EVALUATION OF THE OVICIDAL ACTIVITY OF CALCIUM HYPOCHLORITE ON THE EGGS OF *Bulinus globosus*, INTERMEDIATE HOST OF *Schistosoma haematobium* IN NIGERIA

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Abstract

The ovicidal activity of calcium hypochlorite was investigated in the laboratory using eggs of *Bulinus globosus* collected from a fresh water stream in Ipogun Village, Ondo State, Nigeria. Calcium hypochlorite concentrations of 3mg/l, 4mg/l, 6mg/l, 9mg/l, and 15mg/l were prepared using untreated well water. One-egg-mass was introduced into 60 ml each of the test solution concentrations and also into untreated well water in separate 100 ml beakers. The assays were conducted in three replicates and were observed for 48 hours at room temperature. Results showed that calcium hypochlorite had positive ovicidal effect with an LC_{95} of 12.86mg/l at 48 hours. This suggests that calcium hypochlorite has effective ovicidal activity, which may be used in the control of the schistosome bearing snail, *Bulinus globosus.*

Keywords: calcium hypochlorite, ovicidal, snail host, schistosomiasis, control.

Introduction

Schistosomiasis continues to plague various communities in developing countries with little or no access to safe potable water. There are recent global concerns on the attention given to its control and eradication as it is currently one of the Neglected Tropical Diseases [NTDs] (WHO 2007), enjoying a low operational and rationalized budget, particularly in tropical Africa. Chemotherapy still remains the major control option mounted in the global war against the scourge, but this alone cannot solve the problem thus growing concerns about an integrated approach in the control of the disease is becoming popular.

Early control programmes are necessary in the absence of any anti-schistosomal drugs focussed on the elimination of snails using synthetic molluscicides (Sturrock, 2001). Perhaps one of the major setbacks about the latter option, is that such water bodies are used in the endemic communities for various domestic and recreational purposes (Oniya 2007). The need for a safe molluscicide has led into extensive research into vegetable alternatives and many potential candidates have been identified (Farnsworth *et al* 1983; Adewunmi, 1984; Mkoji *et al* 1989; Baptista *et al* 1992; Azare *et al* 2007). Though there have been reported successful field trials (Adewunmi *et al* 1990; Baptista *et al* 1992; Takougang *et al* 2006), it still does not prevent repopulation of the snail species after focal applications. Prolonging repopulation of snail species as much as possible will promote the use of chemo-therapeutic agents in endemic communities, as it reduces the transmission potential, particularly the aquatic phase. Oniya *et al* (2006) reported the molluscicidal potential of calcium hypochlorite, a synthetic chemical used in the treatment of municipal water. Further exploration of calcium hypochlorite as an ovicidal agent, targeted at the eggs of the intermediate snail host is presented in this paper.

Materials and methods

Snail collection

Adult *B. globosus* snails were collected from Aponmu Stream in Ipogun Village (7°19′ N; 5°05′E), Ondo State, Nigeria. The village is about 14 kilometres from Akure, the Ondo State capital and is also endemic for

This study was accepted on 29th December 2010.
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Zoological Society of Nigeria.

schistosomiasis. The snails were collected between 08.00 and 10.00 hours with the aid of a scoop net made from a kitchen sieve and mounted on a 1.5 m wooden handle. They were transferred into a transparent bottle containing about 60 mls of the source-water and transported to the laboratory.

Egg retrieval

In the laboratory, the snails were introduced into five rectangular glass tanks measuring 60 cm x 30 cm x 30 cm, lined with transparent polyethylene bags. The glass tanks contained untreated well-water. The snails were fed with dried lettuce once daily, and they laid eggs overnight. The water and the polyethylene bags in the snail aquarium were changed daily. The egg masses were retrieved every morning by cutting off the area on the polyethylene where eggs were deposited by the snails. The eggs retrieved were then transferred into a beaker containing untreated well water.

Preparation of test solutions

Test solutions were prepared six (6) hours prior to the commencement of the experiment using various concentrations of calcium hypochlorite (NAAFCO, Nigeria). The concentrations used were: 3mg/l, 4mg/l, 6mg/l, 9mg/l, and 15mg/l. Each was dissolved in a bottle containing one litre of untreated well-water. A control was set up using untreated well-water. The hypochlorite solution was allowed to stand for 6 hours for dissolution and homogeneity.

