

Assessment of the Molluscicidal Activities of Sasanqua Saponin

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Abstract: This study is aimed at finding out the molluscicidal activities of Sasanqua Saponin extracted from the residue of *Camellia oleasa* (tea-cake seeds). The sasanqua saponin was obtained from Chenxi Chemical plant, Peoples' Republic of China. The molluscicidal activities were tested in the laboratory and against the eggs, juvenile and adult snails of *Oncomelania hupensis* and the intermediate host of *Schistosoma japonicum* (intestinal schistosomiasis). The immersion technique was adopted in the experiments. Sasanqua saponin extracted from the residue of *Camellia oleasa* (tea-cake seeds) was provided by Chenxi Chemical Plant, Peoples' Republic of China. The mollusciciding activities of the sasanqua saponin against eggs. Juvenile and adult *Oncomelania hupensis* snails (intermediate host of *Schistosoma japonicum*) were carried out in the laboratory and the field using immersion technique. The mortality rate of adult snails exposed to 10 ppm of sasanqua saponin at 20°C for 48 h was 96% and Lc90 were 2.76 and 10.34 ppm respectively. At 5 ppm exposure concentration for 48 and 72 h, the mortality rate was 96 and 100%, respectively. Lc50 and Lc90 after 48 h were 1.31 and 3.10 pm, respectively. The hatching rate snail eggs immersed in 5 ppm of sasanqua saponin after 72 h were 14%. There was on hatching of eggs at 10 ppm of sasanque saponin while the blank or control had 78% hatching rate. The mortality rates for juvenile snails exposed to 10 ppm of sasanqua saponin were 93.3 and 100% respectively after 48 and 72 h of incubation. Toxicity test showed that sasanqua saponin do not have any adverse effects on silver carp.

Keywords: Adult snails, juvenile snails, molluscicidal, oncomelania hupensis, sasanqua saponin, schistosoma japonicum

INTRODUCTION

Schistosomiasis (bilhareiasis) is one of the major public health problems in the people's republic of china (cheng, 1971; Mao and Shaq, 1982).

Snail host control measure is one of several possible supportive procedures in integrated schistosomiasis morbidity control activities. However, the control strategy is dominated by population based chemotherapy schedules (McCullough, 1992).

Snails may be controlled or eradicated by environmental modifications, use of molluscicides (chemical and plant origin). Adequate water supply and sanitation, proper management, of nightsoils (faeces and health education (Birley, 1991; WHO, 1993).

Snail control by the use of molluscicides has been shown to be a rapid and effective means of reducing schistosomiasis transmission. Mollusciciding in combination with control method have been shown to greatly reduce the incidence, prevalence and intensity of schistosomiasis (McCullough, 1992). Natural molluscicides from plant extracts, microorganisms and

biological agents have been found to be very promising but not fully developed (Birley, 1991). The control of the intermediate snail hosts of schistosomiasis is of secondary importance in terms of morbidity and transmission control. In china, it has been shown that without snail control, the prevalence of schistosomiasis cannot be maintained at a very low level for a long period of time; no matter how frequently chemotherapy is given (Chen and Lu, 1993).

Research on plant molluscicides have increased in recent years. This is due to its advantage of being more readily available, less expensive (cheaper), more aligned to self reliant control strategy and less polluting than the synthetics (Webbe and Ducan, 1978; Kloos and McCullough, 1981; Hostettmann and Marston, 1987; Kuo, 1987; McCullough, 1992).

In China, more than 500 species of plants have been screened for molluscicidal effects. Of the 500 species, 20 demonstrated high molluscicidal effect with snail mortality reaching 90 to 100% at concentrations of 30 to 10.000 ppm. Twenty-eight species were shown to have moderate effect, with mortality of 51 to 89% at

concentrations of 10.000 ppm, 114 species had only slight effect, killing 1 to 50% of snails at 10.000 ppm. The other species (338) were not toxic to *Oncomelania* snails (Kuo, 1987; Thiiborg *et al.*, 1996; Jayasinghe *et al.*, 1998).

Various classes of compounds have been found to be responsible for the molluscicidal activities of plants. However, saponin seems to hold the greatest promise for the control of snail vectors of schistosomiasis. This is due to the fact that saponins are strongly molluscicidal as synthetic agents and the content of saponins in vegetable material is often high, as much as 30% in some plants (Hostettmann and Marston, 1987; Abdel-Gawad *et al.*, 2000; Gao and Wang, 2006; El-Wakil, 2007; Hassan *et al.*, 2010).

