Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original article doi: 10.12980/jclm.3.2015j5-98

Enzyme inhibition by molluscicidal agents of *Bauhinia variegata* and *Mimusops elengi* in the nervous tissue of *Lymnaea acuminata*

Kanchan Lata Singh, D.K. Singh, Vinay Kumar Singh*

Malacology Laboratory, Department of Zoology, Deen Dayal Upadhyay Gorakhpur University, Gorakhpur 273009, U.P., India

ARTICLE INFO

Article history:
Received 27 Jun 2015
Received in revised form 23 Jul 2015
Accepted 25 Aug 2015
Available online 29 Sep 2015

Keywords:
Fasciolosis
Quercetin
Saponin
Enzyme
Fresh water snail

ABSTRACT

Objective: To evaluate the effect of enzymatic activity in the nervous tissue of snail *Lymnaea acuminata* (*L. acuminata*) by *Bauhinia variegata* (*B. variegata*) and *Mimusops elengi* (*M. elengi*) and their active molluscicidal components quercetin and saponin.
Methods: Treatment of sublethal concentration (40% and 80% of 96-h LC_{50}) *in vivo* of column-purified fraction of *B. variegata* leaf and *M. elengi* bark and their molluscicidal agents quercetin and saponin inhibit the acetylcholinesterase (AChE), acid and alkaline phosphatase (ACP and ALP) activities in the nervous tissue of *L. acuminata*.
Results: AChE activity was more inhibited than ACP and ALP in snail exposed to column-purified fraction of *M. elengi* bark and saponin. Among all the treatments the highest inhibition in AChE activity (27.77%) was noted in snail *L. acuminata* exposed to 80% of 96-h LC_{50} of saponin at 96-h exposure period.
Conclusions: It can be concluded from the present study that inhibition of AChE, ACP and ALP by *B. variegata* leaf (quercetin) and *M. elengi* bark (saponin) in snail *L. acuminata* could be the cause of snail mortality.

1. Introduction

Water-borne disease fasciolosis with gastropods as intermediate hosts is still a burden for mankind, especially in tropical countries. Fasciolosis is caused by two trematodes *Fasciola hepatica* (the common liver fluke) and *Fasciola gigantica* (the large liver fluke) [1,2]. Fasciolosis is a common disease of many wild and domestic animals, especially sheep, goat, cattle[2,3]. The total global economic loss such as reduction of milk and meat yields, attributed to fasciolosis has been estimated earlier to be more than $3 billion per year[4]. Fresh water snail of *Lymnaea acuminata* (family: Lymnaeidae) (*L. acuminata*)is the intermediate host of liver fluke *Fasciola gigantica*. It causes endemic fasciolosis in cattle population in the eastern region of the state Uttar Pradesh, India[1]. Snail control is one of the best approaches of combating the disease. Snails can be controlled indirectly by destroying their habitat or directly by killing them. Singh *et al.*[5] have noted that *Bauhinia variegata* (*B. variegata*) leaf and *Mimusops elengi* (*M. elengi*) bark and their active components quercetin and saponin are potent molluscicides. Both plants have great pharmacological and biological aspects[6,7]. The mode of action by which they cause snail mortality is not known. Present study was to explain our previous study aimed at elucidating the effect of plant molluscicidal agent on certain enzyme acetylcholinesterase (AChE), acid phosphatase (ACP) and alkaline phosphatase (ALP) in the nervous tissue of snail *L. acuminata*.

2. Materials and methods

2.1. Test materials

Fresh leaves of *B. variegata* Linn. (Kachnar, family: Fabaceae) and bark of *M. elengi* Linn (Moulsari, family: Sapotaceae) were collected from the campus of Deen Dayal Upadhyay Gorakhpur

*Corresponding author: Vinay Kumar Singh, Malacology Laboratory, Department of Zoology, DDU Gorakhpur University, Gorakhpur 273009, U.P., India.
Tel: +91-551-2202187(O), 09415855488
E-mail: vinaygkpuniv@gmail.com; drvksingh_gpu@yahoo.in
Foundation Project: Supported by University Grants Commission (Grant No. F. No. 39-590/2010).

