and remove an essential link in the life cycle of the flukes. In the present study, toxic effect of acetone extract of stem bark of plant *Euphorbia tirucalli* were performed against two harmful snails *L. acuminata* and *I. exustus* in the experimental pond. The toxicity of acetone extract was time as well as dose dependent and there was a significant negative correlation between LC values and exposure periods, thus the LC_{50} value was decreased from 53.58 mg/L (24h) to 19.47 mg/L (96h) in pond. Similar results were obtained in the case of snail *I. exustus*. Sub lethal doses of the plant extract changed the biochemical profiles of both snails significantly. So, it can be concluded that this extract was toxic against both the snails in pond. Therefore, it can be said that acetone extract of *E. tirucalli* plant is toxic against both snails and can be helpful in management of fascioliasis disease eco-friendly.

Key words:*Lymnaea acuminata* · *Indoplanorbis exustus* · *Euphorbia tirucalli* · Fascioliasis · Acetone Extract · Toxicity

INTRODUCTION

Parasitic diseases are among the Third World's three great killers. *Fasciola hepatica* is a parasite that infests humans and many species of animals. The freshwater snails are intermediate hosts of helminthes worms causing diseases in domestic animals [1-3]. Aquatic snails *Lymnaea acuminata* and *Indoplanorbis exustus*, are intermediate hosts of the liver fluke *Fasciola hepatica*, which are found in large numbers in the ponds, ditches, lakes, streams and canals and causes endemic fascioliasis in human and herbivores animals. One of the method for the control of this disease is the use of molluscicides and the urge for the use of plant molluscicides has received increased interest primarily because it could be an appropriate and inexpensive technology for snail control in endemic poor nations of the world. The success of *Fasciola hepatica* as a parasite is related to its ability to infect and complete its lifecycle in wide range of mammalian hosts. A large variety of animals, such as sheep, goats, buffaloes, horses, donkeys, deer, rats, camels and rabbits, show infection rates that may reach 90% in some areas [4,5]. Destroying the snails which harbour the developing *Fasciola* larvae is a way to interrupt the parasites life cycle and prevent human and livestock infections is regarded by many experts [2, 6-9]. Today, use of botanical molluscides is considered as an important strategy in control of the snail hosts. The use of plants with molluscicidal properties appears to be a simple, inexpensive and safe alternative [10]. In present investigation molluscicidal properties of acetone extract of stem bark of medicinal plants *Euphorbia tirucalli* were evaluated against these snails in pond.

MATERIALS AND METHODS

Plant: The stem bark of the plant *Euphorbia tirucalli* was collected from the botanical garden of DDU, Gorakhpur University, Gorakhpur, Uttar Pradesh, India.

Corresponding Author: Saroj Chauhan, Department of Zoology, Natural Product Laboratory,
D.D.U. Gorakhpur University, Gorakhpur-273009 (U.P.) India.

First of all, stem bark was washed with water and then dried in shade. Then dried stem bark was powdered with the help of mechanical device.

Extraction of Compounds: 300 grams powder of stem bark was subjected to extraction through Soxhlet apparatus in acetone solvent for about 50 hours, after that a concentrated solution was obtained. After evaporation of solvent, the extracted compound in dried form was obtained. This extracted compound was stored in air-tight desiccator and further used for experiments.

Animals: The two fresh water harmful snails *Lymnaea acuminata* (3.65±1.00 cm total shell length) and *Indoplanorbis exustus* (0.85±0.037 cm in shell length) were collected from the fresh water bodies of Gorakhpur district (India). Prior to experiment, snails were allowed to acclimate to laboratory conditions for 72h.

Toxicity Experiment: The experiment was conducted in cemented experimental ponds. Each pond was stocked with 100 snails with a size difference not greater than 1.5 times [11]. The experimental animals were exposed continuously from 24h up to 96h to four different concentrations. Control groups were kept under similar conditions without any treatment. 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 pond.

Lethal concentration (LC) values for different exposure periods, lower confidence limits (LCL) and upper confidence limits (UCL), slope value, 't' ratio and heterogeneity were calculated by using probit log analysis of POLO Plus computer programme of Robertson *et al.* [12].

