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Effect of orally administered hydroxypropyl chitosan on the levels of iron, copper, zinc and calcium in mice

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ABSTRACT

Two hydroxypropyl chitosan HPCS samples (HPCS1 Mw 1.6×10^5, HPCS2 Mw 3.5×10^4) were prepared by the reaction of chitosan with propylene oxide under alkali conditions. The median lethal dose of the hydroxypropyl chitosan was greater than 10 g/kg for the laboratory mice. HPCS1 at the 0.1%, 1.0%, 1.5% and HPCS2 at the 1.0% level in diets were used to feed the mice for 90 days respectively. No pathological symptoms, clinical signs or deaths were observed for all mice. The weights of the mice in HPCS groups and control group had no significant difference. The levels of Fe, Cu, Zn and Ca in the mice were measured by atomic absorption spectrophotometry. HPCS had no significant effect on the levels of Fe, Cu, Zn and Ca in the tested mice's livers and hearts. However, hydroxypropyl chitosan at high dose exhibited inhibitory effects on the levels of Fe, Zn and Ca in some organizations.

1. Introduction

Chitosan is derived from the deacetylation of chitin, the second most abundant biopolymer isolated from insects, crustaceans such as shrimp and crab, and fungi. It is composed of D-glucosamine with some degree of *N*-acetyl-D-glucosamine. As a renewable resource, chitosan has a number of unique properties, including biocompatibility, biodegradability, nontoxicity and antimicrobial activity. These properties have aroused great interest of the scholars in fields such as food science, cosmetics, pharmaceutics, biotechnology, wastewater treatment, agricultures and textiles [1]. Chitosan was approved as a feed additive by FDA in 1983. In spite of all these superior properties, plain chitosan has a major drawback: its solubility is poor above pH 6 [2]. Therefore, special attention has been paid to its chemical modification. Many water-soluble chitosan derivatives are potential to be used as a carrier for drug delivery [3,4]. To increase the solubility, chitosan is often modified by carboxylation or quaternization. However, our previous reports showed dietary carboxymethyl chitosan [5] and quaternized chitosan [6] could depress the levels of some trace elements in mice.

Hydroxypropylation of chitosan can produce water-soluble functional derivative [7]. Liquid–crystal phases, foam performance and emulsifying power have been reported in solutions of hydroxypropyl chitosan [8]. Hydroxypropyl chitosan may be potentially suitable for drug carrier and food additive. So far, there is no report about the toxicity of hydroxypropyl chitosan in vivo. The safety evaluation of modified natural products was very important for their applications in foods and drug carriers. So the effect of the hydroxypropyl chitosan on metal elements in vivo should be paid much attention by researchers and users, which is the investigation focus in this paper.

2. Experimental

2.1. Materials and chemicals

Chitosan CS1 (Mw 2.5×10^5) and CS2 (Mw 4.3×10^4) were prepared in our laboratory, and their degree of *N*-deacetylation is around 92%. Nitric acid (65%, v/v) and perchloric acid were guaranteed reagents for metal analysis. Other reagents were of analytical grade. Kunming strain mice (4 weeks old, 16–22 g) were purchased from Hubei Experimental Animal Center.

2.2. Preparation of hydroxypropyl chitosan HPCS1 and HPCS2 samples

Purified chitosan (10 g) was dispersed in isopropyl alcohol (100 mL), and then swollen enough by adding 33% NaOH (w/w) aqueous solution under the condition of stirring for 2 h at room temperature. The suspension was subsequently put into

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refrigerator at −18 °C for 48 h. It was thawed at room temperature. Next, propylene oxide (100 mL) and 10% tetramethyl ammonium hydroxide solution (10 mL) were added into it in constant stirring state at room temperature. After 1 h, the mixture was heated to reflux at 45 °C for overnight under stirring. Finally, the resulting precipitate was neutralized to pH 7.0 with 1:1 (v/v) HCl, and dialyzed using glass paper against distilled water for 3 days to remove the salt. The solution was then concentrated by evaporation under reduced pressure below 60 °C, the product was precipitated by adding excessive acetone into the residue solution. The solid was filtrated and washed with acetone for 3 times. The target hydroxypropyl chitosan was obtained under vacuum at 50 °C for overnight.

HPCS1 was produced from CS1, and HPCS2 was produced from CS2.

FT-IR spectra of the samples were recorded with KBr pellets on a Nicolt Impact 380 spectrophotometer. Gel permeation chromatography (GPC) of the samples was operated by our published method [9]. CHN elemental analysis was measured by Heraeus CHN-O-RAPID Elemental Analyzer, and the degree of substitution (DS) was calculated [7].

2.3. Estimation of water-solubility of HPCS

The pH dependence of water-solubility of the HPCS samples was evaluated by using turbidity measurements. The 0.5 g test sample was completely dissolved in 100 mL 1% HAc (w/v). Then the pH of solution was adjusted with 2 M NaOH aqueous. The transmittance of the solution was recorded on UV-722s spectrophotometer through a quartz cell with an optical path length of 1 cm at 600 nm.

