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Modified pectic polysaccharide from turmeric (*Curcuma longa*): A potent dietary component against gastric ulcer

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ABSTRACT

Native, intact (TrPP) and modified, low-molecular-weight (MTrPP) forms of pectic polysaccharides isolated from turmeric were evaluated for ulcer-preventive potentials in *in vitro* and *in vivo* models. Data indicated that MTrPP possessed significantly better ulcer-preventive property than TrPP; inhibiting ulcer scores up to 85%. Results were substantiated by effective muco-protection, H^+,K^+-ATPase down-regulation, inhibition of *H. pylori* growth/adherence, higher antioxidant/cytoprotective mechanisms. Structural data indicated TrPP and MTrPP differ in their molecular weights and structural characteristics with different sugar compositions and side chain ratios. MTrPP was rich in galacturonic acid (687 mg/g; TrPP—544 mg/g) and galactose (52.9%; TrPP—21.7%). Results were substantiated by NMR/FTIR data indicating the presence of homogalacturonan and rhamnogalacturonam-I containing galactans. By virtue of binding to inflammatory marker (galectin-3), galactans may reduce inflammation induced ulcerations. The low molecular weight of MTrPP (155 kDa; TrPP—13 kDa) may increase its bioavailability than TrPP, thus MTrPP may possess higher antiulcer potential.

1. Introduction

Gastric ulcer is a common and serious upper gastrointestinal disorder that represents one of the leading health problems across the globe (Wallace & Granger, 1996). Although the multi-factorial etiology of the disease that manifests into gastric ulcers has not been understood comprehensively, dynamic imbalance between gastro-defensive and aggressive factors appears to be the root cause.

Oxidative stress (OS) induced hyper-secretion of gastric acid by H^+,K^+-ATPase up-regulation, exposure to pathogenic *Helicobacter pylori*, consumption of non-steroidal anti-inflammatory drugs (NSAIDs), changes in life style including smoking and alcohol consumption - are the main factors causing aggravation of acidity. Excess acid in the stomach is known to erode the protective gastric mucin lining and disrupt overall mucosal integrity of the stomach leading to ulcerations (Odenbreit, 2005; Brunton, Chabner, & Knollmann, 2006).

OS, together with *H. pylori* infection, have been shown to impair mucin turn over (Odenbreit, 2005). Therefore, potentiating mucosal defense via boosting stomach antioxidant capacity, enhancing gastric mucin production, regulating gastric acid secretion and eradication of *H. pylori* can be thought of as the mainstay of ulcer prevention (Tuorkey & Abdul-Aziz, 2011). Though there are different courses and combinations of anti-secretory and anti-*H. pylori* medications used to treat stomach ulcers, side effects outbeat the therapeutic effects of such treatments (Parikh & Howden, 2010; Chubineh & Birk, 2012). Thus, an alternate, less expensive, more rational, non-toxic and effective approach is to explore natural products and their constituents that promote ulcer prevention.

Dietary/plant based compounds can be heavily relied upon to treat gastric disorders owing to their safety and lack of adverse effects, as opposed to synthetic drugs. In Indian Ayurveda and traditional herbal medicine, various herbs and spices have been identified and documented to treat gastrointestinal problems (Chatterjee & Bandyopadhyay, 2014; Kangwan, Park, Kim, & Hahm, 2014). We have previously reported from our laboratory that phenolic compounds from various dietary sources are responsible for the observed anti-ulcerogenic effects (Siddaraju & Dharmesh, 2007a,b). Subsequently, plant derived bioactive carbohydrate polymers such as pectic polysaccharides (PPs) have also been shown

Abbreviations: PPs, pectic polysaccharides; TrPP, native turmeric pectic polysaccharide; MTrPP, modified turmeric pectic polysaccharide; HG, homogalacturonan; RG-I, rhamnogalacturonan-I; GalA, galacturonic acid; Galp, galactose; HA, haemagglutination; NCCS, National Center for Cell Sciences; NSAIDs, non-steroid anti-inflammatory drugs; ELSD, evaporative light scattering detector; TBARS, thiobarbituric acid reactive substances.

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to exert multi-mechanistic gastro-protective effects (Srikanta, Siddaraju, & Dharmesh, 2007; Srikanta, Sathisha, & Dharmesh, 2010).

Pectin is a water-soluble, major structural component of the cell wall of plants, and is regarded as one of the most complex and heterogeneous classes of polysaccharides. Majority of the published literature on bioactivities of pectin attribute the observed activities to RG-I regions rich in side chains composed of galactan, arabinan and arabinogalactan (Ridley, O'Neill, & Mohnen, 2001).

Turmeric (*Curcuma longa*) is a remarkable herb whose bioactive constituents have been proven to exert a series of disease preventive properties (Gupta et al., 2013; Noorafshan & Ashkani-Esfahani, 2013). Recently, our group has patented the bioactive functioning of turmeric PPs, particularly against metastasis (Dharmesh & Jayaram, 2010). Considering these extensively reported ethnopharmacological properties of turmeric, we set the goal of the present study so as to examine the anti-ulcer potentials of pectic polysaccharides isolated from turmeric (turmeric PPs) in the native and modified forms, and to elucidate the plausible mechanism of action involved in blocking ulcer development. We report a highly potent anti-ulcerous modified pectic polysaccharide isolated from turmeric (MTrPP) that could mitigate acid secretion by controlling the action of H^+,K^+-ATPase, both in *in vivo* and cell culture models. In addition, we have also investigated whether MTrPP could effectively abrogate *H. pylori* growth and adherence (colonization) *in vitro*, followed by testing the essential roles of MTrPP in *in vitro* cytoprotection and *in vivo* cellular antioxidant homeostasis.