University, Gorakhpur, U.P., India. Quercetin (3, 3, 4, 5, 7-penta hydroxyl flavone) and saponin (sapogenin: 10%–20%) were purchased from Sigma Chemical Co. USA.

2.2. Bioassay

Snail *L. acuminata* [length (2.30 ± 0.25) cm] were taken from local pools and ponds. Thereafter, they were acclimatized in laboratory condition for 72 h. Glass aquariums containing 3 L of dechlorinated tap water (22–24 ℃) were set up for each treatment. Snails were treated with 40% and 80% of 96-h LC_{50} of column-purified fraction of *B. variegata* and *M. elengi* and their active molluscicidal agents–quercetin and saponin for and 96-h (Table 1). Control group contains only dechlorinated tap water without treatment. Snails were taken out after 24-h and 96-h treatment, and washed with water. Nervous tissue was quickly taken out for the measurement of different enzyme activity. Nervous tissue was placed on filter paper to remove the adherent water and weigh. Enzyme activity was measured in treated as well as control group of test animals.

Table 1
Sublethal concentration (40% and 80% of 96-h LC_{50}) of active component and column-purified fraction of *B. variegata* and *M. elengi* on *L. acuminata*. mg/L.

Treatment	96-h LC_{50}	40% of 96-h LC_{50}	80% of 96-h LC_{50}
B. variegata leaf-CP	5.98	2.39	4.78
Quercetin	5.39	2.15	4.31
M. elengi bark-CP	7.20	2.88	5.76
Saponin	1.30	0.52	1.04

CP: Column-purified.

2.3. AChE activity

AChE activity was estimated by Ellman *et al.*[8] as modified by Tripathi *et al.*[4]. Nervous tissue (50 mg) of *L. acuminata* was taken out around the buccal mass and homogenized in 1.0 mL of 0.1 mol/L phosphate buffer pH 8.0 for 5 min in an ice bath. Homogenates was then centrifuged at 3575 r/min for 30 min at 4 ℃. The supernatant was used as an enzyme source. 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 buffer (0.1 mol/L pH 8.0), 0.1 mL of chromogenic agent 5, 5-dithio-bis-2-nitrobenzoic acid, and 0.02 mL of freshly prepared aetylthiocholine iodide solution in distilled water. The change in optical density at 412 nm was noted for 3 min after every 30 s interval at 25 ℃. Enzyme activity has been expressed as μmol of sulfhydryl (SH) hydrolyzed per mg of protein per min.

2.4. ACP activity

ACP activity in the nervous tissue of *L. acuminata* was estimated by the method of Bergmeyer[9] as modified by Kumar *et al.*[10]. Nervous tissue homogenate (2% w/v) was prepared in ice cold 0.9% NaCl and centrifuged at 7993 r/min for 15 min at 4 ℃. The supernatant was added to 1.0 mL of acid buffer substrate (0.41 g citric acid, 1.125 g sodium citrate, and 165 mg 4-nitrophenyl phosphate disodium salt to 100 mL of double distilled water). The incubation mixture was mixed thoroughly and incubated for 30 min at 37 ℃. And 4.0 mL of 0.1 mol/L NaOH was then added to the incubation mixture. Standard curves were drawn with different concentrations of 4-nitrophenol at 420 nm. The ACP activity has been expressed as μmol of substrate hydrolyzed per mg of protein per 30 min.

