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Protective effect of marine mangrove *Rhizophora apiculata* on acetic acid induced experimental colitis by regulating anti-oxidant enzymes, inflammatory mediators and nuclear factor-kappa B subunits

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ABSTRACT

Ulcerative colitis is a disease that causes inflammation and ulcer in the lining of the large intestine. In this study we investigate the effect of *Rhizophora apiculata* (*R. apiculata*) on acetic acid induced colitis in mouse model. Experimental animals were randomized into four groups: normal untreated, colitis control, *R. apiculata* treated group and sulfasalazine treated group. *R. apiculata* significantly ($p < 0.01$) decreased macroscopic score and wet weight of damaged colon compared to colitis control. This effect was confirmed biochemically by significant ($p < 0.01$) reduction of colitis associated increase in myeloperoxidase activity. *R. apiculata* significantly ($p < 0.05$) increased anti-oxidant enzymes such as superoxide dismutase (SOD) and glutathione (GSH) levels compared to colitis control. *R. apiculata* significantly ($p < 0.01$) reduced lipid peroxides (LPO), nitric oxide (NO) and inflammatory mediators such as myeloperoxidase (MPO), lactate dehydrogenase (LDH), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and tumor necrosis factor-α (TNF-α) expressions compared to colitis control. *R. apiculata* treatment significantly ($p < 0.01$) inhibits the translocation of NF-kB p65 and p50 subunits. Taken together these findings suggest that *R. apiculata* prevents acetic acid induced colitis in experimental mouse model and may serve as an excellent anti-oxidant and anti-inflammatory agent that could potentially be useful as a (natural) therapy for inflammatory bowel disease (IBD).

1. Introduction

Ulcerative colitis (UC) is a common inflammatory bowel disease (IBD) with chronic inflammatory process in the digestive mucosa that causes inflammation and ulcers in the lining of the rectum and colon [1]. The etiology of IBD still remains unclear but environmental factor and genetic susceptibility could be major causes of involvement in the initiation of UC [2]. The prevalence of IBD rapidly increased in the United States, Europe and Asia [3,4]. UC affects approximately 500,000 to 2 million people in the United States and they are most common during adolescence and early adulthood. The symptoms include abdominal pain, diarrhea with bloody and/or mucus diarrhea, dehydration, abdominal cramps, fever, anemia, weight loss and psychosocial consequences [5]. Patients with severe and recurrent UC have an elevated risk of developing colorectal cancer (CRC). CRC is the second leading cause of death in the United States and around 108,000 people were diagnosed with CRC every year [2].

CRC is a feared complication of chronic UC and inflammation which appears to be an important biological risk factor for the development of CRC [6,7]. Chronic inflammation increases oxidative stress, regeneration, repair, and dysplasia in the colon that transform into an invasive CRC [8]. Consumption of alcohol increases the risk of development of ulcer. Excessive ethanol ingestion results in gastritis characterized by mucosal edema, sub-epithelial hemorrhages, cellular exfoliation and inflammation cell infiltration.

To scavenge reactive oxygen species (ROS), gastric cells have several enzymatic and non-enzymatic anti-oxidants including superoxide dismutase (SOD), reduced glutathione (GSH) and catalase (CAT) but excessive generation of ROS enhances lipid peroxides (LPO) (oxidative stress) and could deplete these antioxidant enzymes [9]. Oxidative stress could be a major contributing factor to tissue injury and fibrosis that characterize IBD. The colon mucosa of UC secretes high amount of pro-inflammatory cytokines and inflammatory mediators such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, nitric oxide (NO), inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX) that seem to play an important role in UC in relevant experimental models [10,11]. UC is associated with neutrophil infiltration which is directly correlated with increase in myeloperoxidase (MPO) level that produces hypochlorous acid from hydrogen peroxide and chloride anion that are cytotoxic [12]. Increased level of superoxide and NO during UC increases peroxynitrite formation that mediates oxidation of lipids, proteins and DNA.

Therapeutic strategies for treating IBD now focus on the use of anti-inflammatory agents such as sulfasalazine the most widely prescribed

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drug. Sulfasalazine is converted to 5-ASA in the intestine that disturbs the prostaglandin and leukotriene pathways. The adverse effects of sulfasalazine include vomiting, hypospermia, hepatitis, pneumonitis, hemolytic anemia, chronic nephrosis and encephalitis [13,14]. In rare cases, sulfasalazine can exacerbate colitis, resulting in diarrhea, abdominal cramps and discomfort [15].

Medicinal plant based derivatives possess protective effect with loss or no adverse effects against colitis by causing an elevation of antioxidants and activities of associated enzymes [16]. The use of complementary medicine among patients with IBD has been increasing in the western world [17]. There are even limited plant based control studies indicating the efficacy of these agents for the treatment of UC [18]. Medicinal properties attributed to the *Rhizophora apiculata* (*R. apiculata*) in folk medicine are based to a great extent on the fact that use of its root, leaf or stem extracts imparts an inhibitory effect on the growth of bacterial, viral and fungal pathogens [19,20]. Polysaccharide extracted from the leaf of *R. apiculata* is reported to inhibit HIV strains in various cell cultures [20]. *R. apiculata* has a high content of flavonoids, tannins, catechin, anthraquinone, pyroligneous acid and syringol [21]. Building upon earlier studies of the potential utility of *R. apiculata* against numerous immune based disorders; in this study we investigated the effect of *R. apiculata* in an experimental model of colitis induced by administration of acetic acid in mice.

