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Garlic oil inhibits dextran sodium sulfate-induced ulcerative colitis in rats

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ABSTRACT

Aims: Garlic oil (GO) is used for centuries in folk medicine as a therapy for many diseases including inflammatory disorders. Recently, it has exhibited potent anti-oxidant, anti-inflammatory and immunomodulatory effects. Consequently, we evaluated the possible protective effect of GO in a rat model of colitis, induced by dextran sulfate sodium (DSS).
Main methods: Colitis induced by allowing rats a free access to drinking water containing 5% DSS for 7 days, from day 1 to day 7. GO was administered orally in doses of 25, 50 and 100 mg/kg/day. Mesalazine used as a standard medication in a dose of 15 mg/kg/day. All animals fasted for 2 h, 1 h before and 1 h after giving the treatment, which introduced daily for 7 days, from day 1 to day 7, at 10:00 to 11:00 A.M. Animal body, and colonic weights, colonic myeloperoxidase (MPO), and superoxide dismutase (SOD) activities, colonic reduced-glutathione (GSH), malondialdehyde (MDA), tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-10 levels, macroscopic and microscopic changes of colonic tissues were evaluated.
Key findings: GO treatment significantly suppressed the elevated colonic weight, MPO activity, MDA, TNF-α and IL-1β levels. However, it potentiated the decrease body weight, colonic SOD activity, GSH and IL-10 levels. Moreover, it ameliorated the marked macroscopic and microscopic changes of colonic mucosa in a dose dependent manner.
Significance: Garlic oil inhibits DSS-induced colitis in rats may be through its anti-oxidant, anti-inflammatory and immunomodulatory properties. Therefore, GO could be a promising protective agent recommended for UC patients.

1. Introduction

Ulcerative colitis (UC) is a major type of inflammatory bowel disease characterized by chronic, relapsing intestinal inflammation with extensive damage of colonic mucosa. It is presented by a variety of clinical manifestations, including attacks of abdominal cramps, pain, bloody diarrhea, bleed per rectum, weight loss, fever and easy fatigability, which may begin gradually or start totally all at once [1,2].

Although, the underlying pathogenesis of UC is still unclear, the role of oxidative stress and immunological dysfunction evidenced to be crucial in its development. Disruption of the anti-oxidant system of the colonic mucosa is associated with oxidative damage to mucosal lipids and proteins, resulting into mucosal ulceration. Meanwhile, the damaged mucosal barrier allowed exposure of the mucosal immune system to the luminal antigens, with subsequent alteration and dysregulation of the immune response, and further progression of the disease [3,4].

To date, the standard treatment modalities for UC, including anti-inflammatory and immunosuppressive drugs, mainly targeting down-regulation of immune response as well as inflammatory cascades, which resulted in recovery and sometimes mucosal healing, however, there is no actual cure. Unfortunately, these medications associated with a lot of unacceptable side effects and complications. Therefore, the search for new molecules, preferably of natural origin, with high efficacy and safety is urgent [5].

Garlic (*Allium sativum*) is a bulbous plant belonged to the *Liliaceae* plant family. For centuries, fresh, and aged garlic, its powder and oil used as a nutritional, medicinal and spiritual remedy. In folk medicine, it used for treatment of many conditions including, joint, and tooth pain, cough, constipation, gynecologic disorder, infectious diseases, parasitic infestation, animal, and insect bites [6]. Evidence from recent experimental and human trials showed that garlic and its oil have a potent anti-oxidant, anti-inflammatory, immunomodulatory, hepatoprotective, anti-atherosclerotic, antimicrobial and antineoplastic activities [6–8]. These activities assumed to be caused by the presence of hundreds of active ingredients such as diallyl, dimethyl and allylmethyl, mono to hexa-sulfides, and alliin [6,9,10].

Therefore, the present study aimed to investigate the anti-oxidant, anti-inflammatory and immunomodulatory activities of garlic oil in a rat model of experimental colitis induced by DSS, regarding for colonic oxidative stress state, cytokine levels, myeloperoxidase activity, macroscopic and microscopic changes of colonic mucosa.

