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Chitosan quinapyramine sulfate nanoparticles exhibit increased trypanocidal activity in mice

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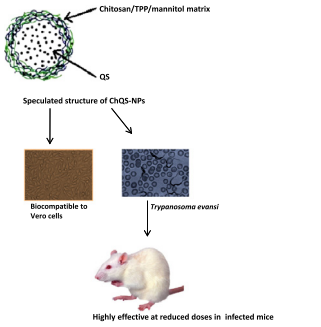
GRAPHICAL ABSTRACT



In the proposed structure of quinapyramine sulfate-loaded chitosan/mannitol nanoparticles (ChQS-NPs), the inner core consists of encapsulated quinapyramine sulfate, surrounded by one or more layers of chitosan/TPP/mannitol matrix. ChQS-NPs are non-toxic to vero cell line and highly effective against parasite *Trypanosoma evansi* in mice

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ABSTRACT

An aminoquinaldine derivative, quinapyramine sulfate (QS) is the commonly used and effective drug for treatment of *Trypanosoma evansi* infection. It produces the trypanocidal effect, but often poorly tolerated causes serious local reactions. The encapsulation of QS in Chitosan/mannitol nanoparticles to provide sustained release and would improve both the therapeutic effect of QS was encapsulated into nanoformulation (ChQS-NPs) prepared from a Chitosan, tripolyphosphate and mannitol nanomatrix. ChQS-NPs showed an initial burst followed by slow release exhibiting quasi-fickian Higuchi diffusion mechanism. Our experiments revealed a dose-dependent safety/cytotoxicity (metabolic activity), in ChQS NPs treated mammalian cells. ChQS NPs were safe at effective trypanocidal doses and even at doses several times higher than the effective dose. ChQS-NPs produced a trypanocidal effect in mice and cleared the parasite at a highly reduced dose (QS 0.85 mg/kg bw) along with the sustained release. The extent of this protection was similar to that observed with the conventional drug with higher dosages (5mg QS/Kg bw). ChQS-NPs are safe, less toxic and effective as compared to the conventional QS and offer a promising alternative for drug-delivery against trypanosomosis in higher animal models.

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1. Introduction

Trypanosomiasis (Surra) is caused by a hemoprotozoan parasite *Trypanosoma evansi*, which is pathogenic to a wide variety of wild and domestic animals, including equines, camels, cattle, buffaloes, goats, sheep, pigs, dogs, tigers, elephants etc. The infection is restricted mainly to animals but ability to infect human beings has also been reported [1]. It causes economic losses to the farmers in terms of morbidity, mortality, treatment cost and also by lowering the efficacy of vaccination due to immuno-suppression in domestic animals [2]. The frequently used and effective drug for treatment of *T. evansi* infection is an aminoquinaldine derivative, quinapyramine sulfate (QS). It produces the trypanocidal effect but also causes side effects like salivation, trembling, diarrhea and even sometimes collapse in sensitive animals within minutes of treatment [2]. The horses are known to be highly sensitive to the drug [3,4]. It has a rapid onset of action and at high doses, it can produce a toxic reaction within two h of administration in equines.

The nanoformulations are known to reduce toxicity and enhance the efficacy at lower doses [5,6]. The undesirable side effects of the drug can also be reduced by its sustained and controlled release [7]. The recent research activities on efficient chitosan-based micro/nanoparticulate delivery systems indicated its usefulness in a wide variety of applications even nowadays [8,9]. Chitosan (CS), a polysaccharide known to be an appreciable pharmaceutical material because of its biocompatibility and biodegradability, forms an ideal hydrophilic carrier system. Chitosan nanoparticles have been used to encapsulate proteins such as insulin, bovine serum albumin, tetanus and diphtheria toxoids, anticancer agents, vaccines, and nucleic acids [10]. In the last two decades, chitosan NPs have been extensively developed and explored for pharmaceutical applications. Little information is available for the use of chitosan nanoparticles in veterinary medicine. Chitosan has been used for the first time for formulating nano-based trypanocidal drugs like QS. QS acts indirectly by inhibition of protein synthesis, displacement of magnesium ions and polyamines from the ribosomes [2,11], but it produces severe side effects in the treated animals. We have previously reported the use of anionic polymer sodium alginate using double emulsion cross-linking method [12,13], although it was safer than the conventional drug QS at the efficacious and higher doses, however, at very higher concentrations, it was more toxic than the conventional drug. We purposed to synthesize a trypanocidal formulation using a highly safe and efficient cationic polymer. We used chitosan NPs as an attractive carrier system for QS as they offer many advantages such as hydrophilic surface particles, nano-size of less than 100 nm, capability to evade reticuloendothelial system (RES) and circulate in the blood for a considerable time [14], and employing ionotropic gelation method, avoiding the use of toxic organic solvents.

