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Antioxidant and cytotoxic effects of vanillin via eucalyptus oil containing self-nanoemulsifying drug delivery system

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ABSTRACT

In this research work, different self-nanoemulsifying drug delivery systems (SNEDDS) of vanillin were developed in order to enhance its antioxidant and cytotoxic effects. Various SNEDDS of vanillin were produced spontaneously by phase titration technique and were characterized for various physicochemical parameters. Optimized SNEDDS having droplet size of 19.0 nm, polydispersity index of 0.108, zeta potential of −28.7 mV, refractive index of 1.337, % transmittance of 99.1% and drug release profile of 97.6% (after 24 h) was selected for in vitro antioxidant and cytotoxicity studies. DPPH scavenging assay showed significant antioxidant activity of optimized vanillin SNEDDS in comparison with free vanillin. However, antioxidant profile of optimized vanillin SNEDDS was comparable with standard ascorbic acid. In vitro cytotoxicity studies on colorectal human cancer cells (HT-29) indicated that vanillin in optimized SNEDDS is around two times more efficacious than free vanillin. The results of this work indicated that developed SNEDDS could be successfully used for enhancement of therapeutic efficacy of vanillin.

1. Introduction

The colorectal cancer is one of the most common types of cancer [1]. Various chemotherapeutic agents used in the treatment of colorectal cancer produce several adverse effects which are not acceptable by cancer patients [2,3]. Recently, scientists are moving to isolation of natural compound from their plant sources for the treatment of cancer with no adverse effects. Vanillin is a natural bioactive compound which is obtained from the bean or pod of *Vanilla* orchid [4,5]. It is very good flavoring agent which showed strong antioxidant activity due to the presence of phenolic groups [5–7]. It has also been investigated as an active anticancer agent in the treatment various tumors such as breast cancer and colorectal cancer etc. both in vitro as well as in vivo but clinical trials are still missing in literature [8–12]. Recently, vanillin also showed antiplasmodial and antidepressant activity in animal models [11–14]. Self-nanoemulsifying drug delivery systems (SNEDDS) are the pre-concentrates of nanoemulsions which are composed of oil, surfactant and cosurfactant [2]. When SNEDDS are administered orally, they form very fine nanoemulsions upon mild agitation with gastrointestinal fluids which had droplet size of less than 50 nm [2,15,16]. In the recent years, the potential of SNEDDS has been investigated extensively in enhancing solubility, bioavailability and therapeutic efficacy of several poorly soluble drugs [2,3,15–20]. Anticancer therapeutic efficacy of several chemotherapeutic agents have also been enhanced by SNEDDS in literature [2,3,18–20]. To the best of knowledge of authors, antioxidant and anticancer effects of vanillin have not been investigated or enhanced by SNEDDS. Hence, in this research work, various SNEDDS formulations of vanillin were developed by aqueous phase titration method in order to enhance its antioxidant and cytotoxic effects. Various SNEDDS formulations of vanillin were prepared using eucalyptus oil (EO), Triton-X100, Transcutol-HP and deionized as oil phase, surfactant, cosurfactant and aqueous phase, respectively. All the components of vanillin SNEDDS are safe and fall under safe category of excipients.

2. Experimental

2.1. Materials

Vanillin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2, 4-disulfophenyl)-2H-tetrazolium (WST-1), EO, Cremophor-EL, ethanol Tween-20 and Tween-80 were obtained from Sigma Aldrich (St. Louis, MO). Triacetin was obtained from Alpha Chemica (Mumbai, India). Labrafil-M1944CS, Labrasol and Transcutol-HP were obtained from Gattefossé (Lyon, France). Triton-X100, cottonseed oil, oleic acid, olive oil, propylene glycol (PG) and polyethylene glycol-400 (PEG-400) were obtained from BDH Laboratories (Liverpool,

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UK). Ethylene glycol (EG), isopropyl alcohol (IPA), 1-butanol and 2-butanol were obtained from Winlab Laboratory (Leicestershire, UK). HT-29 colorectal cell were procured from American type cell culture collection (ATCC, Manassas, VA). Water was collected from ELGA water purification system (Wycombe, Bucks, UK).

2.2. Components screening for development of vanillin SNEDDS

Different components for the development of suitable SNEDDS formulations of vanillin were selected based on solubility profile of vanillin in these components. Hence, the equilibrium solubility of crystalline vanillin in different oils (Triacetin, EO, cottonseed oil, oleic acid, olive oil and Labrafil-M1944CS), different surfactants (Tween-80, Tween-20, Triton-X100, Labrasol and Cremophor-EL), different cosurfactants (ethanol, PG, PEG-400, EG, IPA, butanol-1, butanol-2 and Transcutol-HP) and water was measured by shake flask method reported by Higuchi and Connors [21]. The excess amount of crystalline vanillin was added in known amounts of each oil, surfactant, cosurfactant and water in 4 ml capacity glass vials. All the glass vials were tightly closed with caps and each experiment was performed in triplicates. The vials containing drug suspensions were transferred to a biological shaker (Julabo, MA) for continuous shaking at 100 rpm and 25 ± 0.5 °C for 72 h to reach equilibrium [5,7]. After 72 h, all the glass vials were taken out from the shaker, allowed to settle vanillin particles for 2 h. The supernatants from each samples were taken, centrifuged at 5000 rpm for 15 min in order to remove fine solid particles and subjected for analysis of vanillin content using HPLC method at 220 nm as reported in literature [5].

