Nicotine-Loaded Chitosan Nanoparticles as Dry Powder Inhaler Formulation for Management of Nicotine Dependence
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Purpose
Nicotine-related disorder is one of the leading causes of preventable deaths across the world. However, currently available treatments for nicotine addiction are inefficient due to the substantial required dose or serious withdrawal symptoms. Inhalation is an efficient therapy method which delivers drugs directly into deep lungs and achieves respiratory drug delivery. The pulmonary delivery of nicotine nanoparticles would be expected to mimic the effects of tobacco smoking with the minimum negative health effects due to large surface area of the pulmonary alveoli, and faster dissolution of nicotine products in the lungs at pH 7.4. The aim of this work is to investigate the pharmaceutical activity of novel nicotine nanoparticles as dry powder inhaler (DPI) formulation from the locomotor behaviour test in mice using a nose-only inhalation device from the pulmonary drug delivery method.

Methods
Nicotine hydrogen tartrate (NHT)-loaded chitosan nanoparticles (nicotine nanoparticles) were prepared using a W/O emulsion method. The morphology of nanoparticles was studied by using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Particle size and size distribution were measured by dynamic light scattering. The fine particle fractions (FPFs) of nanoparticle formulations were determined by a twin stage impinger (TSI) using a Rotahaler. The in vitro controlled release of nicotine from chitosan nanoparticles was evaluated using the dialysis membrane diffusion technique. In vivo locomotor test (n=8 each group) in the photocell activity chambers was applied to evaluate the efficiency of nicotine nanoparticles compared with NHT by injection, and saline injection was as control. The other groups of mice (n=8 each group), regulated by locomotor activity test as well, were delivered selected amount of either NHT powder or nicotine nanoparticles, and accompanied with the same amount of blank chitosan nanoparticles as control using a nose-only inhalation device at a flow rate of 0.9L/min for 5 minutes. The lowest dose from the inhalation which made mice travel longest distance was selected as the minimum dose required to stimulate mice locomotion. After drug exposure by inhalation at 1 h and 24 h, the lungs tissues of mice were assessed by histological analysis to determine the lung damage from nicotine nanoparticles at varied doses in mice within short and long terms, respectively.

Results
The nanoparticles appeared spherical. The average particle size of nicotine-loaded chitosan nanoparticles was 400±6.3 nm. The high drug loading (65.97±2.57%) was achieved from formulated nicotine nanoparticles. The in vitro aerosolization study produced FPF of 30.6%, which is comparable to currently available DPI products. The drug was rapidly released from nanoparticles in the initial stage, due to the rapid dissolution of surface adhered/entrapped drug, followed by a slower release because of the penetration of the PBS release medium into the nanoparticles and dissolution of the entrapped drug. The maximum cumulative release was found to be around 65% in 4 days. A dose-related response to nicotine was observed from locomotor activity test from injection, with a longest travelled distance seen at the dose of 0.5 mg/kg on NHT and nicotine nanoparticles, in comparison to saline control groups (one-way ANOVA, P<0.05), indicating the greatest stimulation was produced at such dose. The higher dose caused hypoactive effects for mice confirmed by travelling a shorter total distance. In animals injected by chitosan nanoparticles, the locomotion response was not different from saline controls. Based on the minimum hyperactive dose by injection, the similar locomotion activity from inhalation was achieved by loading 50 mg NHT or equivalent amount of nicotine nanoparticles in the nose-only inhalation device (accommodated 12 mice in a session) with significant difference statistically compared with inhalation of blank chitosan nanoparticles. No allergic airway inflammation was observed from histological analysis of lung sections within 24 hours.

Conclusion
We have established an efficient way to prepare nicotine-loaded chitosan nanoparticles as DPI formulation. The outcomes from mice locomotor activity test confirmed that the novel nicotine nanoparticles were active after modifications on NHT in producing hyperactivity at 0.5 mg/kg and hypoactivity above 0.5mg/kg. The minimum loading dose into nose-only inhalation device was determined at equivalent amount of 50 mg NHT based on the locomotion response from injection. Lung structures were not altered within 24 hours. The novel nicotine nanoparticles in this work offers a potential delivery strategy for management of a global health problem associated with nicotine dependence by developing a controlled release formulation for pulmonary drug delivery of nicotine as an alternative to smoking in the future.