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Molluscicidal activity of chlorophyll extraction against the freshwater snails

Mona Said Mahmoud[1], Peter Richter[2], Hatem Abdel Mawgoud Shalaby[1*], Omnia Mohamed Kandil[1], Donat-Peter Häder[2]

[1]*Parasitology and Animal Diseases Department, National Research Center, Giza, Egypt*

[2]*Biology Department, Ecophysiology of Plants, Friedrich-Alexander-University Erlangen-Nuremberg, Germany*

PEER REVIEW

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Dr. Guiquan Guan, State Key Laboratory of Veterinary Etiological Biology, Gansu Provincal Key Laboratory of Veterinary Parasitology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences (CAAS), Xujiaping, Lanzhou, Gansu 730046, China.
Tel: +8693 1834 2671
E-mail: guanguiquan@caas.cn

Comments

This is an exploratory research work about using plant extracts to control snails for preventing fluke diseases. The authors evaluated the activity of chlorophyllin for killing snails in lab condition. The results showed that the chlorophyllin extracted from spinach has promising efficiency to kill new hatched snails, which suggested that it could be used to prevent fluke diseases via control snails in freshwater.
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ABSTRACT

Objective: To evaluate the molluscicidal activity of chlorophyll extract as a photodynamic substance against the snails *Lymnaea stagnalis, Biomphalaria* spp. and *Physa marmorata*.
Methods: Chlorophyllin was extracted from deep-frozen spinach. Snails were incubated in chlorophyllin containing water with 2.5, 5.0, 10.0 and 15.0 µg/mL. All samples were kept in darkness overnight for incubation. After incubation, three samples were irradiated with simulated solar radiation for 3 h. After irradiation, the vitality of the organisms was determined.
Results: The photodynamically active chlorophyllin, at low concentrations, was able to kill snails within a few hours under exposure of solar radiation. Besides, it had a killing effect by about 70% and 100% on the snails' eggs and the newly hatched snails, respectively, after 3 h exposure to solar radiation.
Conclusion: The derivates of chlorophyll was a very interesting substance for photodynamic freshwater snail control. Hence, it might be a promising and cheap new strategy which probably had the potential to replace the synthetic molluscicides for snail control.

KEYWORDS
Chlorophyllin, Fresh water snail, Photodynamic, Molluscicide

1. Introduction

Fasciola spp. cause one of the most important zoonotic diseases with a global economic impact on livestock production systems and a poorly defined but direct effect on human health[1]. Its life cycle includes freshwater snails (Lymnaeidae) as an intermediate host[2]. The worldwide losses in animal productivity due to fasciolosis were conservatively estimated at over US $ 3.2 billion per annual[3]. Also, bilharziosis (schistosomiasis) is a major problem in Egypt and other countries worldwide caused by the trematode *Schistosoma* (Phylum: Plathelmintes). *Schistosoma mansoni* (Africa and South America) and *Schistosoma japonicum* (Southeast Asia) cause gastrointestinal symptoms, while *Schistosoma haematobium* (Africa) causes mostly urogenital disorders. About 200 million people worldwide are infected with schistosomiasis, and about 200 000 to one million people die each year from the complications[4]. The intermediate hosts for *Schistosoma* are snails (*Biomphalaria* for

*Corresponding author: Hatem Abdel Mawgoud Shalaby, Parasitology and Animal Diseases Department, National Research Center, Giza, Egypt.
Tel: 02 0337 1615
Fax: 02 0337 0931
E-mail: shalaby85@gmail.com
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Schistosoma mansoni, *Bilinus* for *Schistosoma haematobium*, or *Tricula aperta* for *Schistosoma japonicum*).