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2. Materials and methods

2.1. Chemicals and reagents

The following chemicals and reagents were commercially purchased and used: dextran sulfate sodium, garlic oil, sulfuric acid, Ellman's reagent, phosphotungstic acid, methanol & o-dianisidine dihydrochloride (Sigma, St. Louis, MO, USA); sodium pentobarbitone (Abbott Lab., Chicago, IL, USA); mesalazine (Pharaonia Pharmaceuticals Co., Burg Al-Arab, Alex., Egypt); phosphate buffer, phosphate-buffered saline, hydrogen peroxide & Tris–HCl buffer (El Gomhuria Co., Tanta, El-Gharbeya, Egypt); thiobarbituric acid (Riedel-de Haën, AG., Germany); N-butanol & tetraethoxypropane (VWR International Ltd., Ballycoolin, Dublin, Ireland); TLR-2 polyclonal antibody (Abcam, Kendall Sq., Suite, Cambridge, MA, USA); biotinylated goat anti-polyvalent antisera (Dako North America, Inc., CA, USA).

2.2. Animals

Twelve-week-old male Wistar rats (150–200 g) obtained from Tanta University animal house used in the present study. All animals housed in plastic cages at a temperature of 22 ± 1 °C, with relative humidity of 60 ± 5%, expose to a 12-h light/dark cycle and permitted to acclimatize for a week before initiation of the experiment. The rats fed a standard laboratory diet and water *ad libitum*. All experiments carried out following the guideline for the care and use of experimental animals in Tanta University, Faculty of Medicine with an approval of Animal Experiment Committee of the Faculty.

2.3. The experimental design

The rats divided into 6 groups of 10 rats each. Group I (control) was normal rats served as negative control. Group II (DSS) was DSS-induced UC group, by allowing rats a free access to drinking water containing 5% DSS for 7 days, from day 1 to day 7 [11]. Group III (MS) UC group treated orally for 7 days with 15 mg/kg/day mesalazine (used as a standard drug for UC treatment) [12]. Group IV (GOL): UC group treated orally for 7 days with 25 mg/kg/day garlic oil (GO). Group V (GOM): UC group treated orally for 7 days with 50 mg/kg/day GO. Group VI (GOH): UC group treated orally for 7 days with 100 mg/kg/day GO [13]. All animals fasted for 2 h, 1 h before and 1 h after giving the treatment, which introduced daily, from day 1 to day 7, at 10:00 to 11:00 A.M.

2.4. Animals' body weights assessment

Changes in each rat body weight measured, and noted down daily, from day 1 to day 7, using standard rat-weighing machine (Radwag Balances & Scales, PS 510.R1, Bracka St., Poland).

2.5. Preparation of colonic samples

Twenty-four hours after the last treatment, rats anesthetized by i.p. injection of pentobarbital (100 mg/kg). Then, colonic samples collected and prepared according to Sakthivel and Guruvayoorappan [14]. In brief, a 10 cm segment of the distal colon resected, 3 cm proximal to the anus, the lumen rinsed with ice-cold phosphate-buffered saline (PBS) for clearing of colonic content, and divided into 2 segments of 5 cm each. The proximal one used for macroscopic and microscopic examination, and the distal one weighed wet, and used for preparation of colonic homogenate. The colonic sample homogenized in 1 ml of 0.1 mmol Tris–HCl buffer (pH = 7). The homogenate centrifuged at 5000 rpm for 10 min and the supernatant was stored at −30 °C, and used for the measurement of colonic oxidative stress markers, cytokine levels and myeloperoxidase activity. Results expressed as mg/cm of wet weight colonic tissue [14].