Test procedure

One hundred ml capacity beakers were used for the bioassay. The aim was to determine the LC_{95} after 48 hours. Sixty ml each of the different concentrations were poured into each of the beakers, and the egg masses were introduced into each of the beakers i.e. one egg mass per beaker. Ten egg cells were selected from each mass and used for the assays. Each of the selected masses was observed under the microscope prior to being introduced into the test medium to ascertain that the egg cells were alive. The beakers were labelled accordingly and experiment was conducted in three replicates for each concentration and control under room temperature. After the egg masses were introduced into the various beakers containing different concentrations of chlorine, they were observed under the microscope at different intervals for mortality. Mortality of the eggs was determined by microscopic observation and recorded at 0, 4, 8, 12, 16, 18, 24, 32, 36, and 48 hours.

Results

During the 16th and 18th hours of introduction of egg masses into the various concentrations, the colour of the egg mass became pale in the higher concentrations of 9mg/l and 15mg/l. Between the 24th to 36th hours, the jelly layer in the highest concentration had reduced considerably, development had also become stagnated, and most of the cells had died. Dead embryos remained motionless in their cells, and showed no signs of development, contrary to observations recorded in the control set up. The dead cells were characterized with a marked lightening of the golden colour of the egg cells.

Plate 1: Egg cells of *Bulinus globosus* at 48th hour in 4mg/l test solution.

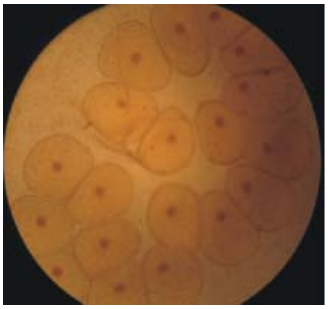

Plate 2: Egg cells of *Bulinus globosus* at 48th hour in 15mg/l test solution.

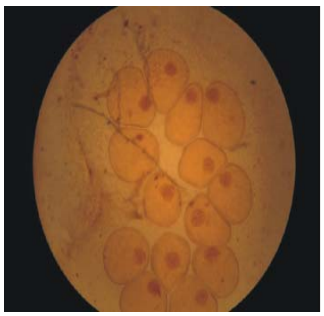

Plate 3: Egg cells of *Bulinus globosus* at 48th hour in control.

Plate 4: Juvenile snails emerge from egg cells after 7 days in control.

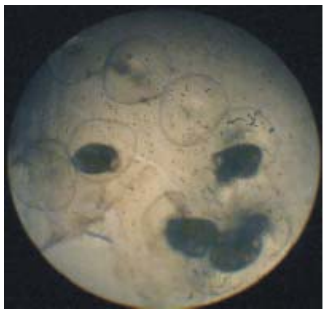

Plate 5: Few juvenile snails emerge from the egg cells after 7 days in 4mg/l test solution.

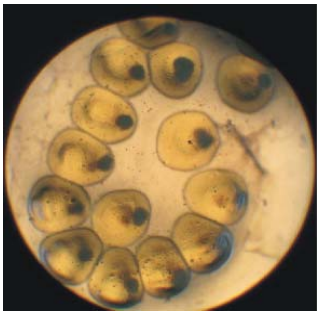

Plate 6: No juvenile snails emerged from the egg cells after 7 days in 15mg/l test solution.

By the 48th hour the egg cells had already become pale except for some of the egg cells in the 3mg/l, 4mg/l (Plate 1) and 6mg/l test concentrations, while most of the cells had died in the higher concentrations. Development had stopped in almost all the egg cells in the 9mg/l and 15mg/l (Plate 2) test concentrations; those that were not dead were no longer developing. Conversely, good development was observed in the egg cells in the control (Plate 3). After seven days, juvenile snails emerged from the control (Plate 4), while few snails emerged from the test concentrations (Plate 5), apart from the 3mg/l where an average of seven snails emerged. On the contrary, no snail emerged from the highest concentration of 15mg/l (Plate 6). The percentage mortalities in the three replicates are presented in Table 1. The LC_{95} is presented in Figure 1.