2. Materials and methods

2.1. Plant collection

R. apiculata (vernacular name — Surapunnai in Tamil) whole plant was collected from Pichavaram mangrove forest which is located in Cuddalore District, Tamil Nadu, India. The plant materials were authenticated by an eminent taxonomist and a voucher specimen (Rhiz-018) was deposited in the Department of Botany, M.E.S. Kalladi College, Mannarkkad, India.

2.2. Animals

Male BALB/c mice (4–6 weeks old) were purchased from the Pasteur Institute of India, Coonoor, Tamil Nadu, India. The animals were kept in a pathogen-free air-controlled room maintained at 24 °C with a 50% relative humidity and 12-hour light/dark cycle, and fed with normal mice chow (Sai Feeds, Bangalore, India) and water ad libitum. All animal experiments were performed according to the rules and regulations of the Institutional Animal Ethics Committee approved by Government of India.

2.3. Chemicals

All chemicals used in these studies were of analytical or reagent grade. Gum acacia was purchased from Hi-Media (Mumbai, India). Formaldehyde solution was procured from Universal Laboratories Pvt. Ltd. (Hyderabad, India). Sulfasalazine was purchased from Wallace Pharmaceutical Pvt. Ltd. Goa. India.

2.4. Extract preparation

The plant material was dried at 45 °C and then powdered using a polarizer. Ten grams of the material was stirred overnight in 70% methanol (100 ml), and then centrifuged at 10,000 rpm for 10 min at 4 °C. The resultant supernatant was collected and methanol was removed by evaporation. The yield of the extract was found to be 12% [w/w]. For in vivo experiments the extract was administered via intraperitoneal (i.p.) injection at a concentration of 10 mg/kg body weight (b.wt). The non-toxic concentration of 10 mg/kg b.wt was selected based on in vivo toxicity studies as reported in [22].

2.5. Induction of colitis to the experimental animals

Animals were divided into four groups of six mice each (n = 6): Group I served as normal untreated and received vehicle (1% w/v) gum acacia; Group II served as ulcerative colitis control and received vehicle along with acetic acid; Group III mice received *R. apiculata* (10 mg/kg b.wt) and acetic acid; and Group IV mice received standard drug sulfasalazine (100 mg/kg b.wt) and acetic acid [23]. Each vehicle/drug treatment was given daily for seven days via (i.p.) injection. On Day 6 of the regimen, the animals were fasted (post-injection) overnight but had ad libitum access to water. On Day 7, 1 h after the final injection of vehicle/drug, the mice were anesthetized by ether inhalation and a polypropylene tube with a diameter of 2 mm was inserted through the rectum into the colon to a distance of 4 cm. For the mice in Groups II, III and IV, a solution of 2 ml acetic acid (3%, v/v) in 0.9% saline (intra-rectal) was instilled into the lumen of the colon. The mice were then maintained in a supine Trendelenburg position for 30 s to prevent the leakage of the intra-colonic instillate. Group I mice received only saline. After 24 h of single dose administration of acetic acid (Day 8), the animals were anesthetized with ether and blood samples were collected into non-coated tubes for isolation of serum for analysis [23,18]. The mice were then euthanized by cervical dislocation and their colons recovered, washed gently with saline and weighed. The colon samples were cut opened longitudinally for macroscopic scoring, histopathological analysis and biochemical measures (see below sections).

2.6. Assessment of colitis

The severity of colitis in each mouse was evaluated by an independent observer blinded to the treatments. The intact colon samples of each mouse were removed and gently cleaned with physiological saline to remove fecal residue and weighed. Macroscopic inflammation scores were assigned based on clinical features; thereafter the tissue was frozen at −80 °C for later measures of biochemical parameters [24]. The macroscopic scoring pattern for evaluation of disease severity index of colitis is presented in Table 1.

2.7. Biochemical assays

Biochemical assays were performed in the colonic tissue samples of the mice. The blood samples that were collected in non-coated tubes were allowed to clot and serum was isolated. Then the serum was used for measurement of NO, COX-2 and lactate dehydrogenase (LDH) contents. A portion of isolated colonic tissue from each mouse was homogenized in 10% (w/v) Tris–HCl buffer (pH 7.0) for use in measurements of SOD, GSH, LPO, MPO, iNOS and TNF-α.

Table 1
Macroscopic scoring pattern for evaluation of disease severity index of colitis.

Pattern for evaluation of disease activity index	Score
No visible damage	0
Focal hyperemia (water oozes out)	1
Ulcerization without hyperemia or bowel wall thickness	2
Ulcerization with inflammation at one site	3
Ulcerization with inflammation at two sites	4
Major sites of inflammation >1 cm along the organ with redness	5
Major sites of inflammation >2 cm along the organ with redness	6
Major sites of inflammation >3 cm along the organ with redness	7
Major sites of inflammation >4 cm along the organ with redness	8
Major sites of inflammation >5 cm along the organ with redness and bleeding	9
Major sites of inflammation >6 cm along the organ with redness, swelling and bleeding	10