Animal Health

Molluscicide Effect of *Sapindus saponaria* Fruit on *Galba cubensis*, an Intermediate Host of Fasciolosis in Cuba

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ABSTRACT

Galba cubensis is the main intermediate host of *Fasciola hepatica* in Cuba. The aim of this paper was to demonstrate the molluscicide effect of *Sapindus saponaria* L. on *Galba cubensis* in laboratory conditions, in order to recommend this plant for ecological control of this snail. Different concentrations of the hydroalcoholic extract of six-month old *S. saponaria* pericarp were tested on *G. cubensis* individuals, based on the methodology of the World Health Organization. The concentration gradient was used to determine LC_{50} and LC_{90}, which then were tested to estimate the heartbeat frequency of these mollusks. The molluscicide effect of the plant extract was considerable ($P \leq 0.01$; $R^2=60.6$), with concentration-dependent mortality and a drop of heartbeat frequency. Significant differences were observed between $LC_{50}=39.8$ mg/L and $LC_{90}=67.9$mg/L, in the test to lower heartbeat frequency ($P \leq 0.01$), and between LC_{90} and the control group ($P \leq 0.01$), but not between the control group and LC_{50} and ($P=0.24$). Molluscicide activity in the plant material was still present after six months of application, which was an important fact to recommend this plant as a candidate to control intermediate hosts of Fasciolosis in our region.

Key words: *plant extracts, Fasciola hepatica, toxicology*

INTRODUCTION

Trematodes cause schistosomiasis (bilharziosis), angiostrongylosis, fasciolosis, paragonimiasis, and other diseases in humans and animals. Before developing and becoming infectious to mammals, these organisms must dwell in different mollusks that serve as intermediate hosts (Perera *et al.*, 1983; Amunarriz, 1991). Today, schistosomiasis is the most significant of all, infecting millions of people in developing countries mainly (Knopp *et al.* 2012; Rollinson *et al.* 2013).

Fasciolosis is also a major zoonosis with a growing interest, especially in livestock raising areas. In Cuba, it is associated to two types of fresh-water mollusks: *Galba cubensis* (Pfeiffer, 1839) (*Lymnaea cubensis, Fossaria cubensis*) and *Pseudosuccinea columella* (Say, 1817) (*L. francisca, L. columella*), as intermediate hosts of *Fasciola hepatica* (liver fluke) (Vázquez *et al.*, 2014). The former caused a major outbreak of Fasciolosis in humans, in the municipality of Esmeralda, Camagüey province, 1999, though it did not kill any person. However, in Cuba and other countries, the most commonly occurring morbidity affects bovines and ovines, with significant economic losses due to reductions in milk and beef productions (Vázquez *et al.*, 2009; Khan *et al.*, 2013), and liver condemnation (Palacio *et al.*, 2017).

Chemical molluscicide substances have been utilized as part of programs to reduce morbidity and mortality caused by schistosomiasis, but these products often produce adverse side effects, which are irreversible on the ecosystems, in addition to the high costs to the developing economies, making them inaccessible (McCullough, 1992). For decades, the World Health Organization (WHO) has recorded a high number of plants with molluscicide potential, which may play an important role to control schistosomiasis and other tropical diseases, and are more environmentally friendly than synthetic products. Notwithstanding, there are few reports about national malacological control programs based on this alternative that offer a more effective and environmentally-friendly choice (WHO, 1983; Marston and Hostettmann, 1985; Singh *et al*., 2010).

Sapindus (Sapindaceae) is a genus that comprises a dozen tree or shrub species, which are abundant in tropical and subtropical regions. Abreu (2005) and Goyal *et al.*, (2014) reviewed the ethnobotany, as well as the biological and phytochemical activities of the three most important species: *S. saponaria, S. mukorossi*, and *S. trifoliatus*. They contain triterpenic saponins, which are referred to have molluscicide activity. *S. saponaria*.L (*S. inaequalis* D.C., *S. emarginatus* Willd.), also known as Wingleaf soapberry, grows in the Caribbean basin (León and Alaín, 1953; Roig, 1974).

