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Short Communication

Molluscicidal Activities of Curcumin-Nisin Polylactic Acid Nanoparticle (PLA) on Adult Snail Intermediate Hosts of *Schistosomes* and *Fasciola* spp.

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Abstract
Digenetic trematode infections including schistosomiasis and fascioliasis have highly neglected statuses but are a menace to people in the poorest countries of the tropics, causing high morbidity and mortality in humans as well as great global losses in livestock production. This has neccesitated the widespread search for better control options for the snail vectors of these diseases. Hence, a novel drug - curcumin and nisin poly lactic acid (PLA) entrapped nanoparticles (CurNisNp) was screened for molluscicidal activity against the adults (> 2 months old) of *Biomphalaria pfeifferi, Bulinus globosus* and *Lymnaea natalensis* vector snails. Mortality was determined after 96-h of exposure at varying concentrations. The snails of the species *L. natalensis* were found to be the most susceptible to the molluscicide (LC_{50} 323.6 ppm). This finding further supports the desirability of curcumin-nisin polylactic acid (PLA) nanoparticles as a molluscicide and therefore shows that it could be a good alternative to conventional molluscicides with prospects in the selective control of fascioliasis. However, more optimization of the drug could ensure a greater molluscicidal potency.

Key words: Curcumin, nisin, nanoparticles, schistosomiasis, fascioliasis, snail intermediate host, molluscicidal activity.

Introduction

The past few decades has witnessed the widespread screening of nano-enhanced strategies for the control of protozoan and helminth infections with promising results, although with a number of challenges limiting their large scale adoption. Many workers have attempted to harness the unique properties of nano-based delivery systems for better treatment [1-3], more reliable diagnosis [4-6], the formulation of novel prophylaxis [7], vaccines [8], and also for improved control of snail hosts of these parasitic diseases [9,10] that cause high morbidity and claim millions of lives in the poor and developing countries of the world.

Digenetic trematode infections including schistosomiasis and fascioliasis for example, have highly neglected statuses but are a menace to people in the poorest countries of the tropics, killing about 200,000 people annually [11], causing global livestock production losses exceeding US$3 billion per year [12], and subjecting about 300 million people to chronic illnesses [12]. Key links in the life cycles of their causative agents are the presence of intermediate snail hosts for which conducive tropical and subtropical climatic conditions including high temperature, high relative humidity and frequent rainfall provide suitable habitats, ensuring the

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completion of the parasites' life cycle [13]. Hence, the best way to break the cycle of infection and re-infection is by an effective control of the snail populations [14]. Many plant source bioactive substances have been explored for freshwater snails control with appreciable results [14]. However, only a few nanoformulations have been screened for anti-snail activities [10,15].

An earlier study [10] reported the molluscicidal activities of curcumin-nisin polylactic acid nanoparticles on different life stages of *Biomphalaria pfeifferi*, the snail intermediate host for *Schistosoma mansoni*. This study however, aimed at evaluating the anti-snail potency of the nanoformulation on adults of *B. pfeifferi, Bulinus globosus* and *Lymnaea natalensis*, the snail intermediate host species for *Schistosoma* and *Fasciola* spp.

Material and Methods

Chemicals

The test substance (drug): Curcumin-nisin poly-lactic acid nanoparticles (Cur-Nis-PLA Ns), is a yellow biodegradable hygroscopic powder of 35% composition by mass of the active ingredient. It was prepared by the double emulsion-diffusion-evaporation method at the National Institute of Immunology, New Delhi, India. Blue hygroscopic crystals of copper sulphate (Sigma-Aldrich, St. Louis, MO, USA) were used as chemical molluscicides for the positive control. Whereas, aged water (dechlorinated) was used for snail culture, diluent and also as the negative control.

Synthesis and properties on noanotized curcumin-nisin poly-lactic acid nanoparticles (CurNisNp)

Nanotized CurNisNp synthesised by double emulsion-diffusion-evaporation method with the properties, size 288.4± 24.3 nm, zeta potential 13.7±0.4 mV, polydispersity index (PDI) 0.232 and entrapment efficiency 35% [10] measured by a Zetasizer Nano-ZS system (Malvern Instruments, UK) was used for the molluscicidal bioassay. The formulation procedures are briefly described according to an earlier report [10]. Five milligram (5 mg) each of curcumin and nisin was dissolved in 200 µL 1% polyvinyl alcohol (PVA). The solution was dispensed into 50 mg of poly lactic acid containing organic solvents. The mixture was sonicated for 1 min to obtain a primary emulsion. The emulsion was inturn added dropwise to 16 mL 2% PVA to form a secondary emulsion. The resulting mixture was sonicated at 30 W, 40% duty cycle for 3 mins to form a nanosuspension. The solvent was evaporated through continuous stirring and was centrifuged at 16,000 rpm for 15 min. The formulation was washed three times and then lyophilized with 5% mannitol as cryoprotectant.

Snail collection

Adults of *Biomphalaria pfeifferi*, *Lymnaea natalensis* and *Bulinus globosus* were collected from Eleyele River (7.420852 N, 3.853825 E) and Awba dam (7.4425 N, 3.8888 E) in Akinyele local government area of Oyo state, as well as Odo Ona River (7.29389 N, 3.86722 E) in Ibadan North local government area of the state. They were properly washed in water and transferred into plastic containers with good ventilation. The snails were brought to the Parasitology Laboratory of the Department of Zoology, University of Ibadan for the study.