Saponins are glycosides of triterpene or steroid (spirostanel) sapogenins or both. Saponins affect surface tension due to their froth-forming ability. The haemolytic activity of saponins is attributed to their formation of complexes with cholesterol in red blood cell membranes, which causes a collapse of the cell and the release of haemoglobin (Hostettmann and Marston, 1987; Thiiborg *et al.*, 1996; Jayasinghe *et al.*, 1998). The molluscicidal activities of saponins have been shown to vary with the position of the glycoside chain (mono and bidesmosidic differences), nature of the sugar chains, the sequence of the sugars, the interglycosidic linkages and the substitution patterns of the aglycone (Hostettmann and Marston, 1987). Since saponins seem to hold the greatest promise for the control of snail vectors schistosomiasis, thus the objective of this study is to test the sasanqua saponin extracted from *Camellia oleosa* for its molluscicidal activities against the eggs, Juvenile and adult snails of *Schistosoma japonicum*.

MATERIALS AND METHODS

Source of sasanqua saponin: The sasanqua saponin used in the mollusciciding experiments was provided by chenxi chemical plant, china. It was extracted from the residue of *camellia oleosa* seeds. This study was carried out between June-August, 1994 at the Department of Vector Control, Human Institute of Parasitic Diseases, Yueyang and Peoples' Republic of China.

- **Laboratory mollusciciding tests:**
 - **Mollusciciding of adult and juvenile (baby) *Oncomelania* snails with sasanqua saponin:** Live adult and juvenile (baby) *Oncomelania hupensis* snails of the same age and size, were selected for the test. The testing procedure used was as described by Komiya *et al.* (1962). For each concentration (2.5, 5 and 10 ppm) of the sasanqua saponin solution, groups of 30 adult and juvenile

snails were used, with 10 snails per bag. In all tests, a 72 h exposure period at temperatures from 20°C to 25°C was applied.

After the exposure period, the snails were washed thoroughly in running or deionized tap water. Snail death or mortality count was determined by tactile stimulation of the operculum followed by crushing (knocking) of the snails. The LC₅₀ and LC₉₀ values were determined by Litchfield and Wiicoxon (1949) method.

- **Mollusciciding of snail eggs:** Immersion method of Komiya *et al.* (1962) was used. Fifty eggs of same age from *Oncomelania hupensis* snails were used for each concentration (2.5, 5 and 10 ppm) of sasanqua saponin. Hatching rate of snail eggs into baby snail was made after 72 h (3 days) of incubation.
- **Field mollusciciding tests:** The mollusciciding effects of sasanqua saponin in the ditch of lakeshore were carried out at the water temperature of 26.6°C. The testing procedure used was immersion method as described by Komiya *et al.* (1962) 30 adult snails (10 per nylon bag) were used for each concentration of sasanqua saponin solution. Mollusciciding by immersion was also carried out in combination with shoveling of earth in the ditch inside embankment.
- Toxicity to fish

RESULTS

The mortality rate of adult snails and Juvenile snails exposed to three concentrations (2.5, 5 and 10 ppm) of sasanqua saponin is as indicated in Table 1 and 2. The mortality rate of snails exposed to 2.5 ppm for 72 h was 100% while at 5 ppm it was 96 and 100% after 48 and 72 h respectively. Lc₅₀ and Lc₉₀ after 48 h were 1.31 and 3.10 ppm, respectively. The mortality for adult snails exposed to 10 ppm of sasanqua saponin for 48 h was 96% with Lc₅₀ and Lc₉₀ being 2.76 and 10.34 ppm, respectively.

At room temperature of 23-26°C, the mortality rate of baby (Juvenile) snails exposed to 10 ppm of sasanqua saponin for 48 and 72 h were 93.3 and 100% respectively (Table 2).

When the eggs were immersed in 5ppm of sasanqua saponin at constant temperature of 25°C for three (3) days, the hatching rate was 14%. No baby snail emerged when the eggs were immersed in 10 ppm of sasanqua saponin for three days, indicating the eggs were killed (Table 3). The dissection of the eggs showed that the eggs were denatured and dead. The hatching rate of snail eggs in the control group not exposed to sasanqua saponin was 78%.