The aim of this paper was to determine the molluscicide action of *S. saponaria* L. on *G. cubensis* in laboratory conditions, in order to recommend this plant for ecological control of this snail.

MATERIALS AND METHODS

Mollusks

The *G. cubensis* specimens were collected at Zanja de Vázquez, a biotope of approximately 50 m long by 90 cm wide, located in the Provincial Unit for Antivectorial Fight and Surveillance, in Camagüey (UPVLA-C). After identification at the Medical Malacology Lab UPVLA), the mollusks were grown in the laboratory for various generations. Their sizes (shell height) varied between 10 and 12 mm. The experiments were conducted under controlled humidity and temperature conditions (78 %±1, 230 C±1).

Plant material

The pericarp of *S. saponaria* was stored in a dry and fresh place for six months. The fruit was collected on Taburete Farm, near Camagüey city, and botanical authentication was performed by Eddy Martínez, curator at the herbarium of the Center of Environmental Research in Camagüey (voucher: HACC-970). The material was dried at 40 °C in oven with air recirculation and spraying. A fluid extract was obtained by fractional percolation with an ethanol solution (800) (Soler *et al.*, 1992).

Bioassay

The molluscicide action of the extract in the laboratory conditions was conducted following the methodology established by WHO (1983). Three experimental series were carried out with 24 mollusks each (72 total) in all the six extract concentrations evaluated. Eight snails were placed in each Petri dish (110 mm of diameter and 18 mm high) on a thin filter paper; the control was placed in dechlorinated water. The final volume of the extracts and the control was 5 ml. Mortality was checked at 24, 48, and 72 h. It was determined by mollusk stillness and confirmed through observation of the heartbeats of snails. The dead snails were disposed of (Malek and Cheng, 1974). To determine the lethal concentrations (LC), different deadly values (5-100%) were run by Probit-Log, through transformation of the $\log_{10}$ of all concentrations (Raymond, 1985).

The effect of the extract on mollusk heartbeat frequency was also evaluated in three groups of ten snails each, to LC_{50}, LC_{90}, and with the dechlorinated water used as control. The heartbeat frequency was determined for 1 min every 20 min, for an overall period of 2 h. Observation of heartbeats was made through the shell of the mollusks by means of a curved incision on the upper side, using a stereoscopic microscope (Malek and Cheng, 1974). The moment of plant extract application was considered time zero.

Statistical analysis

One way ANOVA was performed to determine the significant differences among the heartbeat frequency means of G. cubensis, at LC_{50} and LC_{90}. Statistica-6 software was used for calculations.

RESULTS AND DISCUSSION

Table 1 shows the molluscicide activity of the concentration gradient of ethanol solutions of *S. saponaria* and the concentration-dependent effect pattern. The resulting lethal concentrations were LC_{50}=39.8 mg/l and LC_{90}=67.9 mg/l, with CH_{I2}=12.11; g.l.=4; Prob.=0.9834, and the equation was Y=5.0875+5.6644 (X-10.616), where X is the extract concentration and Y is mollusk lethality.

Table 1. ***In vitro*** **molluscicide action of the ethanol extract from the fruit of Sapindus saponaria L. on** ***Galba cubensis*** **(Pfeiffer, 1839) and lethal concentrations (LC_{50} and LC_{90})**

Concentration (ml/l)	Exposed	Dead	Observed/expected mortality		General probit	Range (95% confidence interval)
20	72	7	9.7	3.22		
30	72	12	16.6	17.4		
40	72	38	52.7	36.2	CL_{50}=39.8	34.1<CL<46.4
50	72	45	62.5	51.1	CL_{90}=67.9	52.7 < CL < 86.9
70	72	69	95.8	66.0		
100	72	72	100	71.1		

Mozle (1939), cited by Ojewole (2004) and Torreaalba *et al.* (1953), in research done in Africa and Venezuela, described *S. saponaria* as a promising plant for the control of *Bulinus africanus* and *Australorbis glabratus*, respectively. Since then, the effect of various parts of this plant and other species of *Sapindus* has been studied as molluscicide. The methanolic extract and saponins from the *S. saponaria* fruit were found to be active against *B. glabrata*, with a LC_{100} of 5–10 ppm in 24 h (Ribeiro *et al.*, 1995), which was similar to the activity reported in this fruit in (Muley, 1978) *Melanoides tuberculata*, a snail of interest for pantropical regions (Iannacone *et al.*, 2013).

