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Original article

Antioxidant mediated antiulcer effect of *Eupatorium triplinerve* Vahl against acetic acid induced ulcerative colitis in mice

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ABSTRACT

Purpose: Ulcerative colitis (UC) is associated with tainted neutrophil infiltration, deregulated pro-inflammatory mediators, characterized by severe oxidative stress of the intestine. In the present study, an effort was made to evaluate the effect of methanolic fractions of *Eupatorium triplinerve* (*E. triplinerve*) (ET) on acetic acid induced ulcerative colitis in male adult mice.

Methods: Colitis in mice was induced with 3.0% acetic acid (v/v) in saline via rectal route. Pre-intervention with *E. triplinerve* extract (100 mg and 200 mg kg^{-1} body weight, oral) and reference drug ranitidine (50 mg kg^{-1} body weight used as reference, oral) 4 days before induction of colitis and was extended up to 8 days.

Results: The phase of inflammation before *E. triplinerve* extract pre-treatment significantly showed attenuated macroscopic damage, argyrophilic nuclear organization regions (AgNORs) count and histological changes. Similarly, extract also effectively detracts the activity of both Myeloperoxidase (MPO) and Malondialdehyde (MDA) levels by enhancing the cellular antioxidant enzyme levels (Glutathione-s-transferase [GST], Glutathione peroxidise [GPx] and Catalase [CAT]) at the site of ulceration.

Conclusions: The *E. triplinerve* based therapy resolved that some constituents in extract have an antiulcer effect against UC at colon specific area through its inviolable radical scavenging activity.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic revenant immunological ailment of gastrointestinal tract associated with austere ulceration, relapse bleeding and diarrhoea [1,2]. Crohns disease and ulcerative colitis (UC) are the two sorts of IBD which eventually lead to mucosal damage and disruption of epithelial cells in large intestine. Hence, the etiology of UC remains vague, considered being multi-factorial such as diet, environment and genetic factors [3,4]. Subject with prolonged (or) chronic ulceration or inflammation at colon site may have the chance of incurring colorectal cancer [5]. Quite a few reports avowed that an overexpression of deregulated immunological mediators (Reactive oxygen species [ROS], neutrophils, macrophages, mast cells and lymphocytes) acts as an intense contribution in pathological events of UC [6–8]. Under physiological conditions, an equilibrate state was maintained between oxidants and antioxidants, but it was impaired under morbid events [9]. Oxidative stress mediated by onset of free radicals through neutrophil activation renders the antioxidant system to be inadequate in epithelial cell, which can lead to cell damage and lipid peroxidation [10,11]. Due to complex pathogenesis of UC, the therapy to potentially reduce the severity or to prevent the incident rate was not yet defined. The present available therapies for UC involve treatment with anti-inflammatory agents (glucocorticosteroids and 5-aminosalicylic acid), immunomodulatory agents (azathioprine, mercaptopurines and cyclosporine), but they will provoke adverse side effects [12]. Hence, plant-based remedies will navigate to find out the unknown bioactive compounds to combat several disorders [13].

Eupatorium triplinerve Vahl, an ornamental erect perennial herb with aromatic leaves belongs to the family of Asteraceae. The whole plant is used as folk medicine for the treatment of various diseases (colic, stomach pain, edema, depurative and snake bite). The biochemical constituent of *E. triplinerve* mainly consists of coumarin family substituted by methyl and hydroxyl side chains. Similarly, it is also reported to possess anticancer [14], antifungal, antibacterial [15], hepatoprotective [16], and neuroprotective activities [17]. Several rodent models have been established to find the effective drug that mimics the inflammatory mediator in ulcerative colitis [18,19]. However, the protective action against ulcerative condition was not yet defined so far in *E. triplinerve*. In this study, we

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investigated the antiulcer effect of *E. triplinerve* extract on acetic acid-induced colitis model in mice.

2. Materials and methods

2.1. Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 5,5 dithiobis (2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), 1-chloro-2, 4-dinitrobenzene (CDNB), *O*-dianisidine dihydrochloride, sodium azide, trichloroacetic acid (TCA), hydrogen peroxide (H_2O_2), ethylene diamine tetra acetic acid (EDTA), hemotoxylin, eosin and ranitidine were purchased from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals are of analytical grade.

2.2. Preparation of plant extract

Healthy plants of *E. triplinerve* were collected from State Forestry Research Institute, Chennai, TamilNadu and deposited in the herbarium of Department of Biotechnology, Periyar University, Salem (PU/BT/*Eupatorium triplinerve* Vahl/voucher specimen No.: 012/2010). The plant leaf was washed thoroughly with running tap water and shade dried for 2–3 weeks to get consistent weight. The dried plants were grounded to coarse powder and extracted using methanol (100%, w/v) in soxhlet apparatus for 6 h. The extract was concentrated by using rotary evaporator at 40–50 °C under reduced pressure.