2.5. ALP activity

ALP activity in the nervous tissue of *L. acuminata* was estimated by method of Bergmeyer[9] as modified by Kumar *et al.*[10]. Tissue homogenate (2% w/v) was prepared in ice cold 0.9% NaCl and centrifuged at 7993 r/min for 15 min at 4 ℃. 0.1 mL of supernatant (enzyme source) was added to 1.0 mL of acid buffer substrate (375 mg glycine, 10 mg $MgCl_2 \cdot 6H_2O$, 165 mg 4-nitrophenyl phosphate disodium salt in 42 mL of mixture was made up to 100 mL with double distilled water). The incubation mixture was incubated for 30 min at 37 ℃. And 10 mL of 0.2 mol/L NaOH was then added to the formation of 4-nitrophenol. Standard curves were drawn with different concentrations of 4-nitrophenol at 420 nm. The ALP activity has been expressed as μmol of substrate hydrolyzed per mg of protein per 30 min.

2.6. Protein

Estimation of protein was in enzyme source measured by the method of Lowry *et al.*[11] using bovine serum as a standard.

2.7. Statistical analysis

Each experiment was replicated at least six times and results were expressed as mean ± SE of six replicates. Student's *t*-test was applied between control and treated groups to locate significant ($P < 0.05$) variations[12].

3. Results

In vivo 24-h and 96-h exposure of 40% and 80% of 96-h LC_{50} of column-purified *B. variegata* leaf and *M. elengi* bark powder and their active component quercetin and saponin significantly ($P < 0.05$) inhibited the activities of AChE, ACP and ALP in the nervous tissue of *L. acuminata* (Tables 2 and 3). In control group 24-h exposure period activity of AChE (0.92 μmol SH hydrolyzed/min per mg protein), ACP (15.95 μmol substrate hydrolyzed/30 min per mg protein) and ALP (12.92 μmol substrate hydrolyzed/30 min per mg protein) in nervous tissue of *L. acuminata* (Table 2). Whereas, exposure of 96-h activity of AChE (0.90 μmol SH hydrolyzed/min per mg protein), ACP (15.90 μmol substrate hydrolyzed/30 min per mg protein) and ALP (12.02 μmol substrate hydrolyzed/30

Table 2

In vivo effect of 24-h exposure to sublethal concentration (40% and 80% of 96-h LC_{50}) of active components and column-purified fraction on AChE, ACP and ALP activity in nervous tissue of *L. acuminata*.

Treatment	Concentration % (mg/L) of 96 h LC_{50}	AChE µmol 'SH' hydrolyzed/min per mg protein (%)	ACP µmol Substrate hydrolyzed/30 min per mg protein (%)	ALP µmol Substrate hydrolyzed/30 min per mg protein (%)
Control	-	0.920 ± 0.050 (100.00)	15.950 ± 0.030 (100.00)	12.920 ± 0.010 (100.00)
B. variegata (column-purified leaf)	40 (2.39)	0.780 ± 0.004* (84.78)	13.860 ± 0.070* (86.89)	11.420 ± 0.010* (88.39)
	80 (4.78)	0.670 ± 0.004* (72.84)	11.180 ± 0.003* (70.09)	9.100 ± 0.010* (70.43)
Quercetin	40 (2.15)	0.530 ± 0.001* (57.60)	10.050 ± 0.002* (63.00)	9.770 ± 0.002* (75.61)
	80 (4.31)	0.430 ± 0.002* (46.73)	8.340 ± 0.040* (52.28)	7.850 ± 0.005* (60.75)
M. elengi (column-purified bark)	40 (2.88)	0.750 ± 0.006* (81.52)	12.900 ± 0.070* (80.87)	10.210 ± 0.070* (79.02)
	80 (5.76)	0.700 ± 0.001 (76.08)	7.590 ± 0.010 (47.58)	8.540 ± 0.007 (66.09)
Saponin	40 (0.52)	0.390 ± 0.004* (42.39)	10.290 ± 0.001* (64.51)	9.570 ± 0.007* (74.07)
	80 (10.4)	0.340 ± 0.001* (36.95)	7.130 ± 0.010* (44.70)	6.800 ± 0.008* (52.63)

Value are mean ± SE of six replicates. Values in parentheses indicate percent enzyme activity with control taken as 100% concentrations (w/v) were expressed as final concentration in aquarium water; *: Significant ($P < 0.05$) when student's *t*-test was used for locating differences between experimental and control groups of animals.

