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Therapeutic efficacy of osthole against dinitrobenzene sulphonic acid induced-colitis in rats

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ABSTRACT

Several mediators were associated with the pathogenesis of inflammatory bowel disease such as oxidative stress through the production of reactive oxygen metabolites, neutrophils infiltration and release of pro-inflammatory cytokines. This study was designed to investigate the therapeutic efficacy of osthole against dinitrobenzene sulfonic acid (DNBS) induced-colitis in rats through its anti-oxidant and anti-inflammatory properties. Colitis was induced in rats by single intracolonic instillation of (250 µl DNBS-25 mg/rat). Then 4 days later, rats were received oral administration of either (osthole 50 mg/kg), (sulfasalazine 500 mg/kg) or both in combination for 7 consecutive days. Body weight, some hematological parameters, colonic malondialdehyde (MDA) and myeloperoxidase activity (MPO), antioxidant parameters, colon injury and mucosa architectures were assessed. T helper (Th1)-related cytokines [Tumor necrosis factor alpha (TNF-α) and interferon-gamma (INF-γ)], Th2-relarted cytokines (interleukin-4 [IL-4 and IL-10], and Th-17 related cytokines [IL-17] were determined by ELISA. Osthole significantly improved the loss in body weight. That was accompanied with a remarkable amelioration of the disruption of the colonic architecture as well as a significant improvement in the antioxidant defense system. A reduction in MPO and MDA was observed in flamed colon. Treatment with either osthole or combination therapy showed suppressive activities on pro-inflammatory Th2-related cytokines and upregulation of anti-inflammatory Th2-related cytokines The results of this study suggest that osthole exert beneficial therapeutic effect in experimental colitis and improved the efficacy of the synthetized drugs such as sulfasalazine. Therefore, osthole may have a valuable sound in the treatment of inflammatory bowel disease.

1. Introduction

Inflammatory bowel disease (IBD) is considered as the most deteriorating chronic idiopathic intestinal inflammatory disease of the gastrointestinal tract affecting the person's life quality. The etiology of IBD is unclear but involves multifactorial interactions of genetic susceptibility, dysregulated immune responses and environmental factors [1]. Where, the dysfunctional barrier of the intestinal epithelium, immune response to the gut microbiota are involved [2]. IBD is categorized mainly into ulcerative colitis and Crohn's disease, in which their clinical symptoms varied between abdominal pains, diarrhea, mucous bloody, recurrent attacks and relapse. [3].

Prolonged disturbance of the colonic mucosa during colitis caused loss of normal absorptive function that lead to severe diarrhea, which is the most troublesome symptom of the disease. The most hallmark of colitis inflammation is continual bleeding from the ulcerated surface of the colon due to chronic blood vessels erosion that subsequently leads to iron deficiency anemia [4].

The pathogenesis of intestinal inflammation has been related to infiltration of neutrophils, macrophages, lymphocytes, mast cells and oxidative stress through the production of reactive oxygen metabolites (ROS) eventually giving rise to mucosal disruption and ulceration [2,5].

Overproduction of ROS such as hydroxyl radicals, H_2O_2 and O_2^- participated with a vital role in mucosal damage in colitis through lipid peroxidation and cellular antioxidant capacity degradation. Endogenous antioxidant defenses system could protect the mucosal tissues against ROS production by enzymatic (SOD, CAT and GPx) and non-enzymatic antioxidants such as glutathione (GSH). It was reported that the effect of ROS and secretion of inflammatory mediator caused a loss of epithelial barrier integrity and exaggerates the mucosal inflammatory response [6]. On the other hand, neutrophil infiltration is an indicator of oxidative stress that could be assessed by myeloperoxidase (MPO) activity, is one of the most abundant enzymes in azurophilic granule of neutrophils and monocytes, determination [7]. In colitis, the activity of MPO in colonic tissue is a marker of neutrophil infiltration that damage the mucosal macromolecules and increase the

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mucosal disruption and ulceration [8].

The phagocytic cells such as dendritic cells, macrophages, and intestinal epithelial cells, as important cells of innate immunity due to their activation lead to immediate consequences of inflammation. T helper (Th) cells play cial crucial role in the adaptive immune system. Upon differentiation of naive T cells into various effector T cells, such as Th1, Th2, or Th17 cells, a vast array of cytokines related to each subtype of helper T cells have been secreted [9]. The dysregulated T cell responses shared into the secretion of inflammatory cytokines and chemokine characterized the chronic inflammation in IBD. The balance between pro-inflammatory and anti-inflammatory cytokines has been disturbed in the colonic mucosa of IBD patients, in which the level of pro-inflammatory cytokines such as tumor necrosis factor (TNF-α) are increased [10].

Currently, anti-inflammatory agents such as sulfasalazine and mesalamine, antibiotics, 5-aminosalicylic acid (5-ASA), corticosteroids, immunomodulatory, and biological agents are considered to be the approved drugs for treatment of IBD. However, these medical treatments are associated with side effects that add to the disease-related complications [11], including increased exposure to infection and resistance to the medications [12]. Hence, there is a need for additional approaches to cure or minimize the side effects accompanied the available treatments.

