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Synthesis, characterization and evaluation of antioxidant properties of catechin hydrate nanoparticles

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ABSTRACT

Catechin hydrate (CH), is an important phyto compound, reported to have potential therapeutic activity for prevention and treatment of various central nervous system (CNS) disorders. However, its therapeutic action is limited by their low oral bioavailability, poor stability and intestinal absorption, therefore, development of a targeted nanoparticle based carrier system which can overcome its physicochemical limitations and can enhance its biological activity is required. The objective of the present study was to formulate nanoparticle based formulation by ionic gelation method for catechin hydrate. After optimizing the formulation by statistical tool, further, characterization results showed zeta average particle size of 68.76 ± 1.72 nm along with polydispersibility index of 0.174 ± 0.081 and zeta potential of −5.32 mV. Moreover, TEM analysis also confirmed its nanometric size range (range of 61.8–128 nm) and FT − IR scan showed no bond formation between polymers and loaded extract (CH). The *in vitro* compound release kinetics showed a typical linear diffusion profile and cytotoxicity analysis done on NB41A3 cell lines results exhibited the cell viability of 89.5 ± 0.25% in catechin loaded nanoparticles (CH NPs) whereas, it is 82.7 ± 0.34% in CH indicating negligible toxicity in nanoparticle based formulation. The stability testing was done for CH NPs after 8 weeks, and results revealed minimal degradation of catechin. Lastly, the antioxidant activities estimated through DPPH (2, 2–Diphenyl-1-picrylhydrazyl-hydrate), Nitric oxide (NO) and Hydrogen peroxide (H_2O_2) scavenging assays revealed that CH NPs have higher and prolonged anti-oxidant activity in comparison with CH.

1. Introduction

Phytopharmaceuticals have recently become an area of great interest owing to their diverse applications. Medicinal plants are the richest resource of bioactive compounds that have a broad application in modern medicines and chemical entities for synthetic drugs [1]. These bioactive compounds have been known to relieve various diseases and hence, in recent decades more emphasis is given on evaluation and characterization of various plants and its constituents for their potential therapeutic role [2]. Catechin hydrate (CH), one such important natural flavonoid derived as plants secondary metabolite has gained considerable attention due to its potential therapeutic activity like antioxidative and anti-inflammatory properties, apart from having its immense role in prevention and treatment of diseases caused due to oxidative damage. It also exhibits certain potential biological effects including cardio protective, neuroprotective and anti-cancer effects [3]. Besides all this, it has been reported to enhance reduction of lipids on arterial walls, provide protection against induced DNA damage, get adhered to cellular wall, disrupting the microbial growth on the same and has been found to be very effective in scavenging down the alkyl peroxyl radical. Further, The therapeutic efficiency of CH depends on its bioavailability and stability via oral route of administration and it is prone to lose its potency due to low solubility, degradation at different pH of gastrointestinal tract and enzymatic activity, poor intestinal absorption, instability in highly acidic pH and excretion etc [4]. Chemically, it has two benzene rings, representing catechol and resorcinol groups, both of which are highly pH-dependent and even at very low positive potential the catechol groups get oxidized followed by resorcinol group. Additionally, it gets metabolised by glucuronidated and sulphated derivatives, especially in jejunum (GIT) and liver.

Therefore there is a need of formulating CH so as to surpass the mentioned problems of reduced absorption and rapid metabolism. Therefore, developing a novel drug delivery system for CH can help

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in enhancing the shelf life, improved absorption and minimal degradation [5]. Nanoparticle formulation can be of potential use as this carrier system [6] has an advantage such as stability, protects drug against chemical and enzymatic degradation, sustained drug release at targeted site and easy penetration into small capillaries [7], thus crossing the biological barriers easily. In the present study attempt was made to prepare CH loaded nanoparticles by ionic gelation method, where cross linkage was formed between two ionic polymers of opposite charge (anionic and cationic) bound to each other by ionic bond. It is formulated by drop wise infusing the anionic polymer (tripolyphosphate) in to drug loaded polymeric cationic solution of chitosan, finally leading to the three dimensional lattice formation. The polymers used were chitosan and TPP [8] as they were biocompatible, biodegradable, non-immunogenic, non-toxic and water soluble [9]. This formulation can enhance the bioavailability and stability of CH.

2. Materials and methods

Catechin hydrate and dialysis membrane (9777) of cut size 12000 Da were purchased from Sigma Aldrich, USA. Chitosan, tripolyphosphate, monosodium phosphate anhydrous, disodium phosphate anhydrous were obtained from Himedia, Mumbai, India. Phosphoric acid, hydrogen peroxide and all other chemicals used in experiments were of analytical grade.

