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Evaluation of Some Lectins as Anti-protozoal Agents

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Ten lectins from different sources were screened for cytotoxic activity against *Acanthamoeba* sp. (a keratitis-causing amoeba) and *Tetrahymena pyriformis* (a ciliate). Lectins from *Schefflera odorata* ("lima-lima" plant) [molecular weight = 271 kDa], *Swietenia macrophylla* (large leaf mahogany) [molecular weight = 295 kDa] and *Lenzites* sp. (a mushroom) [molecular weight = 184 kDa] were found to possess high cytotoxic activity against the test organisms. These results indicate that lectins may be further exploited as potential chemotherapeutics against certain parasitic diseases.

Key words: Lectins, protozoans, *Acanthamoeba* sp., *Tetrahymena pyriformis*, cytotoxic

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INTRODUCTION

Lectins are carbohydrate-binding proteins or glycoproteins of non-immune origin that can agglutinate cells or precipitate glycoconjugates and polysaccharides[1]. Lectins have also been defined as proteins possessing at least one non-catalytic domain that binds reversibly to specific mono-or oligosaccharides[2]. However antibodies and proteins with enzymatic activity related to carbohydrates are not considered lectins[3]. Because of their chemical properties, lectins have become useful tools in several fields of biological research such as immunology, cell biology, membrane structure, cancer research and genetic engineering.

Lectins are present in a wide range of organisms from bacteria to animals, being present in all classes and families although not in all kinds and species[4]. With their binding capacity, they have the capability to serve as recognition molecules within a cell, between cells or between organisms and are assumed to play fundamental biological roles in different organisms.

In this study, the cytotoxic activity of ten lectins against *Acanthamoeba* sp. and *Tetrahymena pyriformis* was evaluated. *Acanthamoeba* sp. is an amoeba that caused keratitis, a serious and potentially devastating corneal infection generally seen in soft contact lens wearers[5], while the ciliated *Tetrahymena pyriformis* is a widely used as a test organism in the assessment of the cytotoxicity of different compounds and therapeutic agents[6]. Results from this study can be used as preliminary data in the possible utilization of lectins against pathogenic microorganisms such as those that are found in our water supply.

MATERIALS AND METHODS

Isolation of lectins: The lectins used in the experiment were isolated from different organisms listed in Table 1. These were isolated during the period 2000-2003 using the method of Pisueña *et al.*[7].

Microorganisms: *Acanthamoeba* were grown at 28°C on Monoxenic Non-nutrient Agar (MNA) medium and trophozoites collected from 24 to 48 h old cultures. The agar surfaces were flooded with 5 mL of PBS and gently scraped with an inoculating loop. Trophozoites were harvested from suspension by centrifugation at 350×g for 10 min. The supernatant was aspirated out and the sediment was washed twice in PBS. The trophozoites in the resultant suspension were optically counted in a hemocytometer, adjusted to a final concentration of 10^4 amoeba per mL and used immediately for testing.

Table 1: Sources of lectin

Scientific name	Common name	Part used
Schefflera odorata	lima lima plant	leaves
Swietenia macrophylla	mahogany	leaves
Carica papaya	papaya	seeds
Artocarpus blancoi	antipolo	seeds
Phaseolus vulgaris	red mungbean	seeds
Lenzites sp.	(a mushroom)	fruit bodies
Polyporus sp.	wood rotter	fruit bodies
Holothuria atra	sea cucumber	body wall
Holothuria atra	sea cucumber	internal organs
Holothuria atra	sea cucumber	internal organs

On the other hand, *Tetrahymena pyriformis* were grown axenically at 25°C in tissue culture flasks to a density of approximately 10^5 protozoa per mL in 1% proteose peptone medium enriched with 0.1% yeast extract. The protozoa were then centrifuged at 200×g for 1 min. The supernatant was diluted with sterile distilled water to a final density of 10^4 cell per mL.

Anti-protozoal assay: The purified lectins were then tested for anti-protozoal properties against *Acanthamoeba* sp. and *Tetrahymena pyriformis* using microplate-based *in vitro* bioassays. This was done by mixing 50 μL of cell suspension with the same volume but varying concentrations of the purified lectin in microtiter plate wells. Mortality of the cultured cells was examined under a microscope 1 and 24 h after addition of the lectin. Ara-C and Etoposide were used as positive controls while sterile distilled water served as negative control.

RESULTS

All lectins, with the exception of those from marine invertebrates, showed activity against the protozoans at 500 ppm. The growth of both protozoans upon exposure to the lectins was inhibited after 24 h of incubation. *Lenzites* sp. lectin was found to be active against both protozoans after one hour of incubation. These were also observed in the two seed lectins, where *C. papaya* lectin exhibited activity against *Tetrahymena pyriformis* while *A. blancoi* lectin showed activity against *Acanthamoeba* (Table 2).

For the *Acanthamoeba*, lectins sourced from the leaves of *S. macropylla* and *S. odorata*, notably both medicinal plants, showed the most potent activity with lectin from the former inhibiting the growth of cells at a concentration as low as 25 ppm (Table 3). Fungi lectins and seed lectins also inhibited the growth of *Acanthamoeba* cells at concentrations higher than the lectins from medicinal plants.