2.4. Oral acute toxicity in mice

Prior to dosing, the mice were housed in cages in a temperature-controlled animal room (22–26 °C) for 7 days, and then were fasted overnight but given water ad libitum before dosing. The mice were divided into two groups with five males and five females each at random. HPCS1 or HPCS2 was mixed evenly with feedstuff by half and half, and the mixture was administered by oral gavage until the dosage of HPCS was up to 10 g/kg body weight of each mouse within 1 h. The observation of general status, toxic symptom and mortality in mice were continued for 7 days after treatment. Finally, the maximum tolerant dosage and acute toxicity classification were determined.

2.5. Ninety days feeding study in mice

Fifty healthy Kunming strain female mice were divided into four hydroxypropyl chitosan groups with ten mice/each group, and a control group with ten mice. The control group was fed (5/cage) only with basic mice diet: crude fiber 48.0 g/kg, crude fat 42.5 g/kg, crude protein 191.0 g/kg, amino acid 121.3 g/kg, 41.77 mg/kg P, Ca 5.47 g/kg, Cu 7.7 mg/kg, 526.0 mg/kg Fe, and 85.2 mg/kg Zn.

The HPCS1 groups were fed with the basic mice diet containing HPCS1 at 0.1%, 1.0% and 1.5% (w/w), respectively. The HPCS2 group was fed with the basic mice diet containing HPCS2 at 1.0% (w/w). Diets and water were given ad libitum for a continuous period of 90 days. All the animals were observed daily and weighted weekly to check for any sign of toxicity.

The mice were killed at the 90th day, and the vital organs of each mouse were excised and observed grossly. Heart, liver, kidneys, spleen, thymus and lung were weighted and the percent ratios of organ to body weight were calculated, respectively.

The livers were fixed in situ with 10% formalin in 0.1 mol/L phosphate buffer, dehydrated with alcohol and embedded in paraffin.

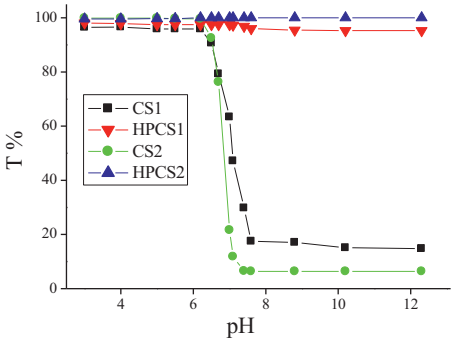

Fig. 1. The pH dependence of solubility of chitosan and its derivatives.

Thin tissue sections were stained with hematoxylin and eosin, and observed under microscope.

2.6. Measurement of trace elements

Each organ (0.1–0.2 g) was digested with 5.0 mL nitric acid and 1.0 mL $HClO_4$. The left mixture was diluted with triple-distilled water to 10.00 mL. The mineral concentrations were analyzed on a TAS 986 atomic absorption spectrometry (Beijing Purkinje General Instrument Co., Ltd., China) under standard conditions (Fe 0.2 nm, 4.0 mA and 1.6 L/min C_2H_2, Cu 0.4 nm, 3.0 mA and 1.6 L/min C_2H_2, Zn 0.4 nm, 3.0 mA and 1.6 L/min C_2H_2, Ca 0.4 nm, 3.0 mA and 1.6 L/min C_2H_2) and excitation lamps (Fe 248.3 nm, Zn 213.9 nm, Cu 324.8 nm, Ca 422.7 nm). The element contents were expressed as microgram of the element per gram of wet tissue weight (μg/g organ). Mean values and S.D. were determined by the SPSS statistics 19.0 program, and the significance of difference was estimated by the Student-Range. A significant difference was accepted with $P < 0.05$.

3. Results and discussion

3.1. Preparation of HPCS samples

The hydroxypropyl chitosan was produced by chemical modification of chitosan with propylene oxide. The pH dependence of transmittance of these samples in solution was shown in Fig. 1. The pH has different effect on solubility of chitosan samples and their HPCS derivatives. All tested samples have good solubility under pH 6. The dissolved chitosan CS1 and CS2 in acidic solution were easily transferred to floc with addition of NaOH whereas the hydroxypropyl chitosan HPCS1 and HPCS2 were water-soluble over a wide pH range. The results showed the chitosan samples had been modified.

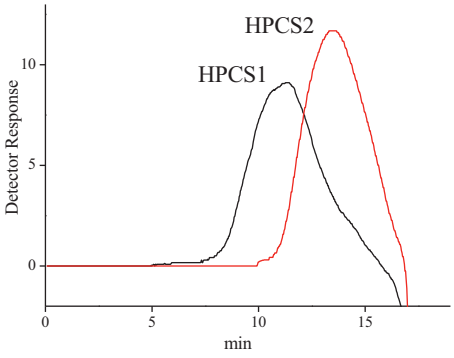

Fig. 2. GPC profiles of hydorxypropyl chitosan samples.