2.6. Determination of colonic myeloperoxidase activity (MPO)

The colonic MPO activity evaluated by the method described by Bradley et al. (1982). Briefly, 0.1 ml of the supernatant mixed with 2.9 ml 50 mmol of phosphate buffer (pH = 6) containing 0.167 mg/ml o-dianisidine dihydrochloride and 0.0005% (wt/vol) hydrogen peroxide (H_2O_2). The change in absorbance recorded at 460 nm using Biosystems semiautomatic analyzer (BTS-350, Barcelona, Spain). One unit of MPO defined as that degrading 1 μmol of H_2O_2 per min at 25 °C. Myeloperoxidase activity expressed as units/mg weight of colonic tissue [15].

2.7. Determination of colonic oxidative stress markers

Superoxide dismutase activity (SOD), reduced glutathione (GSH) and malondialdehyde (MDA) levels determined to check the colonic oxidative stress state. SOD measured by using a colorimetric assay kit (Northwest Life Science Specialties, LLC, Vancouver, WA, USA) with minimal detection limit 5 U/ml. Optical densities assessed and analyzed using an automatic plate reader (Stat Fax-2100, Fisher Bioblock Scientific, BP., Illkirch Cedex, France). SOD activity expressed as units/mg weight of colonic tissue.

GSH assayed according to the method of Jollow et al. [16]. In brief, 0.3 ml of the supernatant was deproteinized by adding an equal amount of 4% sulfuric acid (H_2SO_4), centrifuged at 5000 rpm for 10 min, then 0.5 ml of the resultant supernatant added to 4.5 ml of Ellman's reagent. Its absorbance was determined at 412 nm using Biosystems semiautomatic analyzer (BTS-350, Barcelona, Spain). The level of GSH expressed as pg/mg weight of colonic tissue [16].

MDA content evaluated by the method of Yagi [17]. Briefly, 50 μl of the supernatant added to 1 ml of PBS and shook gently. 0.5 ml of the diluted supernatant added to 4 ml of 40 mmol/l H_2SO_4, then 0.5 ml of 10% wt/vol phosphotungstic acid added and mixed. The mixture centrifuged at 5000 rpm for 10 min, the supernatant discarded, and the sediment mixed with 2 ml of 40 mmol/l H_2SO_4 and 0.3 ml of 10% wt/vol phosphotungstic acid. The mixture centrifuged again at 5000 rpm for 10 min. The sediment suspended in 4 ml of distilled water and 1 ml of 0.33% wt/vol thiobarbituric acid reagent added. The mixture heated for 60 min at 95 °C in a water bath, then, 5 ml of n-butanol added and the mixture vigorously shook. The butanol layer took after centrifugation at 5000 rpm for 10 min and its absorbance determined at 532 nm against blank using Biosystems semiautomatic analyzer (BTS-350, Barcelona, Spain). 0.5 nmol of tetraethoxypropane used as a standard solution. The MDA expressed as mmol/mg weight of colonic tissue [17].

2.8. Determination of colonic cytokine levels

The amount of cytokines in the homogenate's supernatant measured using enzyme-linked immunosorbent assay (ELISA) kits. Kit for tumor necrosis factor alpha (TNF-α) obtained from Assaypro, LLC., Charles, MO, USA, with minimal detection limit 15 pg/ml and kits for interleukin-1 beta (IL-1β) and IL-10 obtained from BioVendor-Laboratorní medicína a.s., Karasek, Czech Republic, with minimal detection limit 4 pg/ml and 1.5 pg/ml respectively. Optical densities estimated and analyzed using an automatic plate reader (Stat Fax 2100, Fisher Bioblock Scientific, BP., Illkirch Cedex, France). Measured TNF-α, IL-1β and IL-10 expressed as pg/mg weight of colonic tissue.

2.9. Histological and immunohistochemical study of colonic mucosa

The colonic tissue segment used for histological and immunohistochemical study prepared according to Eltoum et al. [18]. Briefly, specimens immediately fixed in 10% formalin buffered saline, dehydrated, cleared and impregnated into paraffin, then sections of 5 μm prepared [18].