ISBN 978-93-84422-22-6
Proceedings of 2015 International Conference on Chemical, Mettalurgy and Environmental Engineering (CMAEE 2015)
Istanbul (Turkey), June 3-4, 2015 pp. 277-280

Antiparasite Activity of Chitosan

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***Abstract*:** *Chitin, which after cellulose is the most abundant polysaccharide in nature, is found especially in the structure of the shell of crustacean, cuticles of insects and cell walls of fungi. Chitin is a linear polysaccharide joined by β-(1,4)-linked N-acetylglucosamine units. Chitosan is obtained by the thermochemical deacetylation of chitin in the presence of alkali and naturally it occurs only in certain fungi (Mucoraceae). It has been proved to be biologically renewable, biodegradable, biocompatible, non-antigenic, non-toxic and biofunctional. Chemical extraction of chitin from shells, of the white shrimp Parapenaeus longirostris (Lucas, 1846), produces a chitin with a high viscosity, a low molecular weight and a high degree of deacetylation. In the present study, chemical deacetylation of chitin produced chitosan. Also, antileishmanial activities of chitin and chitosan were evaluated using Leishmania infantum LIPA 137 and Leishmania infantum LIPA 155/10, two reference strains isolated from patients in Pasteur institute from Algeria. The results showed effective antileishmanial activities of chitin and chitosan against Leishmania infantum LIPA 137, but no antileishmanial activity against Leishmania infantum LIPA 155/10. Further studies are necessary to determine the in vivo activities and applications of chitin and chitosan, in particular, in the design of new lines of drugs for use in the treatment of leishmaniasis and hopefully eradication.*

Keywords: *antileishmanial, chitin, chitosan, Leishmania infantum, shrimp shell waste.*

1. Introduction

W Morbidity and mortality because of the leishmaniasis, a parasite disease, cause an estimated 2.4 million disability-adjusted life-years. Globally, there are 1.5–2 million new cases estimated and 70 000 deaths each year, and 350 million people are at risk of infection and disease [1]. In northern Africa, Algeria is one of the eight countries that constitute 90% of cutaneous leishmaniasis in the World. Leishmaniasis contributes significantly to the propagation of poverty, because treatment is expensive and hence either unaffordable or it imposes a substantial economic burden, including loss of wages. Leishmaniasis is endemic in 88 countries on five continents. Surveillance data indicate that the global number of cases has increased during the past decade and the emergence of antileishmanial drug resistance. To date, there is no vaccine in routine use against leishmaniasis [2]. The genus Leishmania is parasitic protozoa responsible for the leishmaniasis, a group of diseases affecting human and various animal populations. Co-infection leishmaniasis/HIV is a fatal synergy characterized by both infections mutually reinforcing their impact on the immune system. The major clinical syndromes found in human beings are cutaneous, mucocutaneous and visceral leishmaniasis, but these can present in a wide variety of forms [3].

On the other hand, much research has focused on chitin and derivatives as a source of bioactive material during past few decades [4, 5, 6]. The purpose of this work is the determination of the antiparasite activities of

chitin and chitosan using *Leishmania infantum* LIPA 137 and *Leishmania infantum* LIPA 155/10, two reference strains isolated from patients in Pasteur institute from Algeria. Up to now, chitin has not been used as an antileishmanial active drug against *Leishmania infantum* strain.

2. Materials and Methods

All chemicals used in this study were analytical grade and purchased from Sigma Chemical Co. (St. LouisMo).

2.1. Test Materials

Shrimp shells were obtained from a seafood restaurant. It was confirmed that all shells were from a single species of shrimp *Parapenaeus longirostris* (Lucas, 1846).

Chitin was extracted from shell waste of the white shrimp *Parapenaeus longirostris* (Lucas, 1846) by sequential treatments with HCl (demineralisation) and NaOH (deproteinisation) [7]. Chitosan was prepared by deacetylation of chitin [8].