Table 3

In vivo effect of 96-h exposure to sublethal concentration (40% and 80% of 96-h LC_{50}) of active components and column-purified fraction on AChE, ACP and ALP activity in nervous tissue of *L. acuminata*.

	Concentration % (mg/L) of 96-h LC_{50}	AChE µmol 'SH' hydrolyzed/min per mg protein (%)	ACP µmol Substrate hydrolyzed/30min per mg protein (%)	ALP µmol Substrate hydrolyzed/30min per mg protein (%)
Control		0.900 ± 0.050 (100.00)	15.900 ± 0.030 (100.00)	12.020 ± 0.010 (100.00)
B. variegata (column-purified leaf)	40 (2.39)	0.630 ± 0.001* (70.00)	10.490 ± 0.006* (65.97)	8.800 ± 0.002* (73.21)
	80 (4.78)	0.580 ± 0.001* (64.44)	8.480 ± 0.004* (53.33)	7.780 ± 0.004* (64.72)
Quercetin	40 (2.15)	0.500 ± 0.010* (55.55)	9.050 ± 0.004* (56.91)	7.060 ± 0.005* (58.73)
	80 (4.31)	0.380 ± 0.070* (42.22)	6.450 ± 0.001* (40.56)	5.960 ± 0.007* (49.58)
M. elengi (column-purified bark)	40 (2.88)	0.640 ± 0.007* (71.11)	10.680 ± 0.054* (67.16)	8.260 ± 0.001* (68.71)
	80 (5.76)	0.590 ± 0.001* (65.55)	6.340 ± 0.001* (39.87)	5.760 ± 0.004* (47.92)
Saponin	40 (0.52)	0.300 ± 0.002* (33.33)	8.350 ± 0.004* (52.51)	7.180 ± 0.004* (59.73)
	80 (10.40)	0.250 ± 0.001* (27.77)	5.810 ± 0.007* (36.54)	4.980 ± 0.008* (41.43)

Value are mean ± SE of six replicates. Values in parentheses indicate percent enzyme activity with control taken as 100% concentrations (w/v) were expressed as final concentration in aquarium water; *: Significant ($P < 0.05$) when student's *t*-test was used for locating differences between experimental and control groups of animals.

min per mg protein) have been observed in the nervous tissue of *L. acuminata* (Table 3). Saponin, the active agents of *M. elengi*, is more pronounced than quercetin (*B. variegata*). Maximum inhibition of AChE activity (27.77%) was in 96-h exposure with saponin (80% of 96-h LC_{50}). In 96-h saponin treatment (80% of 96-h LC_{50}) inhibition of ACP activity (36.54% of control) was more than 24-h ACP activity (44.70% of control). Whereas, the inhibitory effect of 96-h saponin treatment (80% of 96-h LC_{50}) on ALP activity (41.43% of control) is more significant than that of 24-h saponin treatment (52.63% of control) (Tables 2 and 3).

4. Discussion

In vivo treatment with 40% and 80% of 96-h LC_{50} of column-purified fraction of *B. variegata* leaf and *M. elengi* bark and their active agents' quercetin and saponin caused a significant inhibition of AChE, ACP and ALP activity in the nervous tissue of *L. acuminata*. These three enzymes are inhibited by the plant molluscicide as well as synthetic molluscicides in the treated snails[13,14]. Column-purified fraction of *M. elengi* bark and saponin caused more inhibition in AChE than ACP and ALP. Inhibition of AChE resulted in accumulation of acetylcholine at the nerve synapses, so that the post synaptic membrane is in a state of permanent stimulation causing paralysis, ataxia and general lack of co-ordination in neuromuscular system, which ultimately caused death of snails[15,16]. Saponin is haemolysis agent[7,17]. Quercetin acts as AChE inhibitor, an important aspect for neuroprotector[18]. It is potential delivering to central nervous system[19]. AChE in hippocampus and neocortex, caused by degeneration of cholinergic synapse, is currently the most established approach to treat Alzheimer's disease[20].