One of the major preventive steps against trematode infection is the control of the vector snail population by use of molluscicides[5,6]. The investigation of the molluscicidal properties of plants has recently been greatly expanded, with more than 1 400 species studied thus far. Plant extracts have been used to control the snail population with the advantage that, besides being less toxic in nature, they can be degraded faster than the synthetic molluscicides that have also high cost, possible build up of snail resistance, and toxicity in non target organisms[7,8]. Experiments conducted with photodynamic substances extracted from different plant sources, yielded very promising results. In a work performed by Abdel-Kader *et al.*[9], it was demonstrated that larvae of *Culex* were sensitive to hematoporphyrine (0.07 μmol/mL). Also, larvae of *Musca domestica* could be killed with this substance (10 μmol/mL). In the course of his PhD thesis, Allah el Tayeb demonstrated the effectiveness of hematoporphyrine against *Culex* and eggs of the snail *Lymnea natalensis* (vector of the trematode *Fasciola hepatica*)[10]. In a promising work, Wohllebe *et al.* reported that water-soluble chlorophyllin (resulting from chlorophyll after removal of the phytol) and pheophorbid (produced from chlorophyllin by acidification), when used at low concentrations and added to the water body, were able to kill mosquito larvae and other small animals within a few hours under exposure of solar radiation[11]. They added that chlorophyllin was about a factor of 100 more effective than methylene blue or hematoporphyrine, which were tested earlier for the same purpose. In this work the molluscicidal activity of chlorophyll extract as a photodynamic substance against the snails *Lymnaea stagnalis* (*L. stagnalis*), *Biomphelaria* spp. and *Physa marmorata* (*P. marmorata*) have been evaluated.

2. Materials and methods

2.1. Test animals

Adult *L. stagnalis, Biomphelaria* spp. and *P. marmorata* snails, collected locally from lakes in botanic garden in Erlangen, were used as the test animals. Snails were acclimatized in dechlorinated tap water and directly used for experiments.

2.2. Plants and chlorophyll extraction

Different sources for isolation of chlorophyll were used, *e.g.* weeds with algae and freezed spinach. The deep-frozen spinach delivered the highest concentration of chlorophyll. It was extracted in a water bath at 55 °C from the leaves with 96% methanol after adding $CaCO_3$, which prevented formation of pheophytin as recommended by Wohllebe *et al*[12]. The extract was filtered and petroleum benzene was added. The upper lipophilic phase was taken and saponified with methanolic NaOH. This treatment converted chlorophyll into water-soluble chloropyllin. NaOH precipitation yielded high chlorophyllin concentrations. To calculate the concentration, this fallout was measured with a spectrophotometer. The extract was stored in a dark flask at room temperature.

2.3. Sample treatment and experimental design

Defined amounts of chlorophyllin dissolved in methanolic NaOH were evaporated in darkness. Each concentration was applied in three repetitions and three independent approaches. Water and NaOH-treated (corresponding to highest chlorophyllin concentration) samples served as controls. Each chlorophyllin sample was dissolved in 10 mL of water, and subsequently, three snails were added. Snails were incubated in chlorophyllin containing water with 2.5, 5.0, 10.0 and 15.0 μg/mL. All samples were kept in darkness overnight for incubation. After incubation, three samples were irradiated with simulated solar radiation (Sol 180 1 200 W, mercury lamp 0383, Dr. Hönle, Martinsried, Germany) for 3 h. The lamp output was PAR 480 W/m^2, UV-A 32.67 W/m^2, and UV-V 0.77 W/m^2 (determined with spectroradiometer OL 754, Optronics, USA). The emission spectrum of the lamp is shown elsewhere[13]. Three parallel samples were kept in darkness as dark controls. This scheme was performed for all concentrations. After irradiation, the vitality of the organisms was determined. Individuals showing no vital signs (movements, reflexes after tipping with a needle) were counted as dead, while not affected organisms and organisms with reduced viability (compared to untreated control) were counted as survivors. The viability was observed under the microscope for movement of the animal inside.

