Veterinary Parasitology 164 (2009) 206–210

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Molluscicidal activity of *Saraca asoca* and *Thuja orientalis* against the fresh water snail *Lymnaea acuminata*

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ARTICLE INFO

Article history:
Received 23 November 2008
Received in revised form 1 May 2009
Accepted 11 May 2009

Keywords:
Saraca asoca
Thuja orientalis
Saponin
Thujone
Molluscicidal activity
Lymnaea acuminata

ABSTRACT

The molluscicidal activity of bark powder of *Saraca asoca*, leaf powder of *Thuja orientalis* against the snail *Lymnaea acuminata* was studied. The molluscicidal activity of all the plant products was found to be both time and concentration dependent. The 96 h LC_{50} of *T. orientalis* leaf powder against *L. acuminata* was 250.5 mg/l. Ethanol extracts were more toxic than other organic extracts. The ethanol extract of *T. orientalis* leaf (24 h LC_{50}: 32.74 mg/l) was more effective than that of *S. asoca* bark (24 h LC_{50}: 82.38 mg/l). The 24 h LC_{50} of column purified fraction of *T. orientalis* leaf and *S. asoca* bark powder was 29.25 and 64.89 mg/l, respectively. Saponin and thujone were identified as active molluscicide components in the bark of *S. asoca* and leaf of *T. orientalis*, respectively. The product of *S. asoca* and *T. orientalis* may be used as potent molluscicides.

1. Introduction

Fascioliasis is a worldwide zoonosis (Mas-Coma et al., 2005; WHO, 2006; Lewin, 2007) disease, caused by digenetic trematode *Fasciola hepatica* and *Fasciola gigantica*. The incidence of fascioliasis in cattle population of eastern region of Uttar Pradesh state of India is significantly high (Agarwal and Singh, 1988; Singh et al., 1996). Ninety-four percent of buffaloes slaughtered in local slaughter houses are infected with this disease (Singh and Agarwal, 1981; Shukla et al., 2006; Jaiswal et al., 2008). It causes low fertility, abortion and progressive loss in milk production in infected animals (Phiri et al., 2006; Shukla et al., 2006). Fresh water snail *Lymnaea acuminata* is the intermediate host of *F. gigantica*. One way to reduce the incidence of fascioliasis is to de-link the life cycle of flukes by killing the snails (Singh and Singh, 2001; Jaiswal et al., 2008). Synthetic molluscicides have been widely used for the effective control of harmful snails (Agarwal and Singh, 1988), but it has now been realized that these cause serious environmental hazards. Consequently, more research is now being focused on molluscicide of plant origin (Singh and Singh, 1995, 2008; Singh et al., 1996), as these are easily biodegradable and, therefore, safer to use than their synthetic counterparts (Singh et al., 1996; Srivastava and Singh, 2005; Kumar and Singh, 2006; Jaiswal and Singh, 2008). The present study describes the molluscicidal activity of *Saraca asoca* (Caesalpinaceae) and *Thuja orientalis* (Cupressaceae) against the snail *L. acuminata*. Even though a large number of pharmacological effects of both the plants have been reported (Chen et al., 1989; Varghese et al., 1992; Dabur et al., 2004; Ju-Hyun et al., 2005; Guleriaa et al., 2007; Shahid et al., 2007; Monica et al., 2008). Their molluscicidal activity is not reported.

2. Materials and methods

2.1. Test animals

Adult *L. acuminata* snails (2.30 ± 0.25 cm in length), collected locally from lakes and low lying submerged fields in Gorakhpur, were used as the test animals. Snails were acclimatized for 72 h in dechlorinated tap water. Ten experimental animals were kept in glass aquaria containing

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doi:10.1016/j.vetpar.2009.05.008

3 l of dechlorinated tap water at 23 ± 1 °C. The pH, dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were 7.1–7.3, 6.5–7.3 mg/l, 5.2–6.4 mg/l and 103.0–105.0 mg/l, respectively. Dead animals were removed at each observation to avoid any spoilage of the aquarium water.

2.2. Plants

Bark of *S. asoca* and leaves of *T. orientalis* were collected locally from D.D.U Gorakhpur University campus, Gorakhpur and identified by retired Prof. S.K. Singh, plant taxonomist, Department of Botany, D.D.U. Gorakhpur University, Gorakhpur (UP) India.

2.3. Experimental design

2.3.1. Crude plant products

Bark of *S. asoca* and leaves of *T. orientalis* were kept in an incubator at 45 °C for 24 h. Dried bark and leaves were pulverized separately with grinder and the crude powders thus obtained were used for the toxicity experiments.

