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Original article

Novel solvent-free gelucire extract of *Plumbago zeylanica* using non-everted rat intestinal sac method for improved therapeutic efficacy of plumbagin

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ABSTRACT

Introduction: Various shortcomings of the available methods of extraction of plumbagin from *Plumbago zeylanica* using non-edible organic solvents coupled with the poor aqueous solubility and low bioavailability called for extracting plumbagin in a water soluble form via a single step technique using hydrophilic lipid Gelucire 44/14. **Methods:** Gelucire extract of *P. zeylanica* (GPZ) was prepared and evaluated for extraction efficiency, High-performance thin layer chromatography (HPTLC) and thermal analysis. *In vitro* intestinal absorption and bioavailability of plumbagin from GPZ in comparison with that of aqueous (APZ), ethanolic extract (EPZ) and standard plumbagin studied using non-everted rat intestinal sac model. **Results:** The GPZ showed significantly higher extraction efficiency (3.24 ± 0.12% w/w) compared to ethanolic (EPZ) and aqueous (APZ) extraction, 2.48 ± 0.16% w/w and 0.07 ± 0.02% w/w respectively. GPZ displayed significantly higher Q_{30min} (cumulative percentage absorption of plumbagin in 30 min) and lower $t_{40\%}$ (time required for 40% w/w drug absorption). The flux and apparent permeability coefficient in duodenum and ileum were 2, 3 and 6 fold higher than EPZ, standard plumbagin and APZ respectively. **Discussion:** Improved therapeutic efficacy of plumbagin may be due to the micellar solubilization and consequent enhanced partitioning of plumbagin through intestinal by Gelucire which was reflected in the *in vivo* anti-inflammatory study conducted in rats. **Conclusion:** Thus extraction using Gelucire can be proclaimed as an efficient, economic and solvent-free technique for extraction of plumbagin and can be utilized for various clinically important water insoluble phytoconstituents in order to improve their biopharmaceutical properties.

1. Introduction

Plumbago zeylanica Linn (family Plumbaginaceae), is an erect semi-climbing shrub and grows throughout Asia, Africa and Australia. Traditionally, the aerial part of plant has long been used as medicine to treat rheumatic pain, scabies, skin diseases, dysmenorrhea, injury by bumping, wounds and even cancer. The roots of the plant and its constituents have been reported to possess potential anti-atherogenic, cardiotonic, hepatoprotective, neuroprotective, anti-microbial and anti-ulcer activity (Bothiraja, Joshi, Dama, & Pawar, 2011). Plumbagin (5-hydroxy-2-methylnaphthalene 1,4-dione) is a yellow crystalline naphthoquinone abundantly present in the roots of *P. zeylanica* (Gupta, Siddiqui, & Singh, 2000). It has been explored for its anti-cancer (Aziz, Dreckschmidt, & Verma, 2008; Wang et al., 2008), anti-inflammatory (Sheeja, Joshi, & Jain, 2010), anti-bacterial, anti-fungal (Yen, Lei, & Tung, 2006), CNS stimulant (Bopaiah & Pradhan, 2001) and anti-ulcer (Wang & Hung, 2005) activity. Animal studies suggested that orally administered plumbagin produces only 39% of bioavailability owing to its limited biopharmaceutical properties such as high lipophilicity (log *P* 3.04) and insolubility in water (Yen et al., 2006). Consequently, a large and frequent dose is needed to achieve optimum therapeutic efficacy and is the reason for severe side effects (Singh & Udupa, 1997).

The methods available for extraction of plumbagin from *P. zeylanica* utilized non-edible organic solvents such as pet ether, chloroform, alcohol and acetone and do not provide advantage of high extraction yield (Kapadia, Isarani, & Shah, 2005; Patil, Patil, & Salunkhe, 2011; Saraswathy, Pradeep Chandran, Murali Manohar, & Vairamuthu, 2006). Moreover, the large portion of inflammable volatile organic solvent required for the extraction process, although removed, raises doubt about the safety of the extract for consumption. The extraction processing conditions such as temperature, pressure and extraction

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period often degrade, alter or cause loss of the bioactive constituents of *P. zeylanica* leading to decrease in their therapeutic efficacy (Fuentes et al., 2009). A recent supercritical fluid extraction procedure utilized carbon dioxide for the extraction of *P. zeylanica* and can be regarded as safe for consumption (Rodrigues, Viana, & Baumann, 2006). However, it requires expensive high-pressure equipment and it has been reported to reduce the stability of phenolic compounds (Vatai, Skerget, & Knez, 2009). A need was sensed to develop a solvent-free extraction technique that would maximize yield of plumbagin in water soluble and consequently more bioavailable form.

Gelucires are non-ionic wax excipients consisting of mixture of mono, di and tri-glycerides and mono- and di-fatty acid esters of polyethylene glycol. Gelucire 44/14 is a semi-solid hydrophilic lipid from this group possessing a melting point of 44 °C and a HLB value of 14. Chemically it is lauroyl polyoxyl-32-glycerides (Gattefosse, 1999). It has been employed to enhance the dissolution rate, membrane permeability and intestinal absorption of poorly-water soluble drugs (Damian et al., 2000; Koga, Kawashima, & Murakami, 2002), to improve bioavailability of absorbed drug by inhibiting p-glycoprotein and thereby decreasing intestinal efflux (Yoksel et al., 2003) and to stabilize amorphous drugs (Chauhan, Shimpi, & Paradkar, 2005). It was hypothesized that Gelucire 44/14 by way of increasing solubility and permeability of plumbagin shall increase its bioavailability.