In the field, mollusciciding trials by immersion in 10 ppm sasanqua saponin for 72 h were 100% mortality

Table 1: Mollusciciding effect of three concentrations of sasanqua saponin on adult *Oncomelania hupensis* snails at 20 to 25°C

Concentration of sasanqua saponin (ppm)	Exposure times (hours)								
	24			48			72		
	No of adult snails	No of dead snails	Mortality rate (%)	No of adult snails	No of dead snails	Mortality rate (%)	No of adult snails	No of dead snails	Mortality rate (%)
2	30	18	60	30	20	67	30	30	100
5	30	22	73	30	29	96	30	30	100
10.0	30	24	80	30	29	96	30	30	100
Control (water)	30	0	100	30	1	100	30	2	7

Table 2: Mollusciciding effect of three concentrations of sasanqua saponin on juvenile *Oncomelania hupensis* snails

Concentration of sasanqua saponin (ppm)	Exposure times (hours)								
	24			48			72		
	No of juven snails	No of dead snails	Mortality rate (%)	No of juven snails	No of dead snails	Mortality rate (%)	No of juven snails	No of dead snails	Mortality rate (%)
2.5	30	12	40	30	17	56.6	30	19	61.3
5.0	30	18	60	30	19	61.3	30	25	83
10.0	30	24	80	30	28	93.3	30	30	100

Table 3: Mollusciciding effect of three concentrations of sasanqua saponin on eggs

Concentration of sasanqua saponin (ppm)	Exposure times (days)					
	3			5		
	No of eggs	Accumulated no of hatched eggs	(%) Hatching of rate	No of eggs	Accumulated no of hatched eggs	(%) Hatching of rate
2.5	50	23	46	50	19	38
5.0	50	7	14	50	0	0
10.0	50	0	0	50	0	0
Control (water)	50	39	78	50	46	92

Table 4: Mollusciciding effect of sasanqua saponin alone and in combination with earth shoveling in the field on adult *Oncomelania hupensis* snails

Concentration of sasanqua saponin (ppm)	Exposure times (hours)								
	24			48			72		
	No of snails	No of dead snails	Mortality rate (%)	No of snails	No of deadsnails	Mortality rate (%)	No of snails	No of dead snails	Mortality rate (%)
2.5	30	5	17	30	6	20	30	9	30
5.0	30	5	17	30	18	60	30	22	73
5.0 + earth shoveling	30	28	93	30	30	100	30	30	100
10.0	30	21	70	30	30	100	30	30	100
Control	30	0	0	30	0	0	30	1	4

Table 5: Comparative mollusciciding effects of 10 ppm sasanqua saponin with 10 ppm of sodium pentachlorophenate (NaPcp)

Molluscicide (10 ppm)	Snail no	Mortality rate (%)
Sasanqua saponin	30	100
NaPCP	30	99.8
Control (blank)	30	7.3

(Table 5). Sasanqua saponin had no adverse effects on fish (silver carp). TLM of silver cap for 48 h were 3.42 and 3.49 ppm, respectively.

DISCUSSION

rate while the control group had only 4% mortality rate (Table 4).

The combination of earth shoveling with immersion indicated a mortality rate of 100% at 5 ppm of sasanqua saponin after 48 h exposure. The average density of living snails in the ditch after 5 and 30 days of mollusciciding declined by 93.1 and 99.5%, respectively. In contrast with sasanqua saponin, the average density of living snails in the test with 10 ppm of NaPcP (Sodium Pentachlorophenate) and the blank control group declined by 99.8 and 7.3%, respectively

The laboratory and field tests indicated that sasanqua saponin has a high mollusciciding effect on the eggs, juvenile and adult *Oncomelania hupensis* snails. The mollusciciding effect is at par with the chemical molluscicide, NaPcP. However, NaPcP can pollute bodies of water when applied; hence the plant molluscicide sasanqua saponin could be more preferable as it is less polluting than the synthetics. No plant molluscicide has been found to be specific to snails and none has been employed widely in any endemic country (McCullough, 1992; Abdel-Rahman and Hassan, 2008; Bakry, 2009).

Sasanqua saponin was found to be an effective molluscicide at low concentration of 10 ppm. This is in agreement with report of Kuo (1987) in which *Camellia oleosa* 100% mortality had at 2000 ppm after 24 h of exposure. This plant is known to grow in ten provinces of South China. The seeds are oleaginous and after extraction of the oil, the dregs are made into tea cake. This *Camellia oleosa* seeds from which sasanqua saponin was got has been reported to kill leeches, *Oncomelania* and *Lymnaea* snails (Kuo, 1987). This plant could become an effective and economic plant molluscicide when used in combination with chemotherapy. This is in view of its being non-toxic to fish (Kuo, 1987; Abdel-Gawad *et al.*, 2000; El-Wakil, 2007; Hassan *et al.*, 2010).

Mollusciciding operations, health education and community involvements, coupled with population-based chemotherapy in China would go a long way in schistosomiasis control.

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