2.3.2. Organic solvent extract

Five gram of bark powder of *S. asoca* and leaf powder of *T. orientalis* were extracted with 100 ml, each of 95% of ethanol, 98% ether, 99.5% carbon tetrachloride, and 99% acetone at room temperature for 24 h. The solvents were removed under vacuum and the following remaining dried parts were used for the determination of molluscicidal activity; *S. asoca* ethanol, 260 mg; carbon tetrachloride, 220 mg; ether, 290 mg; and acetone, 230 mg. *T. orientalis* ethanol, 570 mg; carbon tetrachloride, 220 mg; ether, 470 mg; and acetone, 430 mg.

2.3.3. Column purification

One hundred milliliters of ethanol extract fraction of *S. asoca* bark powder and leaf powder of *T. orientalis* was subjected to silica gel (60–120 mesh, Qualigens, Glass Precious, Electrochemindus, Private Limited, Mumbai, India) Chromatography through a 5 cm × 45 cm column. Thirty fractions of 5 ml were eluted with ethanol (95%). Ethanol was evaporated under vacuum and the remaining solids were used for determination of molluscicidal activity in each fraction.

2.3.4. Pure compound

Saponin (Sapogenin—10%) and thujone (1-isopropyl,4, methyl by cyclo [3.1.0] hexan-3-one), were purchased from Sigma Chemical Co. USA.

2.3.5. Thin layer chromatography

Thin layer chromatography (TLC) was done according to the method of Jaiswal and Singh (2008), for identification of active molluscicidal components present in bark powder of *S. asoca* and leaf powder of *T. orientalis*. TLC was done on 20 cm × 20 cm precoated silica gel (Precious Electrochemical Industry, Pvt. Ltd., Mumbai, India). The solvent used was benzene/ethyl acetate (90/10, v/v). Co-migration of column purified fractions of the plant with pure component saponin and thujone was done for identification of the molluscicidal components. TLC plates were developed by iodine.

Table 1
Concentration of different plant products and their active components used for the toxicity determination against *Lymnaea acuminata*.

Treatments	Concentrations (mg/l)
Bark powder of *S. asoca*	1500
Carbon tetrachloride extracts	70, 90, 120, 150
Ether extracts	30, 50, 70, 90
Acetone extracts	30, 50, 70, 90
Ethanol extracts	30, 50, 70, 90
Column purified	30, 40, 50, 60
Saponin	.3, 3, 5, 10
Leaf powder of *Thuja orientalis*	200, 300, 400, 500
Carbon tetrachloride extracts	20, 25, 30, 35
Ether extracts	50, 60, 70, 80
Acetone extracts	20, 25, 30, 35
Ethanol extracts	20, 25, 30, 35
Column purified	15, 20, 25, 30
Thujone	1, 3, 5, 10

2.3.6. Treatment: protocol for concentration–response relationship

Toxicity experiments were performed by the method of Singh and Agarwal (1984). Ten experimental animals were kept in a glass aquarium containing 3 l of dechlorinated tap water. Snails were exposed to continuously for 96 h different concentrations of *S. asoca* bark and *T. orientalis* leaf (Table 1). Six aquaria were set for each concentration of plant derived molluscicides. Control animals were kept in an equal volume of water under similar condition without treatments. No response to a needle probe was taken as evidence of snail death. LC values, upper and lower confidence limit (UCL and LCL), slope values, *t*-ratio, *g*-values and heterogeneity factor were calculated by using the POLO computer software of Russell et al. (1977). The regression coefficient between exposure time and different values of LC_{50} was determined by the method of Sokal and Rohlf (1996).

3. Result

The toxicity of different organic solvents of *S. asoca* bark powder and *T. orientalis* leaf powder, against *L. acuminata* was time and concentration dependent. Treatment of 1500 mg/l of *S. asoca* bark powder caused no mortality in treated snails. The LC_{50} of leaf powder of *T. orientalis* at 24 h was 465.8 mg/l (Table 3). There was a significant negative correlation between the LC_{50} and exposure time.

Among the organic solvent, ethanol extract of *S. asoca* bark powder (24 h LC_{50}: 99.18 mg/l) and *T. orientalis* leaf powder (24 h LC_{50}: 32.74 mg/l) was more toxic (Tables 2 and 3). Maximum molluscicidal activities of both plants were observed in between the 16th to 20th of the 5 ml fraction eluted from silica gel column. The 96 h LC_{50} of column purified fraction of *T. orientalis* leaf powder (17.69 mg/l) was higher than that of *S. asoca* bark powder (38.34 mg/l) (Tables 2 and 3). The thin layer chromatography analysis indicates that the *Rf* value of saponin (0.059) was equivalent to the *Rf* value of column purified fraction of *S. asoca* bark powder (0.059) and the *Rf* value of thujone (0.65) was equivalent to the *Rf* value of column purified fraction of *T. orientalis* leaf powder (0.65). Ninety-six hours LC_{50} of saponin and thujone was 0.80 mg/l and