2.3. Methods

2.3.1. Preliminary plant antioxidant screening

2.3.1.1. DPPH radical scavenging assay. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical was assayed by the method of Liyana-Pathiranan and Shahidi [20]. The reaction mixture containing 0.135 mM DPPH in methanol followed by 1.0 mL of plant extract (20–100 μg/mL) in methanol. The mixture was left in dark at room temperature for 30 min. The absorbance was measured at 517 nm using quercetin as standard in UV-spectrophotometer (Systronics-2203, India). The DPPH percentage inhibition was calculated by the following equation: DPPH radical (%) = $[(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$.

2.3.1.2. Ferric Reducing Antioxidant Property Assay (FRAP). FRAP was assayed as described by the method of Oyaizu Yen and Duh [21]. The reaction mixture contains different concentrations of plant extracts (20–100 μg/mL) in 0.2 M phosphate buffer (pH 6.6) and 1.0% ferrocyanate. The temperature of the mixture was raised to 50 °C for 20 min and 2.5 mL of 10% TCA was added after centrifugation at 3000 g for 10 min. To the supernatant, equal volume of distilled water and 1.0% ferric cyanide was added. The absorbance was measured at 700 nm using quercetin as standard. The intensity of the absorbance determines the antioxidant activity of plant extract.

2.3.2. Experimental design

Adult male Swiss albino mice (6–8 weeks old, weight varying from 22–26 g) were housed in polycarbonate cages at Central Animal Facility, Periyar University, Salem. They were kept in room with a 12-hour day-night cycle, temperature of 25 ± 3 °C and humidity of 45–65%. They were fed with commercial mice feed and water *ad libitum*. The experiment was conducted after obtaining institutional animal ethical committee clearance (1085/ac/07/PU-IAEC/2012/11).

2.3.3. Induction and treatment schedule

Animals were randomly divided into five groups ($n = 6$). Group I served as normal animals which received oral administration of saline (0.9% w/v) for 7 days. Group II animals on 4th day received intrarectal induction of 0.2 mL of 3.0% acetic acid (v/v) and allowed it for 30 seconds then 0.5 mL of saline (0.9%) was injected in order to withdraw the prior solution from the colon. Group III animals were pre-treated with standard ranitidine (50 mg/kg., p.o.) for 7 days and 0.2 mL of 3.0% acetic acid induced intrarectally on 4th day. Groups IV and V were pre-treated with *E. triplinerve* extract (100 mg and 200 mg/kg., p.o., based on acute toxicity study [16]) for 7 days and acetic acid was induced as per group III. Treatment in all groups was continued till 8th day. The animals were euthanized on 8th day; colon of each mouse was removed and weighed. The tissues of all groups of animal were used for analyzing damage scores, histological patterns and biochemical parameters.

2.3.4. Determination of E. triplinerve on colonic damage

The severity of ulcer area on colon tissues was assessed according to the scaling grade of Morris et al. [22]. Absence of macroscopic damage: score 0, localized hyperaemia with devoid of ulcers: score 1, elongated ulcers without inflammation: score 2, ulcers with inflammation at one site: score 3, more than one site of inflammation with ulceration: score 4, extension of more than 1.0 cm length of ulcers with erosions in colonic region: score 5, respectively.

2.4. Biochemical analysis

2.4.1. Assessment of myeloperoxidase (MPO) levels

Myeloperoxidase, an index of neutrophil infiltration, was assayed according to the modified method of Goldblum et al. [23]. The reaction mixtures contained 0.1 mL of tissue homogenate, 0.59 M *O*-dianisidine dihydrochloride (w/v) in 50 mM phosphate buffer (pH 6.0) and 0.0005% H_2O_2 (v/v). The change in absorbance was read at 460 nm for one minute by using spectrophotometer. One unit of MPO activity is defined as the change in absorbance per min by 1.0 at room temperature. It was calculated by using the formula MPO activity (U/g) = X/weight of tissue taken, where X = 10 × change in absorbance/min/volume of supernatant taken.

2.4.2. Evaluation of lipid peroxidation and antioxidant enzyme contents

The level of lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels by the method of Ohkawa et al. [24]. The amount of MDA was expressed as nmol/g of wet colonic tissues by using molar extinction $1.56 \times 10^5\ M^1\ cm^{-1}$. The levels of glutathione peroxidase (GPx-E.C: 1.11.1.9) were measured according to Rotruck et al. [25]. The enzyme activity was measured at 420 nm and the values are expressed as *n* moles of GSH oxidized/min/mL of enzyme. Glutathione-S-transferase (GST-E.C: 2.5.1.18) was performed as described by Habig et al. [26]. The specific activity was expressed as micromole of CDNB-GSH conjugates formed/min/mg of protein using 9.6 as molar extinction co-efficient. The protein content was measured by the method of Lowry et al. [27]. Catalase (CAT-E.C: 1.11.1.6) was quantified by the method of Luck [28] and the enzyme activity was calculated and expressed in μmoles of H_2O_2 consumed/units/mg of protein.

2.5. Histopathological studies

A portion of the colon tissue samples was fixed in 10% neutral buffered formalin solution. The fixed tissues were dehydrated in graded ethanol, hot infiltrated and embedded in paraffin wax. The embedded blocks were sectioned in microtome at 6.0 μm size. The sectioned tissues were placed on slides followed by deparaffinization, rehydration and then stained with hemotoxylin and eosin. The