Toxicity Against Non-target Organism: Mixed population of snails (*L. acuminata/I. exustus*) and fishes (*Channa punctatus*) were treated in dechlorinated water with the LC_{90} (24h) of acetone extract of the plant *E. tirucalli* stem bark, caused no mortality amongst non target fish *Channa punctatus* which shares the habitat with these snails.

Biochemical Estimations: The biochemical experiments were performed by the method of Tripathi and Singh [13]. The biochemical experiment was conducted in ponds. Each pond was stocked with 50 snails with a size difference not greater than 1.5 times [11]. The experimental animals were treated with two different sub-lethal doses, i.e. 40% and 80% of LC_{50} (24h) acetone extract for 96h. Control groups were kept under similar conditions without treatment for same duration. Diet was not given to the snail during the course of experiment. After the completion of 96h of treatment snails were removed from the treated water and the nervous and hepatopancreas tissues of both the treated as well as control group were quickly dissect out and used for biochemical 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} (24h) of the extract for 96h exposure periods. After 96h animals were transferred to freshwater free from any treatment for the seven days. After completion of 7th day, the nervous and hepatopancreas tissues of both snails were quickly dissect out and used for bio-chemical parameters.

Total Free Amino Acid: Estimation of total free amino acid was made according to the method of Spies [14]. 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.* [15] 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 [16] as modified by Mahendru and Agarwal [17]. 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 [18], 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.

Enzyme Protease: Protease activity was measured according to the method of Moore and Stein [19], homogenate (50 mg/L, w/v) was prepared in cold distilled water (0° C) and optical density was measured at 570 nm. Protease activity is expressed in as micromoles of tyrosine equivalents per milligram of protein /hour.

RESULTS

Toxicity: The toxicity of acetone extract was time and dose-dependent against both the snails *L. acuminata* and *I. exustus*. There was a significant negative correlation between LC values and exposure periods (Tables 1 and 2). Thus with increase in exposure periods the LC_{50} value of extract was decreased from 53.58 mg/L (24h); >42.09mg/L (48h); >27.64mg/L (72h); >19.47mg/L (96h) against *L. acuminata* (Table 1) and from 61.4mg/L (24h); > 47.63mg/L (48h); > 30.44mg/L (72h); >23.94mg/L (96h) in the case of snail *I. exustus* (Table 2) respectively.

There was no mortality in control group. Hundred snails were exposed to four different concentrations of the extract. Concentrations given are the final concentration (w/v) in the de-chlorinated tap 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).

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Biochemical: Sub-lethal doses (40% and 80% of LC_{50} of 24h) of acetone extract of *E. tirucalli*, caused significant alterations in the level of total protein, total free amino acids, glycogen, nucleic acids and enzyme protease in nervous and hepatopancreas tissue of the snail *L. acuminata* and *I. exustus* (Figs. 1 and 2).

Lymnaea Acuminata: Total protein level was significantly reduced to 77% and 66% of control in nervous tissue and 80% and 67% of control in hepatopancreas tissue after exposure to 40% and 80% of LC_{50} (24h) of plant extract

Table 1: Toxicity (LC values) of different concentrations of acetone extracts of *Euphorbia tirucalli* stem bark against freshwater snail *Lymnaea acuminata* at different time intervals in experimental pond.

		Limits (mg/L)				
Exposure periods	Effective dose (mg/L)	LCL	UCL	Slope value	't' ratio	Hetero-geneity
24h	LC_{10} = 9.39	4.43	13.68	1.69±0.30	5.25	0.41
	LC_{50} = 53.58	44.22	73.11			
	LC_{90} = 305.65	170.75	407.76			
48h	LC_{10} = 7.27	3.11	11.14	1.68±0.29	5.98	0.07
	LC_{50} = 42.09	35.38	52.85			
	LC_{90} = 243.49	144.23	697.62			
72h	LC_{10}= 4.64	1.63	7.82	1.65±0.28	6.23	0.58
	LC_{50}= 27.64	22.14	32.96			
	LC_{90}= 164.70	106.29	391.14			
96h	LC_{10}= 4.21	2.88	7.89	2.30±0.30	5.36	0.94
	LC_{50}= 19.47	15.62	22.74			
	LC_{90}= 70.14	56.79	97.83			

Table 2: Toxicity (LC values) of different concentrations of acetone extracts of *Euphorbia tirucalli* stem bark against freshwater snail *Indoplanorbis eustus* at different time intervals in experimental pond.

