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Development of oral site-specific pellets containing flavonoid extract with antioxidant activity

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ABSTRACT

Herbal medicines are recognized as an effective treatment of common diseases, mainly associated with oxidative stress. Therefore developing drug delivery systems of these biological active ingredients are gaining interest. Parsley (*Petroselinum crispum* L.) is a well-known culinary herb and its leaf contains high amount of apigenin, therefore it is suitable as a natural source of this flavonoid. Apigenin possess many health effects such as antioxidant, anti-inflammatory and anticancer activities. Unfortunately, these benefits are limited due to the low water solubility and bioavailability, it was recently classified as BCS II group compound. Therefore the aim of this study was to develop a carrier system for *Petroselinum crispum* extract, containing high amount of apigenin. Microcrystalline cellulose inert pellet cores were chosen and enteric coatings were applied. The produced multiparticulates had spherical shape, narrow size distribution and low moisture content. 10% (w/w) Eudragit® L 30 D-55 and 15% (w/w) Eudragit® FS 30 D coating was adequate for the modified release *in vitro*. The layered pellets demonstrated antioxidant activity. It was concluded that development of oral site-specific pellets containing flavonoid extract successful and the therapeutic effectiveness could be hypothesized.

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1. Introduction

Nowadays, drug delivery of phytochemicals has gained popularity due to their therapeutic effectiveness with low risk of side effects. Based on epidemiological and intervention studies, herbal medicines are recognized as an effective treatment of common diseases (Rashid et al., 2013). However, their effectiveness depends on the amount consumed and bioavailability of these materials (Manach et al., 2004). It is well known that the therapeutic outcome is the result of the synergistic effect of the compounds which is in correlation with genotype and growing conditions (Briskin, 2000). Therefore incorporation of these biological active ingredients could be beneficial in preventing or treating diseases, mainly associated with oxidative stress.

Parsley (*Petroselinum crispum* L.) is a well-known culinary herb in Europe and globally used in the human diet. *Petroselini folium* and *fructus* (leaf and seeds) are applied as herbal medicine since ancient times. This plant exhibits many biological activities like antioxidant (Zhang et al., 2006), antibacterial (Fejes et al., 2000; Wong and Kitts, 2006), anticoagulant (Gadi et al., 2009), anti-hyperlipidimic (Yazicioglu and Tuzlaci, 1996) and anti-hyperglycemic (Yanardag et al., 2003) properties. The essential oil in the seeds possess diuretic, antispasmodic and appetizer effect due to phenylpropene components (apiol and myristicin) (Racz and Laza, 1984). The leaf contains several compounds with health effects, including cancer prevention. This can be mainly attributed to flavonoids, a diverse group of phenolic compounds (Birt et al., 2001). It was proved that diet rich in polyphenols could decrease the risk of cardiovascular, neurological disorders and certain cancer. The majority of the consumed flavonoids are in glycosidic form (a sugar component is attached to the aglycon molecule) and possibly reach unaffected the duodenum. They are further hydrolyzed by β-glucosidase enzymes which can enhance their absorption and antioxidant activity (Hostetler et al., 2012; Papay and Antal, 2014). In the parsley leaf, apigenin and its glycosides (apiin, apigetrin) are the main flavonoids therefore it is suitable as a natural source of apigenin (Merken and Beecher, 2000; USDA Database, 2014) (Fig. 1). Several studies are published about the potential health effects of apigenin, such as antioxidant (Škerget et al., 2005) and anti-inflammatory activities (Choi et al., 2014). It is also able to induce apoptosis by modulating molecular signaling pathways on different cancer cell lines (Hu et al., 2008; Ren and Tang, 2011). Moreover, it has synergistic effect with paclitaxel (Xu et al., 2011). In human colon cancer cell lines, apigenin is able to enhance the activities of anti-metastatic protein, arrest the cell cycle and induce apoptosis (Chung et al., 2007; Lee et al., 2014; Lefort and Blay, 2011; Chidambara Murthy et al., 2012; Shao et al., 2013). It was reported that after oral consumption of parsley leaves apigenin aglycon is able to absorb through the whole intestine, possibly with active and passive transport mechanisms, like other flavonoids (Meyer et al., 2006; Nielsen et al., 1999). This indicates that the glycosides are also