Osthole, (7-methoxy-8-isopentenoxy-coumarin), is documented as an active constitute of natural coumarin derivatives, and was first extracted from the mature fruit of Cnidium monnieri. In addition, it is applied in clinical practice of the Traditional Chinese Medicine due to its safety, reliability, and low toxicity with no severe effects on the gastrointestinal tract. Modern researches have suggested that osthole exhibits antioxidant, anticancer, anti- inflammatory, and immunomodulatory activities [13]. So, it has been used in various types of diseases such as cardiovascular, cancer, infectious, osteoporosis and inflammatory diseases [14]. It was mentioned that, osthole displays anti-inflammatory effect through its selective inhibitory effect on both 5-lipoxygenase (5-LO) and cyclooxygenase-(COX-1) enzymes that are critical during the phase of inflammation [15]. In addition, osthole has a prospective antioxidant mechanism due to its effect on scavenging ROS and inhibition of lipid peroxidation [16]. These studies suggested that osthole could suppresses the production of prostaglandin (PG), nitric oxide (NO), and malondialdehyde (MDA), as well as its role to decrease the releasing of ROS [17]. Also, Osthole suppressed the immune response stimulated by macrophages through the inhibition of interleukin-6 (IL-6), TNF-?? and downregulation of tumor growth factor-? ? (TGF-??) expression [18].

There is no available data regarding the therapeutic effect of Osthole on induced-colitis. Therefore, the present study was conducted to investigate the therapeutic effect of osthole on DNBS-induced colitis in rat model. Hence, it is possible to identify the beneficial mechanism of osthole alone or in combination with sulfasalazine on the intestinal inflammation as well as oxidative stress markers and cytokine production pattern.

2. Material and methods

2.1. Animals

Healthy adult male *Sprague Dawley* rats (250–300 g) were obtained from the National Research Centre (Dokki, Egypt). The rats were housed randomly in groups of 7 in polypropylene cages. They were maintained at (25 ± 3) °C and 12:12 h dark/light cycle. The animals were acclimatized for one week. The animals had free access to standard pellet chow throughout the experimental protocol, while, they were kept fasting overnight before induction of colitis but had access to water. The experimental protocol was approved by Institutional Animal Care and Use Committee (IACUC), Faculty of Science, Cairo University (Egypt) (CUFS/S/PHY/40/15). All the experimental procedures were done following the international guidelines, Guide for Care and Use of Laboratory Animals.

2.2. Chemicals

2, 4, 6-Dinitobenzenesulfonic acid hydrate 98% (DNBS) was purchased from Alfa Aesar®, A ajaohnson Matthey Company, (Germany). Sulfasalazine (Sulfa.) was purchased from Pfizer Co. for Pharm. & Medical (Cairo, Egypt). Osthol (Osth.) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All kits were purchased from a Bio-diagnostic company for diagnostic and research reagents (Dokki, Giza, Egypt). All other chemicals and solvents were of analytical grade and were purchased from local firms.

2.3. Induction of experimental colitis

Colitis was induced by dinitrobenzene sulfonic acid (DNBS) treatment as described previously by [19]. Fasting started for early 12 h before colitis induction with access to water *ad labitum.* Rats were slightly anaesthetized with intramuscular injection of (50 mg/kg) ketamine, combined with (10 mg/kg) xylazine. During anesthesia, animals were monitored for blink, swallowing reflexes and regular respiration rate. Then one ml syringe attached to polyethylene catheter was inserted gently into the colon via the anus until approximately, the splenic flexure (8 cm) from the anus. The DNBS (25 mg/ rat) was freshly prepared and dissolved in 100 µl of 50% ethanol and deposited in the colon [19]. Thereafter, the animals were kept for few minutes in a Trendelenburg position avoiding reflux actions. Rats administered 8% sucrose during the first week after DNBS inoculation to avoid dehydration.

2.4. Experimental groups

Animals were randomly divided into five groups (7 rats/group) as follows, **control group** received single intrarectal instillation of saline and after 4 days rats were treated with carboxy methyl cellulose (CMC) as a vehicle for osthole and sulfasalazine orally for 7 days. **DNBS-colitis group** received single intrarectal instillation of DNBS (25mg/rat) [19], then, after 4 days rats were subjected to CMC orally for 7 days. **(DNBS + Osth) group** received single intrarectal instillation of (DNBS, 25mg/rat) then, after 4 days rats were orally administered osthole (Osth, 50 mg/kg body weight) suspended in CMC for 7 days [20]. **(DNBS + Sulfa) group** received single intrarectal instillation of (DNBS) then, after 4 days rats were orally administered with sulfasalazine (Sulfa., 500 mg/kg body weight) suspended in CMC for 7 days [21]. **(DNBS + Osth + Sulfa) group** received single intrarectal instillation of (DNBS, 25mg/rat) then, after 4 days rats were treated with osthole (50 mg/kg body weight) and sulfasalazine (500mg/kg body weight) simultaneously orally for 7 days with the same previous concentrations. On the day 12, the end of the experimental period, the animals were euthanized under anesthesia. The abdomen was dissected. The colons were removed and processed for biochemical and histological examination, the collected blood samples were centrifuged at (1008 g) for 20 min. to obtain serum, then stored at −80 °C.

2.5. Body weight and disease activity index

Body weight (g) for each rat, was measured daily, clinical features including weight loss, stool consistency, and bleeding from rectum were monitored daily during the whole experiment. The progress of colitis in all the experimental groups was quantitatively measured by the disease activity index as described by [22] with minor modifications. Each score was determined as follows: stool consistency (0: normal, 1 and 2: loose stool, 3 and 4: diarrhea), rectal bleeding (0: normal, 1: Stripes of blood, 2: Obvious blood, 3 and 4: Mostly blood).