3. Results

The molluscicidal activity of chlorophyll at four different concentrations (2.5, 5.0, 10.0 and 15.0 μg/mL) against freshwater snails *L. stagnalis, Biomphelaria* spp. and *P. marmorata* was studied under laboratory conditions. The Photodynamically active chlorophyllin at the four different concentrations was able to kill 100% of snail *L. stagnalis* within few hours under exposure of solar radiation. In snail *Biomphelaria* spp., chlorophyllin was found to be both time and concentration dependent. It was able to kill 100% of the snails within 3 h under exposure of solar radiation. In snail *P. marmorata*, chlorophyllin was found to be time dependent, and the highest lethal effect had occurred after 3 h of exposure that 88.9% and 100% of the snails were killed at 2.5 μg/mL and

Table 1
The effect of different concentrations of chlorophyllin on freshwater snails.

Organism	Amount of chlorophyll (μg/mL)	Mortality %											
		In solar light			In darkness			Control in solar light			Control in darkness		
		1 h	2 h	3 h	1 h	2 h	3 h	1 h	2 h	3 h	1 h	2 h	3 h
L. stagnalis	2.5	100.0	100.0	100.0	0.0	0.0	0.0	0.0	11.1	11.1	0.0	0.0	0.0
	5.0	100.0	100.0	100.0	0.0	0.0	0.0						
	10.0	100.0	100.0	100.0	0.0	0.0	0.0						
	15.0	100.0	88.9	100.0	0.0	0.0	0.0						
Biomphelaria spp.	2.5	66.6	100.0	100.0	0.0	0.0	0.0	0.0	11.1	11.1	0.0	0.0	0.0
	5.0	77.7	88.9	100.0	0.0	11.1	11.1						
	10.0	88.9	100.0	100.0	0.0	0.0	0.0						
	15.0	100.0	100.0	100.0	0.0	11.1	11.1						
P. marmorata	2.5	66.7	88.9	88.9	0.0	0.0	0.0	0.0	11.1	11.1	0.0	0.0	0.0
	5.0	66.7	88.9	100.0	0.0	0.0	0.0						
	10.0	66.7	100.0	100.0	0.0	0.0	0.0						
	15.0	88.9	88.9	100.0	0.0	0.0	0.0						

the other concentrations, respectively (Table 1). This study was extended to study the lethal effect of chlorophyllin on the snails' eggs and the newly hatched snails. The results showed that, it had a killing effect by about 70% and 100% on the snails' eggs (Table 2) and the newly hatched snails, respectively, after 3 h exposure to solar radiation.

Table 2
The killing effect of high concentrations of chlorophyllin on freshwater snails' eggs.

Concentration of chlorophyll	Mortality %	
	L. stagnalis	*P. marmorata*
30 μg/mL	70.3	70.6
Control	0.0	0.0

The results of the *in vitro* experiments showed that the photodynamically active chlorophyllin, at low concentrations, was able to kill the snails and their eggs within a few hours under exposure of solar radiation.

4. Discussion

One way to reduce the incidence of trematode infection is to de-link the life cycle of flukes by killing the snails[14]. Synthetic molluscicides have been widely used for the effective control of harmful snails, but it has now been realized that these molluscicides cause serious environmental hazards[15]. Consequently, more researches are now being focused on molluscicide of plant origin[15,16], as these are easily biodegradable and, therefore, safer to use than their synthetic counterparts[7,17,18]. In this study, the photodynamically active chlorophyllin, at low concentrations, was able to kill snail animals within few hours under exposure of solar radiation. Besides, it had a killing effect by about 70% and 100% on the snails' eggs and the newly hatched snails, respectively, after 3 h exposure to solar radiation. In that sense, natural chlorophyll and its derivates were tested for their ability to control mosquito larvae[11]. The application of very low concentrations of chlorophyllin to water inhabited by mosquito larvae reliably killed these larvae via the photodynamic activity of chlorophyllin in the presence of light. Upon irradiation solubilized chlorophyll transferred its excitation energy to oxygen to produce singlet oxygen ($^1O_2$) and other reactive oxygen species that have the potential to damage and kill specific developmental stages of pest organisms or their vectors[19,20]. In this study, the time-dependent effect of chlorophyllin might be due to the uptake of its active component which progressively increased the lethal photodynamic effects in snail body with increase in exposure peroid. In 2010 Erzinger and Hader developed and patented at INPI (National Institute of Intellectual Property) a new Bioinsecticide Nontoxic Biodegradable from a new semi-synthetic derivative of chlorophyll and in conjunction with a formulation system that was able to get a product with high stability front light and maintained the same lethal power of chlorophyll and chlorophyllin for mosquito larvae described in previous work[21]. Recently, Wohllebe *et al*. demonstrated for the first time the efficiency of the photodynamic substance chlorophyllin to kill different life stages of the protozoan parasite *Ichthyophthirius mulftifiliis* (Fouquet) which caused the white spot disease in many freshwater fish species[15]. They added that experiments with infected fish indicated that the substance was also very effective against parasites on the fish.