ALP, a marker enzyme for plasma and endoplasmic reticulum, is often employed to assess the integrity of plasma membrane. The increase in ALP activities in serum may be attributed to either synthesis of the enzyme molecules or loss of other proteins from tissue[10]. ACPs are its own case in a 'marker' enzyme for the lysosomal integrity. ACP activity after toxin administration may be attributed to an increase in cellular degeneration of other pathological sensitive indicator of injury[10,21]. The lowering of enzyme levels is an indication of hepatoprotective action of quercetin. Quercetin also known to reduce toxicant-induced liver damage[22]. Quercetin has long been used to enhance renal filtration and increase the excretion of xenobiotics and has free radical scavenging activity. This is due to antioxidative action of

quercetin[23,24]. ACP and ALP are indicators of cellular leakage and loss of functional integrity of the cell membrane in liver[23]. ACP is a lysosomal enzyme which plays an important role in catabolism, pathological necrosis, autolysis and phagocytosis[10]. ALP plays a critical role in protein synthesis[25] shell formation[26] and other secretary activities[27], transport of metabolites[28] in gastropods. Both ACP and ALP were also inhibited by treatment with saponin and quercetin in snail *L. acuminata*. It seems that the molluscicidal components of *B. variegata* and *M. elengi* and their active component quercetin and saponin kill snails by inhibiting these enzymes in different ways. In conclusion, it can be stated that plants *B. variegata* and *M. elengi* are potent molluscicides. Both plants are easily available, ecologically safe and culturally more acceptable among native livestock keepers.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

We thank to University Grants Commission (Grant No. F. No. 39-590/2010 (SR) New Delhi, India for funding this study.

References

[1] Singh DK, Singh VK, Kumar P. *Pestiferous gastropods and their control: gastropods pest control methods*. Saarbrücken: LAP Lambert Academic Publishing; 2012.

[2] Rojo-Vázquez FA, Meana A, Valcárcel F, Martínez-Valladares M. Update on trematode infections in sheep. *Vet Parasitol* 2012; **189**: 15-38.

[3] Mas-Coma S, Bargues MD, Valero MA. Fascioliasis and other plant-borne trematode zoonoses. *Int J Parasitol* 2005; **35**: 1255-78.

[4] Tripathi AP, Singh VK, Singh DK. Attraction of *Lymnaea acuminata* against the visible photo stimulant: a new in fasciolosis control. *Research* 2014; **1**: 743.

[5] Singh KL, Singh DK, Singh VK. Characterization of the molluscicidal activity of *Bauhinia variegata* and *Mimusops elengi* plant extract against the *Fasciola* vector *Lymnaea acuminata*. *Rev Inst Med Trop Sao Paulo* 2012; **54**(3): 135-40.

[6] Cechinel Filho V. Chemical composition and biological potential of plants from the genus *Bauhinia*. *Phytother Res* 2009; **23**: 1347-54.

[7] Singh KL, Srivastava P, Kumar S, Singh DK, Singh VK. *Mimusops elengi* Linn. (Maulsari): a potential medicinal plant. *Arch Biomed Sci* 2014; **2**(1): 18-29.

[8] Ellman GL, Courtney KD, Andres V Jr., Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 1961; **7**: 88-95.

[9] Bergmeyer HU, editor. *Method of enzymatic analysis*. 2nd ed. New York: Academic Press; 1967, p. 1129.