Snail culture

Twenty five (25) adult snails each of *B. pfeifferi, L. natalensis* and *B. globosus* were transferred separately into a culture jar (aquarium) lined with transparent polythene bags containing dechlorinated tap water. The snails were fed with blanched dried lettuce (*Lactuca sativa*), and $CaCO_3$ pellets were used as calcium supplements. The egg masses laid by the snails were incubated as described by Salawu and Odaibo [16] with few modifications. Newly hatched snails were transferred into larger containers and maintained as they grew larger.

2.5. Molluscicidal bioassay activity test

The molluscicidal bioassay activity tests was carried out on the adults (8-9 weeks old) of all three snail species in line with the WHO guidelines [17]. Ten (10) clean or uninfected snails were placed in 40 mL of varying concentrations (87.5 ppm, 43.75 ppm and 21.88 ppm) of the nanoparticle formulation and mortality was observed after 96-h exposure. Observation and examination for mortality were done using hand lens or dissecting microscope where necessary. The snails that could move or with an active heart beat (as observed under the microscope) were counted as living and vice versa. The percentage mortality was calculated.

All experiments were performed in duplicate with values expressed as mean ±SD. The negative control groups were placed in dechlorinated water.

Statistical analysis

The data were entered in SPSS version 21 for windows for analysis. Two-way ANOVA was used to test significant differences in snail mortality in different concentrations. Probit regression graph was used to determine the LC_{50} and LC_{90} of the nanotized formulation. Linear Regression analysis and Pearson's correlation were applied to determine the relationship between snail mortality and test concentrations. *P* value less than 0.05 is considered statistically significant.

Results and Discussions

The protective behaviours of the adult snails upon immersion into the test concentrations were surfacing behaviour and partial retraction of their cephalopodal mass with normal crawling activities following after only a few minutes. There was a significant association between mortality and concentration among the adults of *L. natalensis* and *B. globosus* ($P<0.05$). Among the adults of *B. pfeifferi* however, snail mortality was not dependent on the concentrations of the drug ($P>0.05$). The adults of *L. natalensis* snails were the most susceptible to the drug with up to 50% mortality at the test concentration of 87.5 ppm (Table 1). *Biomphalaria pfeifferi* with LC_{50} 9055.2 ppm was most resistant to exposure to CurNisNP while *L. natalensis* with LC_{50} 323.6 ppm was most susceptible to the nanotized drug (Table 2).

Table 1.

Percentage mortality (± SD) of adult snail intermediate hosts (8-9 weeks old) exposed to CurNisNp

	Concentration (ppm)			
Species	**87.5**	**43.75**	**21.88**	**Control**
L. natalensis	50.0 ± 7.07^{a}	25.0 ± 3.54^{b}	5.0 ± 0.71^{c}	0.0
B. globosus	0.0 ± 0.00^{a}	0.0 ± 0.00^{a}	5.0 ± 0.71^{b}	0.0
B. pfeifferi	10.0 ± 1.41^{a}	15.0 ± 2.12^{a}	5.0 ± 0.71^{a}	0.0

Note: Similar superscripts denote no significant difference while different superscripts denote significant difference. Significant differences compared across concentrations of CurNisNp

Table 2.

Probit analysis of lethal concentrations of CurNisNp against *L. natalensis, B. globosus and B. pfeifferi* snails

			Lethal concentration (ppm)	
Species	**Regression equation**	**R^2**	**LC_{50} (95% CI)**	**LC_{90} (95% CI)**
L. natalensis	y=0.0007x + 0.2604	0.209	323.6 (83.2 - 546.3)	863.8 (201.2 – 1274.0)
B. globosus	y=0.0002x+0.0062	0.630	1984.1 (718.2 - 3151.7)	3591.5 (1385.6 - 5326.7)
B. pfeifferi	y = -5E-05x + 0.0271	0.487	9055.2 (8498.1 - 11785.0)	16714.2 (11904.7 - 17783.6)

Discussion

The suggestion on the potential of curcumin-nisin PLA nanoparticles for adoption as a desirable molluscicide is further supported by its selective anti-snail potency on adult snail intermediate hosts of very important digenetic trematodes.

The snails' avoidance behaviours following exposure to CurNisNp indicating its molluscicidal potency is in line with other previous observations [10,18]. Webbe [19] ascerted that this kind of snail behaviour is in response to loss of water balance and more recently, it was opined that this behavior helps to increase their chances of survival and so hinders the action of molluscicides used against them [20].

The species-dependent mortality of snails observed in this study, possibly due to differences in genetics, morphology and general survival rate, has also been reported [21-23]. Although, their observations were different from that of the present study. Higher susceptibilities were reported for *B. pfeifferi* compared to *L. natalensis* by Molla et al. [23], who attributed the observation to the differences in the type of active ingredients present in the different plant parts used.

In addition, the relationship between exposure period and snail mortality was not significant ($P>0.05$) in contrast to an earlier report [24]. However, the mortality of the snails for up to 96 hours demonstrates the benefits afforded to the drug combination (Cur-Nis) by encapsulation with polylactic acid delivery system. This is not unreasonable as curcumin, although in combination with another pharmacologically active substance (nisin), is widely known as an antioxidant, a relaxant and an anti-inflammatory agent [25]. Therefore, the formulation may have had its molluscicidal bioactivity enhanced by presenting a prolonged bioavailability and continuous absorption through the snail teguments with increasing exposure period.

Conclusions

The observed escape behaviour of the snails in response to exposure to CurNisNp and the species dependent susceptibilities of the snails suggest that the nanotized formulation has prospects in the selective control of *L. natalensis*, the intermediate snail host of *Fasciola gigantica*. Moreover, further studies are recommended on

the comparative molluscicidal assessment with optimized nanotized formulation and mechanisms of action in snails.

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