2.2. Analytical Methods

Dried chitin and chitosan samples (1 mg) were dispersed, separately, in 100 mg of anhydrous KBr and pressed. The IR spectra were recorded at room temperature in the wavenumber range of 400–4000 cm^{-1} and referenced against air with a Nicolet 380 FTIR instrument (Thermoelectron Corporation).

2.3. Antiparasite Activity

Antiparasite activities of chitin and chitosan were evaluated using two reference strains, *Leishmania infantum* LIPA 137 and *Leishmania infantum* LIPA 155/10.

Strain *Leishmania infantum* LIPA 137 was obtained from Pasteur Institute of Algeria. *Leishmania infantum* LIPA 137 is a strain sensitive to Glucanthime®. Toxicities of chitin and chitosan against *Leishmania infantum* promastigotes were assessed as previously described by Gosland et al. (1989) [9] after some modifications.

3. Results and Discussion

Preparation of chitin and deacetylation of chitosan were confirmed by FT-IR data as follows (Fig. 1). Deacetylation of chitin to produce chitosan was recognized by increasing of NH_2 functional groups (708.7 cm^{-1}, 1572.4 cm^{-1} and 3111.7 cm^{-1}) and by decreasing of C-O functional groups (1661.7 cm^{-1}) (Fig. 1a and 1b).

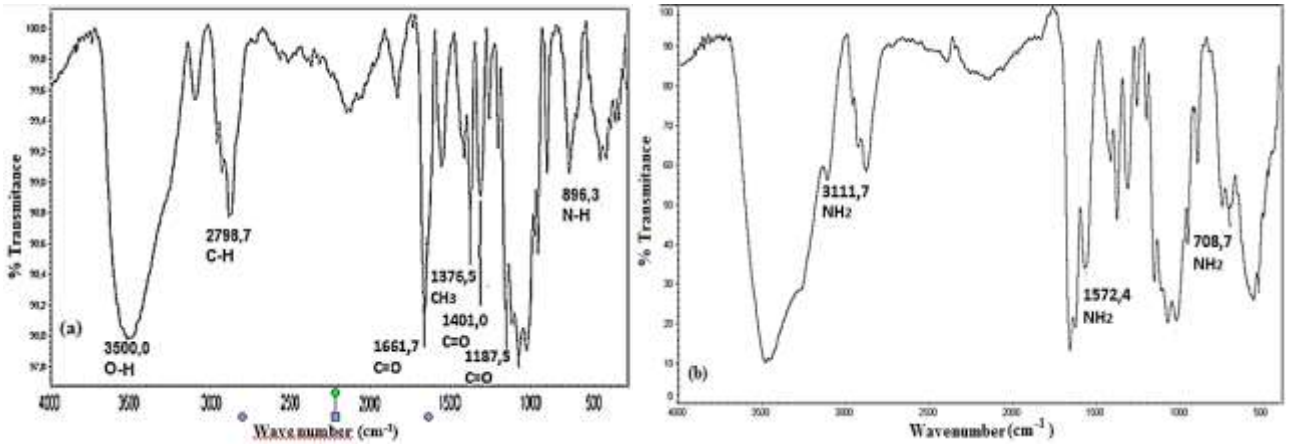

Fig. 1: FT-IR spectra of chitin (a) and chitosan (b).

The cytotoxic effects of chitin and chitosan on *Leishmania infantum* LIPA 137 (glucantime® sensitive) were evaluated. The results (Fig. 2a) indicate that chitin has the potential to suppress 100% of promastigotes growth at concentrations equal or superior to 5000 µg/mL. The IC_{50} value was 600 g/mL. Similarly, chitosan has

the potential to suppress 100% of promastigotes growth at concentrations equal or superior to 1000µg/ml. The IC_{50} value was 240 µg/mL.