The derivates of chlorophyll was a very interesting substance for photodynamic freshwater snail control. Hence, some primarily experiments were conducted in which the effects on *L. stagnalis, Biomphelaria* spp. and *P. marmorata* were tested. It might be a promising and cheap new strategy for freshwater snail control.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Freshwater snails are the intermediate host of trematodes infective to animals and human. The common strategy for prevention of fluke disease is reducing quantity of snails in freshwater. It's a promising research field to explore environmentally friendly medicines to control snails for preventing fluke diseases.

Research frontiers

Plant extracts have been used to control parasites due to their natural advantages, such as less toxicities, low residues and easy degradation. In present study, authors first evaluated the molluscicidal efficiency of chlorophyllin from spinach in the world.

Related reports

It was reported that extracts of chlorophyll had activity for killing mosquitoes, water flea, as well as fungi and bacteria. However, there was not report about control snails using chlorophyll extracts.

Innovations and breakthroughs

Chlorophyllin can be found in any green plants and is easy to extract. It is much cheaper and 100% biodegradable. In this paper, authors demonstrated that it had high efficiency to snails, especially for newly hatched snails. This is the first report using chlorophyllin to kill snails.

Applications

Although this is just a lab evaluation about killing activity of chlorophyllin to snails, it suggests that chlorophyllin has a potential value as a plant medicine to prevent fluke diseases in future.

Peer review

This is an exploratory research work about using plant extracts to control snails for preventing fluke diseases. The authors evaluated the activity of chlorophyllin for killing snails in lab condition. The results showed that the chlorophyllin extracted from spinach has promising efficiency to kill new hatched snails, which suggested that it could be used to prevent fluke diseases via control snails in freshwater.

References

[1] Piedrafita D, Spithill TW, Smith RE, Raadsma HW. Improving animal and human health through understanding liver fluke immunology. *Parasite Immunol* 2010; **32**(8): 572-581.

[2] Imani-Baran A, Yakhchali M, Malekzadeh VR, Paktarmani R. Molecular study for detecting the prevalence of *Fasciola gigantica* in field-collected snails of *Radix gedrosiana* (Pulmonata: Lymnaeidae) in northwestern Iran. *Vet Parasitol* 2012; **189**(2-4): 374-377.

[3] Spithill TW, Smooker PM, Copeman DB. *Fasciola gigantica*: Epidemiology, control, immunology and molecular biology. In: Dalton JP, editor. *Fascioliasis*. Oxford: CAB International Publishing; 1999, p. 465-525.

[4] World Health Organization. Report of the WHO informal meeting on use of triclabendazole in fascioliasis control. Geneva: World Health Organization; 2006.

[5] Kumar P, Singh VK, Singh DK. Combination of molluscicides with attractant carbohydrates and amino acids in bait formulation against the snail *Lymnaea acuminata*. *Eur Rev Med Pharmacol Sci* 2011; **15**(5): 550-555.