2.3. Preparation of vanillin SNEDDS via construction of pseudo-ternary phase diagrams

Based on solubility profile of vanillin in different components, EO, Triton-X100 and Transcutol-HP were selected as oil phase, surfactant and cosurfactant, respectively for the development of vanillin SNEDDS. Deionized water was selected as aqueous phase as it is frequently used aqueous phase in literature [15,16,20]. Pseudo-ternary phase diagrams for development of vanillin SNEDDS were constructed by aqueous phase titration method [15]. In this method, surfactant (Triton-X100) and cosurfactant (Transcutol-HP) were mixed in the mass ratios of 1:0, 1:2, 1:1, 2:1 and 3:1. EO (oil phase) and a particular mass ratio of Triton-X100 to Transcutol-HP (S_{mix}) were further mixed at different mass ratios (1:9 to 9:1). The mixture of EO and particular S_{mix} were titrated by slow addition of deionized water and pseudo-ternary phase diagrams were constructed for each S_{mix} [15–17]. The physical state of SNEDDS was marked on a phase diagram of each S_{mix} ratio with one axis representing the aqueous phase (water), second oil phase (EO) and third representing the specific mass ratio of surfactant (Triton-X100) to cosurfactant (Transcutol-HP).

2.4. Formulation development

The maximum SNEDDS zones were observed in 1:1 mass ratio of Triton-X100 and Transcutol-HP, hence different vanillin SNEDDS formulations were prepared using 1:1 S_{mix} ratio. From the phase diagram, different SNEDDS formulations with formulation codes of V1-V5 were selected. Almost entire range of SNEDDS zones in the phase diagram were considered and varied EO concentrations (6, 12, 18, 24 and 30% w/w) with minimum Triton-X100 (20% w/w) and Transcutol-HP (20% w/w) concentration were selected. 20 mg of crystalline vanillin was dissolved in Transcutol-HP due to highest solubility and rest of the components were added by vortexing at 1000 rpm at 25 ± 1 °C for about 10 min. After 10 min, the vanillin was found to be solubilized completely in each SNEDDS and vanillin-loaded SNEDDS were transferred to glass vials till further use. The compositions of vanillin SNEDDS V1-V5 are listed in Table 1.

Table 1
Composition of vanillin SNEDDS (V1–V5) prepared using eucalyptus oil (EO), Triton-X100, Transcutol-HP and water.

Code	SNEDDS composition (% w/w)[a]				S_{mix} ratio
	EO	Triton-X100	Transcutol-HP	Water	
V1	6.0	20.0	20.0	54.0	1:1
V2	12.0	20.0	20.0	48.0	1:1
V3	18.0	20.0	20.0	42.0	1:1
V4	24.0	20.0	20.0	36.0	1:1
V5	30.0	20.0	20.0	30.0	1:1

[a] Twenty milligrams of vanillin was incorporated into each SNEDDS.

2.5. Thermodynamic stability and self-nanoemulsification efficiency of SNEDDS

Thermodynamic stability tests on developed vanillin SNEDDS were performed to eliminate unstable or metastable formulations of vanillin. These tests on vanillin SNEDDS were performed viz. centrifugation, heating and cooling cycles and freeze-pump-thaw cycles as detailed in our recently published articles [15–17]. The purpose of self-nanoemulsification efficiency test was to investigate phase separation or drug precipitation upon dilution with aqueous media such as water, acidic buffer (0.1 N HCl) and phosphate buffer (pH 6.8). For this test, 1 ml of each vanillin SNEDDS (V1–V5) was diluted sufficiently (1:500) with water, 0.1 HCl and phosphate buffer. The efficiency of each vanillin SNEDDS was evaluated using A–E grading systems as mentioned in literature [15–17].

2.6. Physicochemical characterization of vanillin SNEDDS

Quantitatively, developed vanillin SNEDDS were characterized for droplet size distribution, polydispersity index (PI), zeta potential (ZP), refractive index (RI), percentage of transmittance (% T). Qualitatively, optimized SNEDDS (V1) was characterized for surface morphology using transmission electron microscopy (TEM). The mean droplet size and PI of vanillin SNEDDS (V1–V5) were measured using Brookhaven Particle Size Analyzer (Malvern Instruments Ltd., Holtsville, NY) at 25 ± 1 °C at scattering angle of 90° according to previous reports [22]. The ZP of vanillin SNEDDS (V1–V5) was measured using Malvern Zetazizer (Malvern Instruments Ltd., Holtsville, NY) according to previous reports [15,22]. The RI of vanillin SNEDDS (V1–V5) was measured using Abbes type Refractometer (Precision Testing Instruments Laboratory, Germany) at 25 ± 1 °C [16,17]. The % T of vanillin SNEDDS (V1–V5) was determined spectrophotometrically at 550 nm according to previous reports [16].

TEM analysis (JEOL JEM 2100F, USA) was conducted to evaluate surface morphology and structure of droplets of optimized SNEDDS V1. TEM experiment was performed under light microscopy operating at 100 kV according to previous as reports [17].

2.7. In vitro release of vanillin via dialysis membrane

In vitro release studies on vanillin SNEDDS (V1–V5) were carried out via dialysis membrane in order to compare the release vanillin from different SNEDDS and vanillin aqueous solution, all having 20 mg of vanillin. These studies were carried out in 500 ml of phosphate buffer (pH 6.8) using United States Pharmacopeia (USP) XXIV method using dissolution apparatus [17]. The speed and temperature of dissolution apparatus were maintained at 100 rpm and 37 ± 0.5 °C, respectively. For these studies, 1 ml of each vanillin SNEDDS (V1–V5) and vanillin aqueous solution were transferred to ready-to-use dialysis bag. Three ml of samples from each sample matrices were withdrawn at 0, 0.5, 1, 2, 4, 6, 8, 18 and 24 h and same amount of vanillin free fresh phosphate buffer (pH 6.8) was replaced at each time interval. The amount of vanillin in each sample was analyzed using RP-HPLC method at 220 nm [5].