The cytotoxic effects of chitin and chitosan on *Leishmania infantum* LIPA 155/10 were evaluated. The results (Fig. 2b) indicate that both chitin and chitosan exhibited no cytotoxic effects at concentrations inferior or equal to 5000 µg/mL

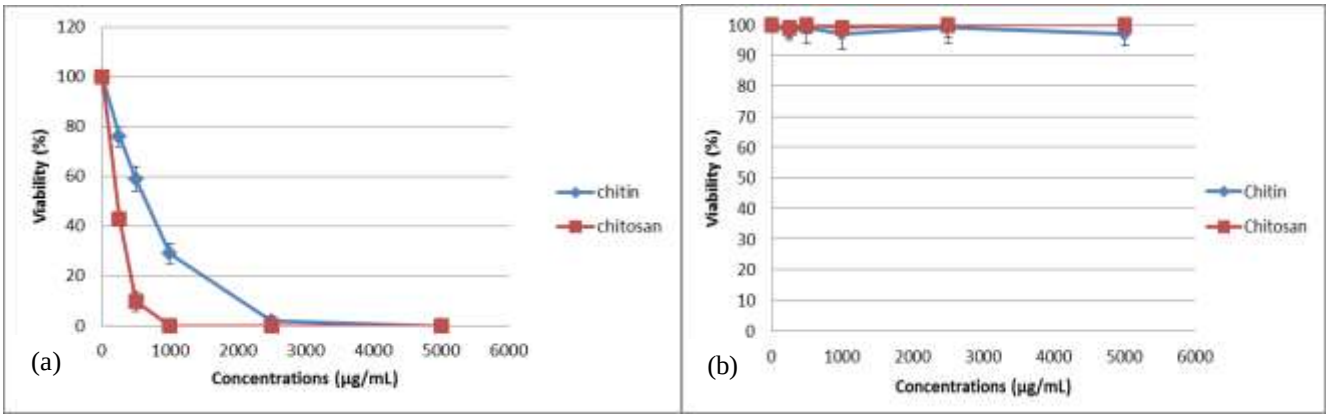

Fig. 2: Antiparasite activities of chitin and chitosan with various concentrations against *Leishmania infantum* LIPA 137 strain (a) and *Leishmania infantum* LIPA 155/10 strain (b).

Previous work has shown that, incubation of promastigotes with chitin microparticles indicated no considerable reduction in the number of viable *Leishmania major*, suggesting of the nontoxicity of chitin microparticles [10].

Some studies evaluated the in vitro activity and cytotoxicity of chitosan. It had a IC_{50} value of 112.64 ± 0.53 mg/mL for promastigotes of *Leishmania infantum* [11, 12]. None of these studies hypothesized a mechanism of the inhibitory effect observed.

Resistance of leishmanial parasites to therapeutic drugs is due to overexpressed ABC efflux pumps, also known as traffic ATPases [13]. The ATP-binding cassette (ABC) transporters play a major role in membrane-associated drug resistance by translocating wide variety of substrates across extra and intracellular membranes including [14].

This study hasn't given a hypothesize on the mechanism of the inhibitory effect observed. Further studies to determine the in vivo activity and the application of chitin and chitosan, in the design of new lines of drugs in leishmaniosis treatment will be of great interest. Indeed, chitin and chitosan could be decent alternatives to other drugs described in the literature [15, 16].

4. Conclusion

From this study, we can conclude that chitin and chitosan are attractive targets for selective antiparasite drug development. In fact, chitin and chitosan prepared from the white shrimp *Parapenaeus longirostris* (Lucas, 1846) showed great and specific antiparasite effects on *Leishmania infantum* LIPA 137 strain.

Thus, chitin and chitosan have promising roles in natural leishmaniasis prevention and treatment. This new result could offer a new pharmacological tool for the treatment of leishmaniosis that reduces the doses required, lowering toxic side effects because of meglumine antimoniate. Further studies are necessary to determine the in vivo activities and applications of chitin and chitosan, in particular, in the design of new lines of drugs for use in the treatment of leishmaniasis and hopefully eradication.

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