[6] He JC, Wang TP, Zhang SQ, Gao FH, Zhang GH, Yang WP, et al. [Evaluation of mid-term effectiveness of medium-and-long-term programme for prevention and control of schistosomiasis in Anhui Province]. *Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi* 2011; **23**(3): 249-252. Chinese.

[7] Jaiswal P, Sing DK. Molluscicidal activity of *Carica papaya* and *Areca catechu* against the freshwater snail *Lymnaea acuminate*. *Vet Parasitol* 2008; **152**(3-4): 264-270.

[8] Al-Daihan S. Effect of plant molluscicides on selected enzymes related to energy metabolism in *Biomphalaria arabica* snails molluscan hosts to *Schistosoma mansoni* in Saudi Arabia. *J Egypt Soc Parasitol* 2010; **40**(1): 187-195.

[9] Abdel-Kader MH, Al-Sherbini AS, El Tayeb TA. 8-Sunlight and photosensitizers for insect control. The 9th International Conference on "Environmental Protection is a must"; 1999 May 4-6; Alexandria: Alexandria University; 1999.

[10] El Tayeb TA. Laser scanning microscopy for determination of the efficiency of hematoporphyrin in control of *Culex pipiens* larvae and the snail vector of *Fasciola gigantica* [dissertation]. Cairo: National Institute of Laser Enhanced Sciences (NILES), Cairo University; 2003, p. 1-216.

[11] Wohllebe S, Richter R, Richter P, Häder DP. Photodynamic control of human pathogenic parasites in aquatic ecosystems using chlorophyllin and pheophorbid as photodynamic substances. *Parasitol Res* 2009; **104**(3): 593-600.

[12] Wohllebe S, Ulbrich C, Grimm D, Pietsch J, Erzinger G, Richter R, et al. Photodynamic treatment of *Chaoborus crystallinus* larvae with chlorophyllin induces necrosis and apoptosis. *Photochem Photobiol* 2011; **87**(5): 1113-1122.

[13] Klisch M, Sinha RP, Richter PR, Häder DP. Mycosporinelike amino acids (MAAs) protect against UV-B-induced damage in *Gyrodinium dorsum* Kofoid. *J Plant Physiol* 2001; **158**(11): 1449-1454.

[14] Jaiswal P, Singh VK, Singh DK. Enzyme inhibition by molluscicidal component of *Areca catechu* and *Carica papaya* in the nervous tissue of vector snail *Lymnaea acuminata*. *Pestic Biochem Physiol* 2008; **92**(3): 164-168.

[15] Wohllebe S, Richter P, Häder DP. Chlorophyllin for the control of *Ichthyophthirius multifiliis* (Fouquet). *Parasitol Res* 2012; **111**(2): 729-733.

[16] Singh V, Singh DK. The effect of abiotic factors on the toxicity of cypermethrin against the snail *Lymnaea acuminata* in the control of fascioliasis. *J Helminthol* 2009; **83**(1): 39-45.

[17] Srivastava P, Singh DK. Control of harmful snails: Tajpat (*Cinnamomum tamala*) a potential molluscicide. *J Appl Biosci* 2005; **31**: 128-132.

[18] Kumar P, Singh DK. Molluscicidal activity of *Ferula asafoetida*, *Syzygium aromaticum* and *Carum carvi* and their active components against the snail *Lymnaea acuminata*. *Chemosphere* 2006; **63**(9): 1568-1574.

[19] He YY, Häder DP. Involvement of reactive oxygen species in the UV-B damage to the cyanobacterium *Anabaena* sp. *J Photochem Photobiol B* 2002; **66**(1): 73-80.

[20] Tominaga H, Kodama S, Matsuda N, Suzuki K, Watanabe M. Involvement of reactive oxygen species (ROS) in the induction of genetic instability by radiation. *J Radiat Res* 2004; **45**(2): 181-188.

[21] Erzinger GS, Häder DP. Inventor; Bioinsecticide nontoxic biodegradable from UNIVILLE. National Institute of Intellectual Property-INPI. No. 020090120220. 20. 2009.