The minimum concentration of lectins needed to inhibit the growth of *Tetrahymena pyriformis* is lower than that for *Acanthamoeba*. Lectins from the medicinal plants *S. odorata* and *S. macrophylla* and from

Table 2: Preliminary assay of the cytotoxic activity of different lectins against *Acanthamoeba* sp. and *Tetrahymena pyriformis*

	Acanthamoeba sp.		*Tetrahymenapyriformis*	
Lectin	1 h	24 h	1 h	24 h
S. odorata leaf lectin	-	+	-	+
S. macrophylla leaf lectin	-	+	-	+
C. papaya seed lectin	-	+	+	+
A. blancoi seed lectin	+	+	-	+
P. vulgaris seed lectin	-	+	+	+
Lenzites sp. lectin	+	+	-	+
Polyporus sp. lectin	-	+	-	+
H. atra body wall lectin	-	-	-	-
H. atra internal organs lectin	-	-	-	-
H. scabra internal organs lectin	-	-	-	-

Table 3: Results of antiprotozoal test against *Acanthamoeba* sp.

Lectin samples/ Concentration (ppm)	500	200	100	50	25	10
S. odorata lectin	+	+	+	+	-	-
S. macropylla lectin	+	+	+	+	+	-
C. papaya seed lectin	+	+	-	-	-	-
A. blancoi seed lectin	+	+	-	-	-	-
P. vulgaris seed lectin	+	+	-	-	-	-
Lenzites sp. lectin	+	+	+	-	-	-
Polyporus sp. lectin	+	+	-	-	-	-
PBS (negative control)	-	-	-	-	-	-
Ara-C (positive control)	+	+	+	+	-	-
Etoposide	+	+	+	-	-	-

Table 4: Results of antiprotozoal test against *Tetrahymena pyriformis*

Lectin samples/ Concentration (ppm)	500	200	100	50	25	10
S. odorata lectin	+	+	+	+	+	+
S. macropylla lectin	+	+	+	+	+	+
C. papaya seed lectin	+	+	+	+	-	-
A. blancoi seed lectin	+	+	+	+	-	-
P. vulgaris seed lectin	+	-	-	-	-	-
Lenzites sp. lectin	+	+	+	+	+	+
Polyporus sp. lectin	+	+	+	+	-	-
PBS (negative control)	-	-	-	-	-	-
Ara-C (positive control)	+	+	+	+	+	-
Etoposide (positive control)	+	+	+	-	-	-

+ inhibition in cell growth observed, - no inhibition in cell growth observed

Lenzites sp. showed activity at concentrations as low as 10 ppm (Table 4). The other lectins, with the exception of *P. vulgaris* seed lectin, showed cytotoxic activity against *T. pyriformis* at much lower concentration than in *Acanthamoeba*. *P. vulgaris* seed lectin appeared more active against *Acanthamoeba* than against *T. pyriformis*. In addition, some lectins showed stronger or the same level of activity in comparison to Ara-C and Etoposide, cancer therapeutic agents that were used as positive control (Table 4).

DISCUSSION

Few studies on antiprotozoal agents from plant-based materials have been done. Of those that have been reported, many used *Entamoeba histolytica* as test organism. Some agents have been tested against *Acanthamoeba* and extracts from plants such as *Ipomoea* sp., *Kaempferia galanga* and *Cananga odorata* were found to be active against three species of this protozoan.

Almost all antiprotozoal agents known are therapeutic agents synthesized in the laboratory. These include ivermectin, fungizone, pentamidine and Distamycin A[8-11], which were found active against *Acanthamoeba*. For *Tetrahymena pyriformis*, the cytotoxicity of several therapeutic agents like cyclosporin-A, cisplatin and doxorubicin[12] has also been assessed. The toxicity of non-ionic surfactants such as Triton X-100[13], some tricyclic antidepressants[14] and food additives[15] like the food color tartrazine, the preservatives sodium nitrate and sodium benzoate and the antioxidant BHT was also evaluated using *Tetrahymena pyriformis*.

In this study, lectins were found to exhibit different levels of cytotoxic activity. The activity of the purified lectins could be due to their interaction with sugars present in the protozoans. The carbohydrate-lectin interactions are known to play important, broad roles in cell recognition, adherence, cell division, among others, in different organisms. It has been reported that Concanavalin A can bind both to the plasma membrane and to intracellular structures of *Tetrahymena pyriformis*[16]. Binding of lectins with some sugars in the protozoans could cause interference in chemical or biological processes that eventually lead to the death of these microorganisms.

Lectins were found to exhibit cytotoxic activity at varying concentrations against *Acanthamoeba* sp. and *Terahymena pyriformis*. Results indicate that lectins may be exploited as potential chemotherapeutic agents against certain parasitic disease and that it might be worthwhile looking into the potential activity of lectins against other microorganisms, particularly those that cause disease.

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