Moreover, they showed important *in vitro* activity levels in *Pomacea canaliculata*, an important agricultural pest, in a mix of aqueous extracts of *S. saponaria* and *Solanum mammosum* (*Solanacaeae*) and *Jatropha curcas* (*Euphorbiaceae*) in Ecuador. The former as a mix with LC_{50} = 17.78 ppm (Quijano *et al.*, 2014). The same authors reported that in field conditions *S. saponaria* (100%) was more active than the formulation with *S. mammosum* (50:50 m/m), in LC_{50}=66.6 ppm and LC_{50} = 192.3 ppm. Nevertheless, the LC_{90} achieved indicated that both formulations might not be considered good candidates for mollusk control programs (Quijano *et al.*, 2016).

Various *Sapindus* spp. have been described to have molluscicide action, *S. laurifolius* against *Bellamya bengalensis*, *S. trifoliatus* against *Lymnaea luteola*, and *S. mukorossi* against *P. canaliculata* (Sukumaran *et al.*, 2008; Huang *et al.*, 2003; Deshmane and Nanaware, 2011). Upadhyay and Singh (2012) said that the fruit of *S. mukorossi* was more active against *L. acuminata* than other less polar extracts, whereas various purified fractions were more active than some commercial products against this mollusk, the main host of *Fasciola gigantica* in the north of India.

The effect of the extracts (LC_{50} and LC_{90}) on heartbeat frequency is shown in Fig. 1. Table 2 shows the significant differences found between the heartbeats using the two lethal concentrations, also between LC_{90} and the control, but not the LC_{50}.

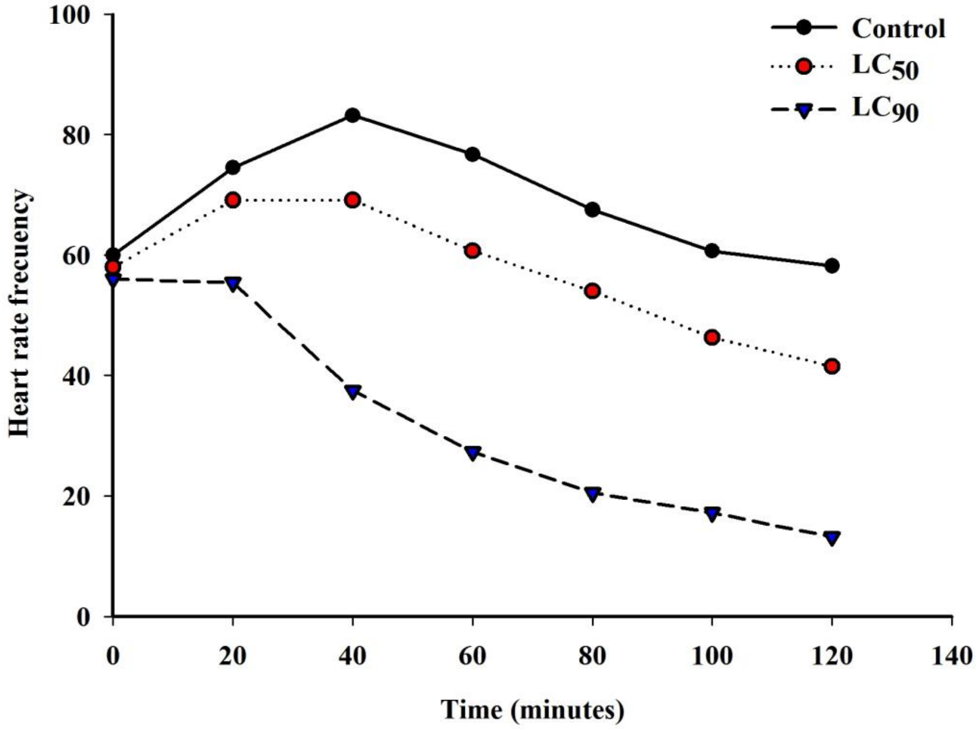

Fig. 1. Heartbeat frequency at LC_{50} and LC_{90} of the ethanol extract from Sapindus saponaria Fruit L. on *Galba cubensis* (Pfeiffer, 1839).