2. Materials and methods

2.1. Isolation, modification and molecular weight determination of pectic polysaccharide

2.1.1. Isolation

PPs were isolated from a high yielding variety of turmeric (Allepey). Briefly, 10 g of defatted powder was subjected to enzymatic digestion to degrade protein (protease) and starch (thermamylase and glucoamylase). The de-proteinized and de-starched residue was suspended in 200 mL of 0.05% ammonium oxalate solution and extraction was carried out at 70 °C for 3 h as per our previously reported procedure (Srikanta et al., 2007).

2.1.2. Modification

Intact turmeric PPs were altered by a simple pH based modification involving alkaline treatment (pH set to 10 using 3 N NaOH, 1 h. at 60 °C) followed by overnight acid treatment (pH set to 3 using 3 N Hcl). Samples were precipitated using ethanol, filtered, washed with acetone, dialyzed and lyophilized. Pectic polysaccharide preparations were designated as—TrPP (native turmeric pectic polysaccharide) and MTrPP (modified turmeric pectic polysaccharide).

2.1.3. Molecular weight

Molecular weights of TrPP and MTrPP were analyzed by performing HPLC using Waters 2695 ultra-hydrogel linear column and Altech ELSD 2424 detector system. A series of dextran standards (T10, T20, T40, T75, T500, and T1000 kD) was used to generate a calibration curve. Isocratic elution was carried out using water as mobile phase at a flow rate of 0.5 mL/min at 30 °C, while the temperature of drift tube of ELSD was set at 120 °C and nitrogen flow rate was 3.5 mL/min.

2.1.4. Fourier Transformed Infrared (FT-IR) and Nuclear Magnetic Resonance (NMR) spectroscopy

FT-IR was carried out using spectral conditions as described by Srikanta et al. (2007). Further, MTrPP with higher anti-ulcer activity was structurally analyzed using NMR spectroscopy. 20 mg of MTrPP was completely dissolved in 500 μL deuterated water and subjected to NMR measurement. ^1H and ^{13}C NMR spectra were measured on a Bruker AMX 400 Spectrometer at 500 MHz at 80 °C.

2.2. Biochemical estimations

Total sugar, uronic acid and other monomeric sugar constituents that make up pectin were determined by GLC (Savitha Prashanth & Muralikrishna, 2014). Since cross-linking of phenolic groups between side chains is a structural feature of pectin, the total phenols were quantified by Folin-Ciocalteu method (Singleton & Rossi, 1965).

2.3. Antioxidant and cytoprotective properties

Antioxidant activities were assessed via measuring reducing power, DPPH radical scavenging and DNA protective abilities, as described previously (Siddaraju & Dharmesh, 2007). Physiological stress, *H. pylori* and NSAIDs are ulcerogenic candidates known to cause OS induced cytotoxic injury to the stomach epithelium, which in turn leads to gastric ulcers. In light of this, the cytotoprotective properties of TrPP and MTrPP were tested by evaluating their protective effects on buccal cells induced with OS using an oxidant system containing H_2O_2—aspirin (Kavitha & Dharmesh, 2014).

2.4. H^+,K^+-ATPase inhibition

2.4.1. Enzyme inhibition using sheep stomach homogenate

H^+,K^+-ATPase inhibitory activity was assayed using an *in vitro* method established by our laboratory (Siddaraju & Dharmesh, 2007).

2.4.2. Effect on gastric cell line

Human gastric epithelial cancer cell line (AGS) was procured from National Centre for Cell Science (NCCS, Pune, India). The cells were cultured as monolayers in Ham's F-12 nutrient medium, supplemented with 10% fetal bovine serum and 100 U/L antibiotic-antimycotic solutions, in a humidified atmosphere of 5% CO_2 in air. The cells (20,000 cells/well) were seeded into 96 well culture plates and further cultured to sub-confluence, following treatment with equal volumes of different concentrations of test PPs in the media.

Semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) of α- subunit of H^+,K^+-ATPase was performed by extracting total RNA from AGS cell lysate using TRIzol reagent according to the manufacturer's protocol. 2 μg total RNA was reverse transcribed using Superscript II RT system. Specific primers were as follows: H^+,K^+-ATPase α-subunit forward, 5′ TCTCTCCGAGCAGCGCA 3′; reverse, 5′ CGTCGCCACTCTTGCTGTCG 3′; GAPDH forward, 5′AGGTCGGAGTCAACGGATTTG3′; reverse, 5′GTGATGGCATGGACTGTGGT3′. RT-PCR was performed using Jumpstart Taq mix. The amplifications were performed in 25 μL reaction volumes with an initial denaturation at 94 °C for 5 min prior to 35 thermal cycles of 94 °C for 1 min, 55 °C for 30 s and 72 °C for 1 min, with a final extension at 72 °C for 10 min. The PCR products were electrophoresed in 1.25% agarose gels at 50 V for 45 min and visualized by ethidium bromide staining.

2.5. *Anti*-H. pylori *tests*

H. pylori was cultured, identified and characterized from endoscopic biopsy samples of infected gastric ulcer patients, following our previously reported method (Siddaraju & Dharmesh, 2007).

2.5.1. MTT micro dilution assay

H. pylori (10^5 cells/well, in Ham's F-12 nutrient medium with 5% FBS) was seeded into 96 well plates at 37 °C for 24 h. The cells were