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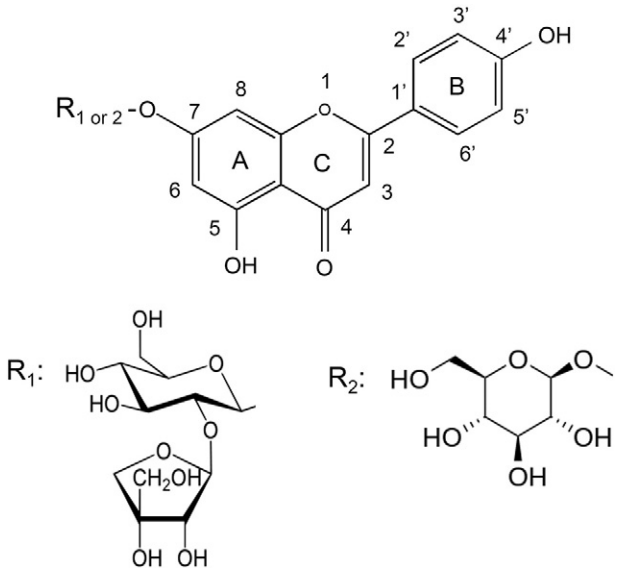

Fig. 1. Structure of apigenin (R_{1or2} = H), apiin (R_1 = apioglucoside) and apigetrin (R_2 = glucose) glucosides.

hydrolyzed by human β-glucosidase in the small intestine and bacterial β-glucosidase enzyme in the colon (Berrin et al., 2003; Day et al., 1998; Hollman et al., 1996). Nevertheless, apigenin was recently classified as a BCS (Biopharmaceutical Classification System) class II compound due to its high permeability but low solubility (Zhang et al., 2012). Importantly, the bioavailability is influenced also by the food matrix and the microbial flora (Birt et al., 2001).

Development of optimal delivery system could be challenging due to the diverse group and low solubility of compounds or the lack of knowledge about the exact dose. Orally administered multiple unit preparations such as pellets offer several advantages over single unit systems *e.g.* matrix tablet. When pellets are taken orally, small particles spread uniformly initially in the stomach which reduce the gastric transit time, the irritation of gastric mucosa and the gastrointestinal transit could be more predictable and reproducible. With better distribution of multiparticulates in the intestine, bioavailability could be improved, moreover, inter- and intra-individual variations and food-effect could be avoided. While the total drug content is split into many units the damage of the coating or the failure of some units do not result dose dumping or the failure of the drug delivery system, resulting a reduction in side effects (Abdul et al., 2010; Varum et al., 2010). They are ideal to control and modify the drug release with polymeric film coatings (Lippold, 1997; Michael et al., 2008). Various resultant drug release profiles can be obtained by simply mixing pellets with different coatings (Bodmeier, 1997; Kállai et al., 2010). Specific coatings are able to control the release of the active ingredient as well as modulate the absorption from the gastrointestinal tract or to provide targeted delivery *e.g.* by colon targeting. The function of pH sensitive polymers is based on the pH differences in the gastrointestinal tract. Besides the physiological environment several factors are influencing the fate of dosage forms and therefore the bioavailability of the drugs after administration. For example, the viscosity of colonic luminal contents and the colonic enzymes are also contributing (Amidon et al., 2015).