Mannitol is used as a cryoprotectant and is being used prior to lyophilization [15]. Based on the properties of biomaterials mentioned above, we aimed to synthesize a novel biocompatible QS nanoparticles encapsulated in chitosan and mannitol (ChQS-NPs). To evaluate the biocompatibility, a concentration-dependent safety/cytotoxicity and genotoxicity of the ChQS-NPs was also determined. Further, the targeting ability of ChQS-NPs to counter the *T. evansi* infection in mice was assessed.

2. Materials and methods

2.1. Synthesis and characterization of quinapyramine sulfate loaded-chitosan NPs (ChQS-NPs)

ChQS-NPs were prepared using ionotropic gelation with tripolyphosphate (TPP) anions. Chitosan (CS;deacetylated chitin, 75%–85% deacetylation) 0.8–1 g was suspended in 100 ml of 2% v/v of acetic acid and stirred for 6 h. QS(50 mg) was added to TPP solution prepared by dissolving 92–138 mg of TPP in 100 ml distilled water. This solution was added drop-wise to previously prepared CS suspension in acetic acid, with continuous stirring. The solution was further stirred for 24 h. The NPs were separated by centrifugation for 20 min at 15 000 rpm. The obtained sediment was washed twice with distilled water at the same speed for 10 min and again centrifuged. The pellet was suspended in 1.5%–5% w/v D-mannitol solution and sonicated using a probe sonicator (on pulse 15 s and off pulse 20 s) for a total 20 min. Finally, ChQS-NPs were lyophilized at −90 °C.

Morphology and size

The morphology and size of the ChQS-NPs were determined by transmission electron microscopy (TEM, Hitachi-H-7650). The samples for TEM were prepared by placing 20–25 μl of the solution onto a 400 mesh copper grid coated with carbon. About 2 min after deposition, the grid was tapped with a filter paper to remove surface water and then viewed under transmission electron microscope under high contrast imaging mode at an acceleration of 80 kV.

Particle size and polydispersity index

Measurement of particle size and polydispersity index was performed by Photon Correlation Spectroscopy (PCS), also known as Dynamic Light Scattering using a Zetasizer Nanoseries ZS90 (Malvern Instruments Ltd., UK). All the samples were diluted with distilled water. The size measurement was performed at 25 °C at a 90° scattering angle, and it was read for 180 s for each measurement. The mean diameter was triplicate for each sample. The mean hydrodynamic diameter was generated by cumulative analysis.

Surface charge

The zeta potential of the ChQS-NPs was observed by the laser light scattering technique using zetasizer (Zetasizer Nanoseries ZS90, Malvern Instruments Ltd., UK). The zeta potential measurements were performed by using an aqueous dip cell in an automatic mode.

Fourier transform infrared spectroscopy

Fourier Transform Infrared Spectroscopy (FT-IR) of ChQS-NPs was performed to determine the interaction of various chemicals used during synthesis. The spectra were obtained for QS, chitosan, and lyophilized ChQS-NPs using an IR spectrophotometer (Perkin-Elmer, Massachusetts, USA).

2.2. Quinapyramine sulfate loading and encapsulation efficiency

The amount of QS in nanoparticles was calculated by the difference between the total amount of QS added and the amount of unbound QS remaining in the supernatant at the time of synthesis of the nanoformulation. The amount of QS loaded in 100 mg of nanoparticles was calculated and represented as % w/w. The supernatant obtained after pelleting the ChQS-NPs was used for determining the residual QS content spectrophotometrically (Shimadzu corporation model UV-2450, Japan), taking unloaded ChNPs as blank. QS concentration in the ChQS-NPs solution was calculated by the regression equation obtained by preparing a standard curve prepared by various QS concentrations (10–50 μg/ml) at 343 nm. Percentage encapsulation efficiency was determined using the formula:

$$\% \text{ encapsulation} = (\text{Total QS} - \text{Unbound QS})/\text{Total ChQS-NPs} \times 100$$

$$\text{QS loading } (\%) = (Wt/Wn) \times 100$$

where Wt is the total amount of QS in the ChQS-NPs (QS added–unbound), Wn is the total weight of ChQS-NPs.

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