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

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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 LC50) 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 LC50 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 (Moul sir, family: Sapotaceae) were collected from the campus of Deen Dayal Upadhyay Gorakhpur
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 °C) were set up for each treatment. Snails were treated with 40% and 80% of 96-h LC₅₀ of column-purified fraction of *B. variegata* and *M. elengi* and their active molluscidal 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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>96-h LC₅₀ (%)</th>
<th>40% of 96-h LC₅₀ (%)</th>
<th>80% of 96-h LC₅₀ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. variegata</em>  leaf-CP</td>
<td>5.98</td>
<td>2.39</td>
<td>4.78</td>
</tr>
<tr>
<td>Quercetin</td>
<td>5.39</td>
<td>2.15</td>
<td>4.31</td>
</tr>
<tr>
<td><em>M. elengi</em> bark-CP</td>
<td>7.20</td>
<td>2.88</td>
<td>5.76</td>
</tr>
<tr>
<td>Saponin</td>
<td>1.30</td>
<td>0.52</td>
<td>1.04</td>
</tr>
</tbody>
</table>

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 treated as well as control group of test animals.

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 (50 mg) of *L. acuminata* was rapidly 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.

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 °C. 0.1 mL of supernatant (enzyme source) was added to 1.0 mL of acid buffer substrate (375 mg glycine, 10 mg MgCl₂, 6H₂O, 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 then centrifuged at 3575 r/min for 30 min at 4 °C. 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 LC₅₀ 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 substrate hydrolyzed/min per mg protein), ACP (12.92 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 (15.95 μmol substrate hydrolyzed/30 min per mg protein) and ALP (12.02 μmol substrate hydrolyzed/30 min per mg protein) in nervous tissue of *L. acuminata* (Table 2).
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 LC50). In 96-h saponin treatment (80% of 96-h LC50) inhibition of ACP activity (36.54% of control) was more than 24-h ACP activity (36.94% of control). Inhibition of ALP activity (41.43% of control) (Tables 2 and 3).

### 4. Discussion

In *vivo* treatment with 40% and 80% of 96-h LC50 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].

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 an increase in cellular degeneration of other 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].

### Table 2

*In vivo* effect of 24-h exposure to sublethal concentration (40% and 80% of 96-h LC50) of active components and column-purified fraction on AChE, ACP and ALP activity in nervous tissue of *L. acuminata*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration % (mg/L) of 96 h LC50</th>
<th>AChE µmol ‘SH’ hydrolyzed/min per mg protein (%)</th>
<th>ACP µmol Substrate hydrolyzed/30 min per mg protein (%)</th>
<th>ALP µmol Substrate hydrolyzed/30 min per mg protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>0.920 ± 0.050 (100.00)</td>
<td>15.950 ± 0.030 (100.00)</td>
<td>12.920 ± 0.010 (100.00)</td>
</tr>
<tr>
<td><em>B. variegata</em> (column-purified leaf)</td>
<td>40 (2.39)</td>
<td>0.780 ± 0.004* (84.78)</td>
<td>13.860 ± 0.070* (86.89)</td>
<td>11.420 ± 0.010* (88.39)</td>
</tr>
<tr>
<td>Quercetin</td>
<td>80 (4.78)</td>
<td>0.670 ± 0.004* (72.84)</td>
<td>11.180 ± 0.003* (70.09)</td>
<td>9.100 ± 0.010* (70.43)</td>
</tr>
<tr>
<td><em>M. elengi</em> (column-purified bark)</td>
<td>40 (2.15)</td>
<td>0.530 ± 0.001* (57.60)</td>
<td>10.050 ± 0.002* (63.00)</td>
<td>9.770 ± 0.002* (57.61)</td>
</tr>
<tr>
<td>Saponin</td>
<td>80 (4.31)</td>
<td>0.430 ± 0.002* (46.73)</td>
<td>8.340 ± 0.040* (52.28)</td>
<td>7.850 ± 0.005* (60.75)</td>
</tr>
</tbody>
</table>

### Table 3

*In vivo* effect of 96-h exposure to sublethal concentration (40% and 80% of 96-h LC50) of active components and column-purified fraction on AChE, ACP and ALP activity in nervous tissue of *L. acuminata*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration % (mg/L) of 96 h LC50</th>
<th>AChE µmol ‘SH’ hydrolyzed/min per mg protein (%)</th>
<th>ACP µmol Substrate hydrolyzed/30 min per mg protein (%)</th>
<th>ALP µmol Substrate hydrolyzed/30 min per mg protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>0.900 ± 0.050 (100.00)</td>
<td>15.900 ± 0.030 (100.00)</td>
<td>12.020 ± 0.010 (100.00)</td>
</tr>
<tr>
<td><em>B. variegata</em> (column-purified leaf)</td>
<td>40 (2.39)</td>
<td>0.630 ± 0.001* (70.00)</td>
<td>10.490 ± 0.006* (65.97)</td>
<td>8.800 ± 0.002* (73.21)</td>
</tr>
<tr>
<td>Quercetin</td>
<td>80 (4.78)</td>
<td>0.580 ± 0.001* (64.44)</td>
<td>8.480 ± 0.004* (53.33)</td>
<td>7.780 ± 0.004* (64.72)</td>
</tr>
<tr>
<td><em>M. elengi</em> (column-purified bark)</td>
<td>40 (2.15)</td>
<td>0.500 ± 0.001* (57.55)</td>
<td>9.050 ± 0.004* (56.91)</td>
<td>7.060 ± 0.005* (58.73)</td>
</tr>
<tr>
<td>Saponin</td>
<td>80 (4.31)</td>
<td>0.380 ± 0.070* (42.22)</td>
<td>6.450 ± 0.001* (40.56)</td>
<td>5.960 ± 0.007* (49.58)</td>
</tr>
</tbody>
</table>

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.
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 secretory 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 mollusccidal 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

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References