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Original article

Aegle marmelos fruit extract abates dextran sodium sulfate induced acute colitis in mice: Repression of pro-inflammatory cytokines during colonic inflammation

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ABSTRACT

Ulcerative colitis (UC) is a common inflammatory bowel disease which on prolongation causes colorectal cancer (CRC) making UC as the highest risk factor for CRC development. Despite the use of *Aegle marmelos* in folk medicine, few studies have reported its colonic healing activity. We exploited the use of dextran sodium sulfate (DSS) in inducing colitis in Swiss albino mice and examine the inflammatory modulating effect of *A. marmelos* fruit extract (AME). HPLC analysis confirmed the presence of two biologically active compounds namely umbelliferon (a coumarin-derivative) and lupeol (triterpenoid). Fourteen days feeding of DSS to mice elicited colitis, with drastically reduced body weight with altered clinical severity score, combined with shortening of colon length. Oral administration of AME (50 mg/kg) evidenced a significant suppression of disease symptoms. The increased mRNA expressions of interleukin (IL) 2, IL-6 and tumor necrosis factor α during colitis, were also reduced significantly. Notable reduction in the NF-κB expression in the colonic region was also noted which is substantiates with docking analysis were UMB and LUP found in AME bounds with NF-κB. Furthermore, DSS-altered histopathological features of colon were also recovered on treatment with AME. Thus the observation revealed the restorative significance of AME in healing the DSS-induced colitis in mice by modulating NF-κB and regulating pro-inflammatory mediators involved in the colonic injury.

1. Introduction

Inflammatory bowel disease (IBD), either ulcerative colitis (UC) or Crohn's disease, results due to chronic/acute inflammation of the intestinal tract with unknown aetiology [1]. UC is a typical inflammatory intestinal disease characterized by erosion, mucosal ulceration, and infiltration of inflammatory cells and shows clinical manifestations that include loss of weight, diarrhea accompanied with blood and mucus, fever, gastric dysmotility, and shortening of the colon [2]. Compared with the normal population, patients with IBD are at a higher risk of developing colorectal cancer (CRC) [3], but the mechanism(s) underlying the frequent development of CRC in patients with UC remains unidentified. The interplay between immune (T-helper cells type 1-Th1) and environmental factors along with the involvement of cytokines including interleukins (IL-1, IL-6, IL-12, IL-23, IL-10), tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) [4] are implicated in the progression of UC. However, a satisfactory therapeutic program targeting UC is yet to be established.

An animal model of intestinal inflammation is vital to understand the pathophysiological mechanisms and different animal models of chemically induced experimental colitis have been developed to study the molecular and cellular mechanisms of inflammation [5]. The most widely used preclinical model is the dextran sodium sulfate (DSS)-induced colitis model, which mimics human UC with some important immunological and histopathological aspects [6] and is frequently used to evaluate the effect of novel anti-inflammatory drugs. DSS causes epithelial toxicity and stimulates lamina propria cells, increasing the production of

Abbreviations: AME, *Aegle marmelos* unripe fruit extract; CRC, colorectal cancer; DPPH, 1,1-Diphenyl-2-picrylhydrazyl; DSS, Dextran sodium sulfate; FRAP, ferric Reducing antioxidant power; HPLC, high liquid chromatography; IBD, inflammatory bowel disease; IL, interleukin; MPO, myeloperoxidase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; RT-PCR, reverse transcriptase polymerase chain reaction; TNF-α, tumor necrosis factor-α; UMB, Umbelliferon; LUP, lupeol; UC, ulcerative colitis.

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pro-inflammatory cytokines [7]. The inflammation is confined to colon which is mainly due to the action of dextran moiety targeting medium-chain length fatty acids present at high concentrations in the colonic lumen and epithelium [8].

In recent years, the importance towards probing the biologically active derivatives of medicinal plants and the possibility in developing them as novel potential therapeutics for several pathologies has increased [1,2].

In Indian traditional medicine, the application of the plant/parts of the plant belonging to the Rutaceae family *Aegle marmelos Correa* (commonly known as beal) has been extensively reported. All parts of the plant have many medicinal properties which have been used as astringent, aphrodisiac, demulcent, haemostatic, antidiarrheal, antidysenteric, antipyretic, anti cancer, antiscourbutic, and as an antidote to snake venom [9–11] and the decoction of the leaves and ripe fruit are used in folk therapy for diabetes mellitus, dysentery and diarrhoea, which is due to the large quantities of mucilage [12]. *A. marmelos* has been shown to be effective against experimental IBD and diarrhoea [13,14]. The dry pulp of the fruit contains chiefly mucilage pectin-like substance. Aegelin, lupeol, skimmianine, fagarine, marmin, marmelide, marmesinin, marmelosin, psoralen, aurapten, cineol, citral, eugenol, cuminaldehyde, luvangetin, citronellal and tannin are some of the bioactive chemical constituents reported from various parts of the plant [15].