2.1. Preparation of chitosan nanoparticles by ionic gelation method

Catechin loaded chitosan nanoparticles (CH NPs) [10] were prepared by ionic gelation method, where chitosan solution (CS) (1–2 mg/ml) and catechin hydrate (1–2.5 mg/ml) of different concentrations were prepared by dissolving it in glacial acetic acid (AA) (1–5% (v/v)) and stirred for overnight, continuously to obtain a clear chitosan solution [11]. Tripolyphosphate (TPP) solution (1–2% w/v) was prepared in distilled water and was added drop wise with a syringe, to the chitosan solution (Fig. 1) at constant stirring for 40 min; this was added with sonication of the same sample for 15 min. This solution was then centrifuged 20,000 g for 30 min and after discarding supernatant, pellet was again dispersed in distilled water. This washing step was further repeated twice and then nanoparticles were lyophilised and stored at 4 °C for analysis.

2.2. Optimization of prepared nanoparticles

The optimization of different process parameters like – chitosan to tri polyphosphate weight ratio, acetic acid concentration, catechin hydrate concentration was performed by mathematical modelling using Design-Expert® software with two level full factorial design [12]. Variations in chitosan to tri-poly phosphate weight ratio and acetic acid concentration during nanoparticle formation were evaluated for their effects on entrapment efficiency (EE) of drug.

The EE was determined by separating the nanoparticles from the aqueous medium containing free catechin [13] by centrifugation at 12750g at 25 °C for 40 min. The amount of free catechin in supernatant was quantified by measuring absorbance at 280 nm using Shimadzu UV spectrophotometer. The EE was calculated using the following equation:

$$EE\ (\%) = (DL_{NP} - DL_{SUP})/DP_{NP}$$

where, EE is entrapment efficiency, DL_{NP} is the total amount of drug loaded, DL_{SUP} is the free drug in supernatant.

2.3. Characterization of optimised nanoparticles

2.3.1. Particle size (PS) and zeta potential (ZP) analysis

The size measurements using dynamic light scattering (DLS) on Malvern Zetasizer (Nano ZS) was performed. Dynamic light scattering (also known as PCS - photon correlation spectroscopy) measures Brownian motion and relates this to the size of the particles (PS) by illuminating the particles with a laser and analysing the intensity fluctuations in the scattered light. Whereas, the zeta potential (ZP) describes the surface charge developed over the particle, dispersed in a liquid. It is the magnitude index of the electrostatic repulsive interaction between particles. Particle's ZP was also carried off to predict dispersion stability [14] of the nanoparticles. The samples were diluted (1:100), filtered (syringe filter membrane −0.40 μm) and then subjected for PS and ZP analysis by zeta sizer.

2.3.2. Transmission electron microscopy (TEM)

Morphology of optimised nanoparticles (A1) was characterized by TEM analysis (Morgagni 268D, AIRF, JNU, Delhi). The optimised formulation was diluted 50 times by 5% acetic acid and sonicated for 15 min [15]. Then sonicated sample was fixed on carbon-coated copper grid (300 meshes). The images of representative areas were taken at suitable magnifications (200 and 500 nm).

2.3.3. Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (IR-810, JASCO, Tokyo in SAIF, Punjab University) measurement was performed to investigate the bonding interactions in catechin nanoparticles (CH NPs). It is used to obtain an infrared spectrum of absorption, emission, and photoconductivity of a solid, liquid or gas. Also, it can be utilized to do the quantifying analysis of an unknown mixture and assumes the intensities of the peaks are directly related to the amount of sample present [16]. Potassium bromide (KBr) disc method was used to prepare the samples and scanned for absorbance from the range of 400–4000 cm^{-1}.

2.3.4. Stability studies

The optimised nanoparticles were divided in to 3 batches. Each of them were kept in small air tight glass vials and stored at different temperatures - 4 °C, room temperature (37 °C) and 45 °C. The CH content was estimated from each batch at different time interval (1–8 weeks) at 280 nm by UV – vis spectrophotometer.

2.3.5. Physicochemical measurement

Different physicochemical parameters like pH, conductivity, viscosity and density of optimised nanoparticles were measured. pH and conductivity of the samples were measured using pH meter (Thermo Orion 420A+). Specific density was calculated using pycnometer i.e., specific gravity bottle (Borosil) and viscosity was estimated by viscometer (LVDV, Brookfield Inc., USA) [17].

2.4. Evaluation of ROS scavenging and antioxidant properties of optimised catechin hydrate nanoparticles

2.4.1. DPPH assay

DPPH (2, 2–Diphenyl-1-picrylhydrazyl-hydrate) is a free radical, which when scavenged by antioxidant molecule, produces colourless ethanol solution, which is detected spectrophotometrically. Catechin (2 mg/ml) and catechin loaded nanoparticles (A1) were taken and ethanol solution of DPPH radical (0.1 mM) was added [18]. The reaction mixture was vigorously vortexed and incubated in dark at room temperature for 30 min. The absorbance was recorded at 517 nm on a micro plate reader (Thermo, Varioskan Flash). Ascorbic acid was used as internal standard. The ability to