Table 2 ANOVA of the effect on heartbeat frequency of *Galba cubensis* (Pfeiffer, 1839) at LC_{50} and LC_{90} of the ethanol extract from Sapindus saponaria Fruit L.

	Sum of squares	Freedom degree	Mean square	F	p
Among groups	4 788.13	2	2 394.06		
Within groups	3 109.76	18	172.76	13.86	0.0002
Total	7 897.89	20			

R^2= 0,61%

Different from the LC_{90} group, the control underwent a slight heartbeat increase to 40 min, as in the LC_{50} treated mollusks during the first 20 minutes. This may have been caused by the influence of stressing factors in the medium and the manipulation of mollusks during the experiment. A decline in the number of heartbeats was corroborated after 40 min in the two groups

Other Cuban plant extracts have demonstrated some effects on the heartbeat frequency of *Biomphalaria havanensis*, a snail with medical interest. It underwent significant lethality levels against extracts of *Agave legrelliana* (Agavaceae), *A. franzosinii* and *A. fourcroydes*, a genus that contains saponins as active metabolite (steroid type) (Díaz and Ferrer, 1996; Hevia *et al.*, 2009). The heartbeat frequency is an important way to determine the molluscicide effect of a product (Malek and Cheng, 1974). In that sense, the extract of *S. saponaria* reduced heartbeat; thus, the vital functions of the mollusk.

One of the active compounds described in the pericarp of *S. saponaria and Sapindus* spp. is hederagenine monodesmosidic saponin types (23-hydroxy oleanolic acid). These are responsible of their detersive effects and biological activity (Lemos *et al.*, 1992; Ribeiro *et al.*, 1995; Abreu, 2005; Goyal *et al.*, 2014). These glucosides are particularly toxic to cold-blooded animals. Water loss is a biocidal action mechanism attributed to *Sapindus* spp., which consists of a modification in the permeability of the membrane of the mollusk that can cause death when the branchial mucosa is affected (Lacaille_Dubois and

Wagner, 1996; Chaieb, 2013). Bidesmosidic saponins are soluble in water, and generally biologically inactive (Lacaille_Dubois and Wagner, 1996).

Besides the hederagenine type saponins, Murgu and Rodríguez (2006), in Brazil, isolated acyclic sesquiterpene oligoglycosides as major components of S. saponaria, and evaluated the accumulation dynamics of these metabolites in different fruit development stages.

An important requirement to succeed in the use of plant molluscicides (other than their biological activity in mollusks, eggs, and juveniles) is the combined application of other techniques for integrated management of diseases caused by trematodes, using local resources (Knopp *et al.*, 2005; Singh *et al.*, 2010; Kiros *et al*., 2014). In that sense, species used for other purposes, like Moringa oleifera, have been suggested (da Silva *et al.*, 2013). Particularly, *S. saponaria*, is a plant with a growing interest in South America, where research aimed to develop related technologies and forest management has been conducted (Sánchez and Silva, 2008; Flechas *et al.*, 2009).

The fact that the plant material of *S. saponaria* used has a laboratory-scale effect on *G. cubensis* (though not recently collected), indicates that the breakdown of active compounds was insignificant or that the compounds generated in the process were active, too. This possibility should be further considered, since a prolonged mean lifespan of this activity is a desired quality for any molluscicide product obtained from plants. New interest prospects may arise in relation to this plant as a candidate to control intermediary hosts of trematode parasitosis in animals and humans. Therefore, it is important to develop additional studies in field conditions.

CONCLUSIONS

Lethality and heartbeat reduction of *G. cubensis* was corroborated in lab conditions, using the ethanol extract from the pericarp of *S. saponaria* fruit after six months of collection.

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