Food Bioscience 12 (2015) 34–46

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Gastro-protective effects of the phenolic acids of *Macrotyloma uniflorum* (horse gram) on experimental gastric ulcer models in rats

Vandana Panda*, Swetha Suresh

Department of Pharmacology & Toxicology, Prin. K.M. Kundnani College of Pharmacy, Jote Joy Building, Rambhau Salgaonkar Marg, Cuffe Parade, Colaba, Mumbai 400005, India

ARTICLE INFO

Article history:
Received 29 December 2014
Received in revised form
23 April 2015
Accepted 13 July 2015
Available online 14 July 2015

Keywords:
Macrotyloma uniflorum seed extract
p-Coumaric acid
Absolute ethanol
Indomethacin
Antiulcer
Antioxidant

ABSTRACT

Macrotyloma uniflorum Lam. (Verdc.) known as horse gram is an underutilized and unexplored food legume distributed throughout Asia and Africa. Its seeds are rich in phenolic acids, *p*-coumaric acid being the most abundant phenolic acid. The present study evaluates the antiulcer and antioxidant activity of the hydroalcoholic extract of the seeds of *M. uniflorum* (MUSE) and *p*-coumaric acid against indomethacin (non-steroidal anti-inflammatory drug) and absolute ethanol (necrotizing agent) induced ulcers in rats. Pre-treatment with MUSE and *p*-coumaric acid showed a dose-dependent decrease in the ulcer index in both models. MUSE and *p*-coumaric acid elicited significant antioxidant activity by attenuating the ulcer elevated levels of malondialdehyde and restored the ulcer-depleted levels of reduced glutathione and the antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase. In conclusion, MUSE possesses potent antiulcer activity which may be attributed to an underlying antioxidant activity.

1. Introduction

Peptic ulcer or Peptic ulcer disease (PUD) is defined as a break in the mucosal lining of the gastrointestinal tract (Yuan, Padol, & Hunt, 2006). It occurs in that part of the gastrointestinal tract which is exposed to gastric acid and pepsin, i.e. the stomach and duodenum. The normal stomach mucosa maintains a balance between defensive and aggressive factors (Dimaline & Varro, 2007). Some of the main aggressive factors are gastric hydrochloric acid, abnormal motility, pepsin, bile salts, leukotrienes, free radicals, alcohol and nonsteroidal anti-inflammatory drugs (NSAID), as well microorganisms such as *Helicobacter pylori*. On the other hand, defensive factors such as the mucus-bicarbonate barrier, surface active phospholipids, prostaglandins, endogenous nitric oxide, endogenous antioxidants and normal tissue microcirculation protect against ulcer formation. Although the etiology of ulcer is still not fully understood, it is generally accepted that peptic ulcers develop when aggressive factors (endogenous, exogenous and/or infectious agents such as *H. pylori*) overcome mucosal defense mechanisms (Tulassay & Herszenyi, 2010). The incidence of PUD varies with age, gender and geographical location and is associated with severe complications including hemorrhage, perforation, gastrointestinal obstruction and malignancy (Brown & Wilson, 1999). Thus, this clinical condition represents a serious health problem worldwide because of its high morbidity, mortality and economic loss.

Modest approaches to control peptic ulcers include potentiation of the mucosal defense, reduction in acid secretion and its neutralization, enhancement of antioxidant levels in the stomach, stimulation of gastric mucin synthesis and inhibition of *H. pylori* growth. Several classes of pharmacological agents have proved to be effective in the management of acid peptic disorders. They include antacids (aluminum hydroxide and magnesium trisilicate), acid suppressive agents such as proton pump (H^+/K^+ ATPase) inhibitors (e.g., omeprazole and lansoprazole), H_2 receptor antagonists (cimetidine and ranitidine), anticholinergic agents (pirenzepine), cytoprotective agents (sucralfate and misoprostol) and antimicrobials for eradication of *H. pylori* (amoxicillin and clarithromycin) (Waller, Renwick, & Hillier, 2005). However, gastric ulcer therapy faces a major drawback because most of the currently available drugs in the market show limited efficacy and are often associated with severe side effects (Lehne, 1998).