		Limits (mg/L)				
Exposure periods	Effective dose (mg/L)	LCL	UCL	Slope value	't' ratio	Hetero-geneity
24h	LC_{10} = 12.51	6.19	17.59	1.85±0.34	4.36	0.10
	LC_{50} = 61.4	51.06	84.58			
	LC_{90} = 301.31	171.84	996.48			
48h	LC_{10} = 10.68	5.40	15.17	1.97±0.33	5.36	0.31
	LC_{50} = 47.63	41.03	58.19			
	LC_{90} = 212.34	135.98	511.30			
72h	LC_{10}= 9.87	6.15	13.15	2.62±0.34	7.12	0.76
	LC_{50}= 30.44	26.40	34.18			
	LC_{90}= 93.87	75.54	133.11			
96h	LC_{10}= 8.09	4.82	11.08	2.72±0.35	4.23	0.56
	LC_{50}= 23.94	19.93	27.29			
	LC_{90}= 70.86	59.35	93.31			

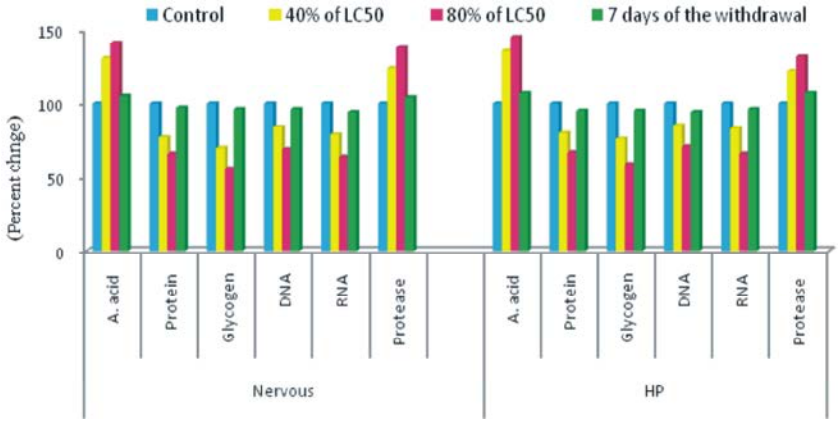

Fig 1: Bar diagram showing the percent change in the level of total free amino acid (µg/mg), total protein (µg/mg), glycogen (mg/g), DNA and RNA (µg/mg) and enzyme protease (µmol tyrosine/mg protein/h) in nervous and hepatopancreas (HP) tissue of the freshwater snail *Lymnaea acuminata* after 96h exposure to 40% and 80% of LC_{50} (24h) of acetone extract of stem bark of *E. tirucalli* and 7 days after withdrawal.

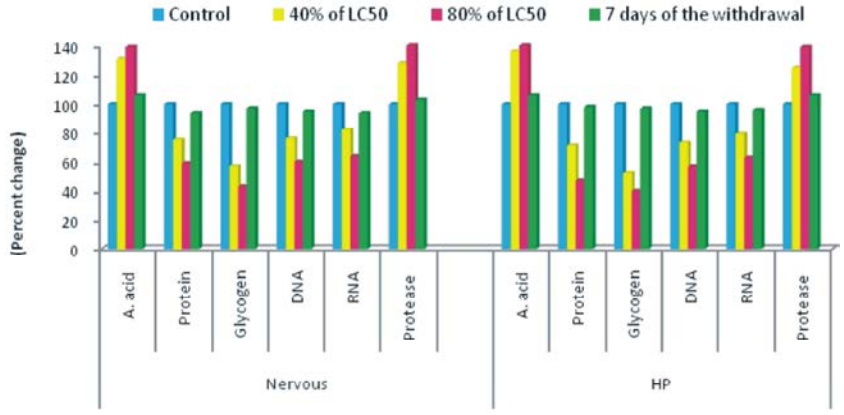

Fig 2: Bar diagram showing the percent level of total free amino acid (µg/mg), total protein (µg/mg), glycogen (mg/g), DNA and RNA (µg/mg) and enzyme protease (µmol tyrosine/mg protein/h) in nervous and hepatopancreas (HP) tissue of the freshwater snail *Indoplanorbis exustus* after 96h exposure to 40% and 80% of LC_{50} (24h) of acetone extract of stem bark of *E. tirucalli* and 7 days after withdrawal.