The objective of the present work was to extract plumbagin in water soluble form via a single step technique using non-ionic hydrophilic lipid Gelucire 44/14 (Gelucire). This extraction procedure is expected to increase extraction efficiency as well as improve the biopharmaceutical properties of plumbagin. The obtained extract was evaluated for plumbagin content using UV spectrophotometer, high performance thin layer chromatography (HPTLC) and differential scanning calorimeter (DSC). The bioavailability of the extracted plumbagin was assessed using non-everted rat intestinal sac model in comparison to reference standard plumbagin and the aqueous and organic extracts of *P. zeylanica*. Further, histopathological studies of the rat intestinal sacs were performed to determine the integrity of drug treated intestine and the pharmacodynamic study was also studied.

2. Methods

2.1. Chemicals

Reference standard plumbagin (99%) was purchased from Research Organic, Chennai, India. Food grade Gelucire 44/14 was a generous gift from Gattefosse, France. Carrageenan was a gift from Scitech Centre (Mumbai, India). Ethanol GR grade was purchased from Merck Chemicals (Mumbai, India). Indomethacin capsules were purchased from local market (Indocap, 25 mg, Jagsonpal Pharmaceuticals Ltd, Uttarakhand). The mixture of Krebs–Ringer phosphate buffer saline pH 7.4 [containing NaCl 0.67%, KCl 0.034%, $MgSO_4$ 0.059%, $CaCl_2$ 0.011%, NaH_2PO_4 0.234% w/v and glucose 0.18% w/v in distilled water] and isopropyl alcohol in the ratio of 7:3 v/v was prepared for *in vitro* intestinal absorption study. All other solvents used for the study were of analytical grade.

2.2. Plant materials

The fresh roots of *P. zeylanica* were purchased from Shri Shail medicinal farm, Nagpur, India, in the month of July–August 2010. The samples were identified by Dr. P. G. Diwakar, Joint Director, Botanical Survey of India (BSI), Pune, Maharashtra, India. A voucher specimen has been deposited to the departmental herbarium, BSI, under the accession number PPJOPLU5. The shade-dried roots were ground, the resulting powder passed through 30 mesh screens and stored in an air-tight container at 15–20 °C until further use.

2.3. Preparation of Gelucire 44/14 extract of P. zeylanica

Gelucire 44/14 extract of *P. zeylanica* (GPZ) were prepared via a single step technique using different amounts of food grade Gelucire as per Table 1. Gelucire was melted at 44 °C to which weighed quantity of ground root powder was added with mixing and further cooled to room temperature to get a solid mass. To this mass, freshly prepared distilled water (20 ml) was added whilst stirring for 30 min to disperse the lipid. The resultant suspension was centrifuged at 10,000 rpm for 1 h at 4 °C (Cryocentrifuge 2810R, Eppendorf, USA), supernatant was filtered through a 0.45 μm membrane filter and the filtrate was dried under reduced pressure (−400 mm Hg).

2.4. Preparation of aqueous and ethanolic extracts of P. zeylanica

The ground roots of *P. zeylanica* (100 g) were cold macerated (refrigerator 2–8 °C) with 250 ml of distilled water to obtain the aqueous extract (APZ) and with 250 ml of ethanol to obtain ethanolic extract (EPZ). After 72 h, the extract was filtered through Whatman filter paper No. 41 (Whatman, Middlesex, UK) and the filtrate was dried under reduced pressure (−400 mm Hg) (Bothiraja, Shinde, Rajalakshmi, & Pawar, 2009; Bothiraja et al., 2011).

The obtained GPZ, APZ and EPZ extracts were stored in amber colored glass vials in a desiccator until required for further use.

2.5. Characterization of extracts

2.5.1. Quantification of plumbagin in extracts

The extracts (10 mg) were dissolved in a suitable quantity of water (for aqueous extract) or methanol (for ethanolic extract) by using a cyclomixer and ultrasonicator. The plumbagin content was estimated by measuring absorbance at 420 nm using a UV spectrophotometer (V-530; JASCO, Japan) after suitable dilutions with distilled water.

2.5.2. High-performance thin layer chromatography

The GPZ, EPZ, APZ extracts and standard plumbagin (1 mg/ml of methanol) were spotted on HPTLC silica gel 60 F_{254} plates (10 × 10 cm, 0.2 mm thickness, E. Merck, Germany) using CAMAG LINOMATE IV automatic spotter (Switzerland). The plates were developed with a twin-touch developing chamber containing benzene: n-hexane (9:1 v/v) as the mobile phase (Bothiraja et al., 2011). Densitometry scanning was performed using TLC scanner III (CAMAG, Switzerland) at 425 nm. CATS 4 (CAMAG) integrative software was used for analysis.

2.5.3. Differential scanning calorimetry

Thermal properties of the standard plumbagin and plumbagin extracts were studied using differential scanning calorimeter (DSC) equipped with an intracooler (DSC 821e, Mettler-Toledo, Switzerland). Indium standards were used to calibrate the temperature and enthalpy scale. Approximately 5 mg of sample was hermetically sealed in an

Table 1
Plumbagin extracted with ethanol, water and different ratio of Gelucire 44/14.

Batches	Crude drug:gelucire	% Plumbagin extracted [a]
GPZ1	1:0.5	0.16 ± 0.02
GPZ2	1:1	1.73 ± 0.14
GPZ3	1:1.5	3.24 ± 0.12
GPZ4	1:2	3.32 ± 0.09
GPZ5	1:2.5	3.47 ± 0.13
GPZ6	1:3	3.52 ± 0.08
EPZ	–	2.48 ± 0.16
APZ	–	0.07 ± 0.02

GPZ, EPZ and APZ; gelucire, ethanolic and aqueous extract of *P. zeylanica*, respectively.
[a] Values were presented as mean ± SD ($n = 3$).