Furthermore, our previous studies on *A. marmelos* fruit extract (AME) have shown anti-microbialand anti diarrheal activities [16–18]. The present study was executed to show experimentally the phytochemical index and the in-vitro antioxidant property of AME. The efficacy of AME as a pro-inflammatory modulator in lessening the intestinal inflammation in an in-vivo mouse model of DSS-mediated colitis was also investigated.

2. Materials and methods

2.1. Ethics statement

No special permission was required for the collection of the fruits, since the study did not involve endangered or protected species and the authority of the Athieetheswara Temple, Vaniyambadi, gave permission to collect the fruits for the study. All animal care and experimental protocols were approved by the Institutional Animal Ethics Committee of the University of Madras, Chennai, Tamilnadu, India (IAEC No. 02/02/2012).

2.2. Chemicals

Folin-Ciocalteu phenol reagent and sodium carbonate were from Merck Chemical Supplies (Damstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), gallic acid, quercetin and guaiacol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). DSS (MW 36–50 kDa) was obtained from MP Bio medicals (Ohio, USA). All the other chemicals used were of analytical grade.

2.3. Collection of fruit and preparation of extracts

Fruits of *A. marmelos* were collected from Vaniyambadi, Tamil Nadu, India, during mid-February (the authority of the Athieetheswara Temple, vaniyambadi, where the plants were collected, gave permission to carry out the study). The fruits were shade dried, seeds were removed and fruits were ground mechanically. 500 g of powdered fruit were extracted with 1 L of water for 12 h. The resulting extract was filtered using Whatman No. 1 filter paper. The filtrate was evaporated under reduced pressure using rota evaporator to give a residue of *A. marmelos* aqueous extract (AME). AME was stored in sterilized containers at room temperature until used for further testing.

2.4. Phytochemical index (DPPH, FRAP, polyphenol and flavonoids content) of AME

DPPH radical scavenging assay, ferric reducing antioxidant power (FRAP) (total antioxidant activity), total flavonoid contents, were determined by the method of Adedapo et al. [19]. Total phenolic content in the extracts was determined by the modified Folin-Ciocalteu method which was adapted from Adedapo et al. [19]. The total flavonoid content of AME was expressed as milligrams of quercetin equivalents (QE) per gram of extract, where quercetin was used as the standard. The total phenolics content of AME was expressed as mg of gallic acid equivalents (GAE) per gram of extract, where gallic acid was used as the standard.

2.5. High-performance liquid chromatography (HPLC) analysis

Qualitative and quantitative analysis of the chemical constituents in the AME was carried out on HPLC system (Shimadzu, USA). The chromatographic separations were performed using C 18 Shim-pack XR-ODS II (150 mm (L) × 3 mm (i.d.)), with a flow rate of 1 mL/min. 20 μl of extracts (2 mg/mL in methanol) and the standards (umbelliferon and lupeol) were prepared to obtain a final concentration of 100 μg/mL in methanol. Samples were filtered with a 0.45 μm syringe filter and collected in 2 mL glass screw-top vials. They were injected by auto sampler and eluted through the column with a mobile phase consisting of water containing 0.1% formic acid (A) and acetonitrile (B) at a ratio of 1:9 (A:B, v/v). The samples were monitored with UV detection at 254 nm at room temperature. Peak purity was checked by the software contrast facilities, and the quantitative estimations of chemical constituents were achieved by the absorbance recorded in the chromatograms and relative external standards using the following equation: $C\boldsymbol{C} = \{A\boldsymbol{C}/A\boldsymbol{ST}\} \times C\boldsymbol{ST}$, where C***C*** is the concentration of the constituent in the AME, A***C*** is the peak area of the constituent in the AME chromatograms, C***ST*** is the concentration of the standard in the reference solution and A***ST*** is the area of the peak for the standard in the reference chromatograms [20].

2.6. Experimental animals

Male Swiss albino mice, 6–8 weeks of age, weighing 18–20 g were obtained from the Laboratory Animal Maintenance Unit, Tamilnadu Animal Science and Veterinary University, Chennai, India. Animals were acclimatized under a 12 h light/dark cycle at 22 °C and 60% humidity for 7 days before the experiments, and fed with a standard laboratory rodent diet and water *ad libitum*.

2.7. Induction of colitis

Colitis was induced by providing drinking water *ad libitum*, containing 2% DSS in drinking water (w/v) from day 0 for 14 days. Colonic inflammation was assessed 7th day after DSS treatment. AME was suspended in distilled water and administrated orally at a dose of 50 mg/kg body weight every day from 7th day after colitis induction till the end of the experimental period.

The animals were randomly divided into four groups ($n = 6$ per group). Group I (control), group II (AME 50 mg/kg body wt in distilled water, orally), group III (2% DSS in drinking water), and group IV (DSS as in group III and AME as in Group II). The time schedule and administration of DSS/AME are given in Fig. 1. At the end of the experimental period, animals were euthanized with overdose of ketamine (90 mg/kg b.w) and zyalazine (10 mg/kg b.w), after