Only a few studies reported previously the incorporation of plant material into multiparticulates. One prepared pellets with the mixture of *Thymus vulgaris*, *Salvia officinalis* and *Urtica dioica* dry extracts and investigated the dissolution profiles of different coating levels of Eudragit® FS 30 (Kaledaite et al., 2014). In another study chitosan pellets were loaded with rutin to treat inflammatory bowel disease (Rabišková et al., 2012) and sustained release pellets were prepared to increase the short half life of naringenin (Wang et al., 2013). The objective of our study was to develop a carrier system for *Petroselinum crispum* extract therefore enhance the apigenin intake with the synergistic effect of compounds, reduce the risk of diseases associated with oxidative stress and colon cancer. Pellets as a multiparticulate dosage form are optimal in this developmental phase due to the lack of knowledge about the exact dose and toxicity. Eudragit® L 30 coated pellets allow targeted delivery to the small intestine thus increase the concentration therefore bioavailability of apigenin at the site of absorption. Furthermore, colon targeting therefore tumor suppression could be achieved with Eudragit® FS 30 coated cores. The produced pellets were further characterized and antioxidant activity measurement was also conducted to monitor the radical scavenging activity.

2. Materials and Methods

2.1. Materials

The commercially available dried parsley leaves (Kotányi Hungária Kft., Hungary) were purchased in the local grocery store (Budapest, Hungary). Microcrystalline cellulose inert pellets in the size range of 500–710 μm (MCC, Ethispheres® 600, NPPharm Ltd., France) and hydroxypropyl methylcellulose (HPMC, Pharmacoat® 606, Shin-Etsu Chemical Ltd., Japan) as a binder material for the layering process were chosen. The extract-layered pellets were further coated with 30% (w/w) aqueous dispersion of Eudragit® L 30 D-55 and Eudragit® FS 30 D (Evonik Industries AG, Germany) enteric polymers. The additives were triethyl citrate (TEC) as a plasticizer (Fluka Chemie AG, Switzerland) and micronized talc as an antisticking agent (Sigma–Aldrich Ltd., Germany). Apigenin and its glycoside standards, 2,2-Diphenyl-1-picrylhydrazyl (DPPH*) free radical, 37% , w/w hydrochloric acid, ammonium acetate, acetonitrile, ethanol (EtOH) and methanol (MeOH) were purchased from Sigma Aldrich (Sigma–Aldrich Ltd., Germany). The water was purified by using Milli-Q water system (Millipore, Germany) for HPLC-UV and HPLC-MS measurements.

2.2. Preparation of Petroselinum crispum Extract

Dried parsley leaves were milled with Retch Mixer Mill MM 400 (Germany) at 25 1/s frequency with 10 pieces of 1 cm diameter balls for 4 min. The particle parameters of D_{10}, D_{50} and D_{90} of the analyzed samples were estimated by laser diffraction (Malvern Mastersizer 2000, Malvern Industries, Germany). The extraction procedure was carried out using same solid-to-solvent ratio as described previously (Justesen, 2000; Luthria et al., 2006). Under constant stirring (500 rpm, ARE Heating magnetic stirrer, VELP Scientifica, Italy) by using 50:50 (%, v/v) ethanol:water mixture as the extraction solvent. To optimize the duration and the temperature of the extraction procedure the apigenin content was measured every 15 min at 25 °C, 40 °C, 60 °C, 80 °C for 2 h. The samples were hydrolyzed to break the glycosodic bond and determine the total apigenin (aglycon) content (Liu et al., 2008). 1 mL of samples were filtered (0.20 μm pore Sartorius filter, Sartorius AG, Germany) and hydrolyzed for 1 h at 90 °C with 0.5 mL 37% HCl prior to the further analysis. The efficacy of the hydrolysis was verified by mass spectrometer and the total apigenin content was measured by HPLC-UV method. To ensure that the apigenin remain stable during hydrolysis, 37% HCl was added to apigenin stock solution for 1 h at 90 °C and controlled again with LC-MS method. All of the prepared extracts were stored in the fridge (4 °C) before use. The 3D figure of the results was plotted by using Origin 2015 software (OriginLab Corporation, USA).

2.3. Analytical Conditions

The measurement of apigenin content was performed on Agilent 1100 Series HPLC equipped with diode array detector. A Supelco C18 (Sigma–Aldrich Ltd., Germany), 15 cm × 4.6 mm, particle size 3 μm

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