Table 1: Percentage mortality in the 3 replicates at 48th hour.

Conc. (mg/l)	I	II	III
		Mortality (%)	
Control	0	0	0
3	30	30	40
4	60	30	50
6	50	60	60
9	80	100	90
15	100	100	100

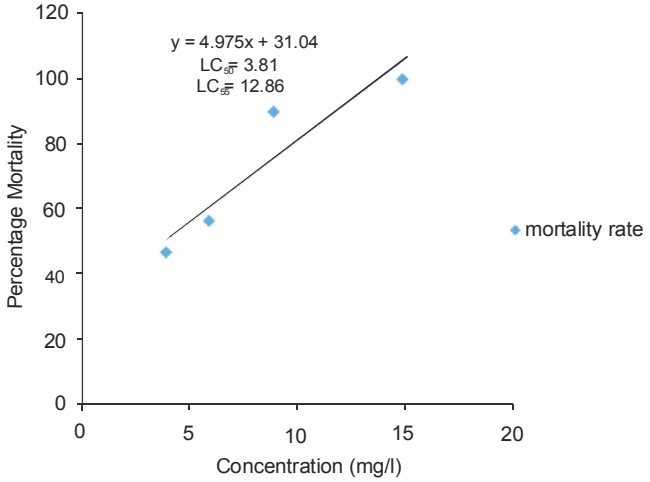

Figure1: LC_{95} of calcium hypochlorite on eggs of *B. globosus* after 48th hour.

Discussion

The need for the eradication and control of parasitic diseases, as well as associated problems was reviewed by Ukoli 1987, 1991, and 1992. The first fruits of the WHO programme were molluscicides which became available in the mid 1950's and one of which, niclosamide (bayluscide), is still in use and effective till today (Sturrock, 2001). At present the scope of molluscicide is only limited to the various snail hosts of the *Schistosoma* species. Each focal application does not however, prevent repopulation of snails soon after application, vegetable or synthetic not withstanding.

The present results showed that calcium hypochlorite had very positive ovicidal activity against the eggs of *B. globosus*. Almost 100% mortality was observed in the two highest concentrations from 32nd to 48th hours post treatment of the egg masses. The characteristic stagnation of development which was clearly evident in the higher concentrations of the test chemical, followed by paling and eventual death of the egg cells are suggestive of its potential use in field applications. Oniya, *et al* (2006) reported calcium hypochlorite as a potent molluscicide at low concentration (LC_{50} 8.32mg/l), being readily available and also used in the treatment of communal drinking water.

These results also confirm the protective role of the jelly layer of the egg mass during adverse conditions. This in essence made it impossible for most of the egg cells in the 3mg/l, and some in the 4mg/l concentration to be totally destroyed. On the other hand, the jelly layer could not tolerate higher concentrations of chlorine and that was responsible for the high mortality rate observed in the two highest concentrations of 9mg/l and 15mg/l.

Effective ovicidal control of eggs of *B. globosus* would make it impossible for the snails to repopulate

soon after any molluscicide activity as the eggs of the snails in the water or along the vegetation on the river banks would also be destroyed. It is thus advisable to integrate the concept of ovicidal agents into molluscicide to promote a more effective snail-kill. Thus, epidemiologists should see the importance of preventing potential snail eggs from hatching as another laudable step to achieving control. This might in time lead to an effective control of schistosomiasis.

Other measures to be integrated along with this option should include chemotherapy and regular community-based enlightenment programmes as previously suggested (Oniya 2007). However, it also requires genuine political commitment by the government in designing intervention programmes that are based on long term strategies and finally uninterrupted implementation of designed programmes until they are completed as planned (Oniya and Olofintoye 2009).

The chemical may be applied directly into water bodies without initial dilution. However, further investigations on dilution factor and effect on non-target organisms are necessary before a final adoption of the procedure. The next phase therefore should involve a field trial, having determined the lethal concentration (LC_{95}=12.86mg/l) in the laboratory.

Acknowledgements

The authors acknowledge with thanks, the support provided by Mr. S.B. Jimoh of Biology Department, Federal University of Technology, Akure, Nigeria, during snail collection and Mrs. M.B Oniya for financial assistance and logistics.

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