In this context, the use of medicinal plants is on a global rise for the prevention and treatment of different pathologies and natural products are regaining importance in the pharmaceutical industry as inspiring sources of new potentially bioactive molecules. Clinical research has confirmed the efficacy of several plants for the treatment of gastroduodenal diseases (Schmeda-Hirschmann & Yesilada, 2005). Most of these plant derived drugs augment the mucosal defensive factors which are thought to be important for

* Corresponding author. Fax: +91 22 22165282.
E-mail address: vspanda@rediffmail.com (V. Panda).

http://dx.doi.org/10.1016/j.fbio.2015.07.004
2212-4292/

protection of gastric mucosa (Tovey et al., 2011). The medicinal properties of plants are attributed mainly to the presence of flavonoids, but they may be also influenced by other organic and inorganic compounds such as coumarins, alkaloids, fatty acids, terpenoids, tannins, phenolic acids and micronutrients, such as copper, manganese, iron, zinc and selenium among others.

Horse gram [*Macrotyloma uniflorum* Lam. (Verdc.)], previously [*Dolichos biflorus*] belonging to the family *Fabaceae* is a lesser known drought resistant legume grown throughout Asia, Africa and Australia and primarily utilized as a feed to animals and horses. In India it is known as the "poor man's pulse" and used as a staple food. The horse gram seed is reported to be high in tannins and polyphenols when compared with other legumes (Kadam & Salunkhe, 1985). It is prescribed for persons suffering from jaundice or water retention and as part of a weight loss diet. It is useful in iron deficiencies and is considered helpful for maintaining body temperature in the winter season.

The seeds of *M. uniflorum* are used in traditional medicine as bitter, thermogenic, astringent, anthelmintic, diaphoretic, diuretic, expectorant, ophthalmic and tonic. The seeds are also useful for hemorrhoids, tumors, bronchitis, splenomegaly and in asthma (Marimuthu & Krishnamoorthi, 2013). Reports of lipids obtained from horse gram in healing of peptic ulcers in rats successfully are available in literature (Jayaraj, Tovey, Lewin, & Clark, 2000). Seeds of *M. uniflorum* contain varying amounts of carbohydrates, proteins, amino acids, lipids, phenolic acids (3,4-dihydroxy benzoic acid, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid, chlorogenicacid, syringic acid and sinapic acid), flavonoids and tannins (quercetin, kaempferol and myricetin), fatty acids (hexanoic acid and hexadecanoic acid), phytosterols (stigmasterol and β-sitosterol), anthocyanidins (cyanidin, petudin, delphinidin and malvidin), saponins and minerals like iron, calcium and molybdenum (Kawsar, Huq, Nahar, & Ozeki, 2008). Phenolic acids obtained from *M. uniflorum* are considered to be potent antioxidants which act by scavenging free radicals and reactive oxygen species (Siddhuraju & Manian, 2007; Sreerama, Sashikala, & Pratape, 2010).

Oxidative damage is considered to be a major mechanism in the pathogenesis of ulcer. Several phenolic acids such as caffeic, *p*-coumaric, ferulic and cinnamic acids have been documented to possess gastroprotective activity (Barros et al., 2008). Since, horse gram contains all these acids, the present study was undertaken to evaluate a polyphenol rich fraction of *M. uniflorum* seed extract for antiulcer activity. *p*-Coumaric acid being the most abundant phenolic acid, its antiulcer activity was also evaluated separately and compared with the extract.