respectively. Glycogen level was reduced to 70% and 56% of control in nervous tissue and 76% and 59% of control in hepatopancreas tissue respectively. DNA level was reduced to 84% and 69% and 85% and 71% of control and RNA level was reduced to 79% and 64% and 83% and 66% of control in nervous and hepatopancreas tissue respectively. While total free amino acid level was significantly increased and it was 131% and 141% of control in nervous tissue and 136% and 145% of control in hepatopancreas tissue respectively. Protease activity was significantly increased to 124% and 138% of control in nervous tissue and 122% and 132% of control in hepatopancreas tissue of snail after exposure to 40% and 80% of LC_{50} (24h) respectively (Fig. 1). Control group was taken as 100%.

Indoplanorbis Exustus: Total protein level was significantly reduced to 75% and 59% of control in nervous and 71% and 47% of control in hepatopancreas tissue respectively after exposure to 40% and 80% of LC_{50} (24h). Glycogen level was reduced to 57% and 43% of control in nervous tissue and 52% and 40% of control in hepatopancreas tissue respectively. DNA level was reduced to 76% and 60% of control in nervous and 73% and 57% of control in hepatopancreas tissue and RNA level was reduced to 82% and 64% in nervous and 79% and 63% of control in hepatopancreas tissue respectively. While total free amino acid level was significantly increased and it was 131% and 139% of control in nervous tissue and 136% and 140% of control in hepatopancreas tissue respectively. Protease activity was significantly

increased to 128% and 140% of control in nervous tissue and 125% and 139% of control in hepatopancreas tissue of snail after exposure to 40% and 80% of LC_{50} (24h) respectively (Fig. 2). Control group was taken as 100%.

Students 't' test showed that these biochemical changes were significantly (p<0.05) time and dose dependent. Seven days withdrawal experiment shows, there was significant (p<0.05) recovery in about all the above biochemical parameters in both the tissues of snails *L. acuminata* as well as *I. exustus*.

DISCUSSION

From the above result, it is apparent that there is a positive correlation between exposure period and mortality. Mortality of experimental animals increased with time. The uptake of the active moiety of acetone extract of plant *E. tirucalli* could be time dependent leading to a progressive increase in the titre of the active ingredients and it effects in both the snails [20, 21]. In natural condition, many factors such as temperature, sunlight, adsorption by soil particles etc. influences on toxicity and toxicant degradation. The toxicity of the experimental plant extract was decreased about four times in the pond than laboratory condition. Similar result was also obtained by Chauhan *et al*. [22] of the plant *Lantana indica* against snail *L. acuminata*. Chauhan and Singh [23], reported LC_{50} value of same plant extract in laboratory and LC_{50} value is 12.27mg/L (24h) against *L. acuminata*. Many researchers found that toxicity of toxicants were degraded in natural conditions [22, 24-26].

From biochemical results, it is clear that this extract alters the different biochemical parameters of both snails. 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 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. 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 [27]. 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 activity has been reported by several workers in different animals as *Tilapia mossambica*, *Pilaglobosa* and Various mammals, [28-31]. Withdrawal experiment indicates that the toxicity of acetone extract of the plant against both snails was significantly (p<0.05) 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.

CONCLUSION

It can be concluded from this investigation that *E. tirucalli* plant is toxic to both the harmful snails and may be used as an effective molluscicide and their sub lethal doses was enough to alter the biochemical profile of the snails significantly. It is also obvious from the toxicity result that in pond, toxicity of the plant extract was decreased about four times than laboratory condition. Further, it is suggested that experiment in natural circumstances should be performed for determination of actual value of this plant origin molluscicides before using it at large scale for its more effectiveness. Withdrawal experiment indicates that the toxicity of acetone extract of the plant against both snails 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 additional advantage in their use.

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