[10] Kumar P, Singh VK, Singh DK. Feeding of bait to snail *Lymnaea acuminata* and their effect on certain enzyme in the nervous tissue. *ISRN Biochem* 2012; doi: 10.5402/2012/343047.

[11] Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265-75.

[12] Sokal RR, Rohlf FJ. *Biometry: the principles and practice of statistics in biological research*. 3rd ed. New York: W.H. Freeman and company; 1995.

[13] Singh A, Srivastava P, Kumar P, Singh DK, Singh VK. Inhibition of acetylcholinesterase and phosphatases by active molluscicidal components of *Saraca asoca* and *Thuja orientalis* in the nervous tissue of *Lymnaea acuminata*. *The Ecoscan* 2011; (Special 1): 87-92.

[14] Singh DK, Agarwal RA. *In vitro* and *in vivo* studies on synergism with anticholinesterase pesticides in the snail *Lymnaea acuminata*. *Arch Environ Contam Toxicol* 1983; **12**: 483-7.

[15] Matsumura F. *Toxicology of insecticides*. 2nd ed. Berlin: Springer; 1985.

[16] Srivastava AK, Singh VK. Feeding of snail attractant pellets (SAP) containing papain on certain biochemical parameters in the gonadal/nervous tissue of the vector snail (*Lymnaea acuminata*). *Singapore J Sci Res* 2015; doi: 10.3923/sjsres.2015.

[17] Francis G, Kerem Z, Makhar HP, Beeker K. The biological action of saponins in animal systems: a review. *Br J Nutr* 2002; **88**(6): 587-605.

[18] Tong-un T, Muchimapara S, Phacharpai W, Waltanathern J. Nasal administration of quercetin liposomes modulate cognitive impairment and inhibit acetylcholinesterase activity in hippocampus. *Am J Neurosci* 2010; **1**(1): 21-7.

[19] Wang XM, He HB, Leng W, Tang X. Evaluation of brain-targeting for the nasal delivery of estradiol by the microdialysis method. *Int J Pharm* 2006; **317**: 40-6.

[20] Nabeshima T, Yamada K. New therapeutic approaches to Alzheimer's disease. In: Tanaka C, McGeer PL, Ihara Y, editors. *Neuroscientific basis of dementia*. Berlin: Birkhäuser Basel; 2001, p. 287-94.

[21] Verma RJ, Vasu A, Saiyed AA. Arsenic toxicity in mice and its possible amelioration. *J Environ Sci (China)* 2004; **16**: 447-53.

[22] Bansal V, Malviya R, Deeksha, Malviya T, Sharma PK. Phytochemical, pharmacological profile and commercial utility of tropically distributed plant *Bauhinia variegata*. *Global J Pharmacol* 2014; **8**(2): 196-205.

[23] Drotman RB, Lawhorn GT. Serum enzyme as indicators of chemically liver damage. *Drug Chem Toxicol* 1978; **1**: 163-71.

[24] Pandey S, Agrawal RC, Maheshwari S. *In vitro* antioxidant and free radical scavenging activity of *Bauhinia jvariegata* Linn. *Int J Sci Res Publ* 2012; **2**(2): 1-5.

[25] Pilo B, Asnani MV, Shah RV. Studies on wound healing and repair in pigeon liver: III. Histochemical studies on acid and alkaline phosphatase activities during the process. *J Anim Morphol Physiol* 1972; **19**: 205-12.

[26] Timmermans LPM. Studies on shell formation in molluscs. *Neth J Zool* 1968; **19**: 413-523.

[27] Ibrahim AM, Higazi MG, Dexian ES. Histochemical localization of alkaline phosphatase activity in the alimentary tract of the snail *Marisa cornuarieties* (L). *Zool Soc Egypt Bull* 1974; **26**: 95-105.

[28] Vorbrodt A. The role of phosphate in intracellular metabolism. *Postepy Higieny Medycyny Doswiadczaline* 1959; **13**: 200-6.