2. Materials and methods

2.1. Plant material

The seeds of *M. uniflorum* were collected from the Colaba market, Mumbai, India. The seeds were air dried under shade, powdered mechanically and stored in air tight containers. The powder was extracted using a mixture of ethanol: water (80:20) v/v in a Soxhlet apparatus. This extract was dried and stored in a refrigerator for further use. The plant was authenticated at the Blatter Herbarium, St. Xavier's College, Mumbai after matching with the existing specimen (specimen no. AD- 6).

2.2. Isolation of p-coumaric acid by preparative HPTLC

After successful development of TLC, preparative High Performance Thin Layer Chromatography (HPTLC) of the hydroalcoholic extract of the seeds of *M. uniflorum* (MUSE) was carried out on the CAMAG HPTLC System for isolation and identification of *p*-coumaric acid. The absorbance value of different bands in the crude extract after TLC separation was studied using the Desaga Scanner for the most possible wavelength absorption of *p*-coumaric acid. Prior to MUSE application, HPTLC plates (HPTLC Silica gel 60 F254, Merck) of 10×10 cm^2 were activated at 110 °C for 30 min. MUSE (100 mg) was dissolved in 10 ml of methanol and 100 μL of this solution was applied as a single band of 180 mm length on the activated HPTLC plates using a Linomat V applicator (CAMAG, Switzerland). The plates were then developed with 10 ml of the solvent system comprising toluene: ethyl acetate: formic acid (6.8:2.3:0.9) (Medic-Saric, Jasprica, Smolcic-Bubalo, & Mornar, 2004) in a twin trough chromatographic chamber and examined at 315 nm for *p*-coumaric acid. After development, the constituent at R_f 0.55 was marked and scraped out from the plate. The scraped material was mixed with methanol and eluted from silica gel by centrifugation at 3000 rpm. The supernatant was evaporated on a water bath to get *p*-coumaric acid. Further confirmation of the isolated constituent was done by using UV–visible, IR and NMR spectroscopies for major functional groups.

2.3. Quantification of p-coumaric acid using HPLC

2.3.1. Chemicals and reagents

HPLC grade methanol, acetonitrile and analytical grade formic acid were procured from Merck® India Ltd. (Mumbai). All the solvents and solutions were filtered through a membrane filter (Millipore filter paper, 0.45 μm pore size) and degassed before use. Standard *p*-coumaric acid was procured from Sigma-Aldrich Pvt. Ltd., MO, USA.

2.3.2. Instrumentation and materials

Analysis was performed on Jasco HPLC system with Jasco PU-2080 Plus Quaternary Gradient HPLC Pump and in-built Jasco MD-2010 Plus PDA multi wavelength detector. Chromatographic software Chrompass was used for data collection and processing. The analytical column was LC–GC Qualisil BDS C18 (5 μm, 250 mm × 4.6 mm).

2.3.3. Chromatographic conditions

Chromatographic separation of *p*-coumaric acid was carried out using an isocratic mobile phase comprising water: acetonitrile (80:20) v/v at pH 3.5 (adjusted with formic acid) (Mas, Fonrodona, Tauler, & Barbosa, 2007). A well resolved and sharp peak for *p*-coumaric acid with a retention time (Rt) of 8 min was obtained. The flow rate was maintained at 1.0 mL/min and the detection carried out at 315 nm.

2.3.4. Preparation of standards

A stock solution (1000 μg/ml) was prepared by dissolving 10 mg of *p*-coumaric acid in 10 ml of methanol. This solution was further diluted with the mobile phase to give a stock solution of 100 μg/mL. Further dilutions were made as required with the mobile phase.

2.3.5. Preparation of calibration curve

The calibration curve was prepared by injecting various concentrations (10–50 μg/mL) of the standard *p*-coumaric acid solution.

2.3.6. Quantification of p-coumaric acid in MUSE

MUSE (50 mg) was dissolved in 10 ml methanol to yield a solution of 5 mg/mL. This solution (20 μL) was injected and the elution was carried out using the mobile phase mentioned earlier. The amount of *p*-coumaric acid in MUSE was calculated from the calibration curve.