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Oregano essential oil decreased susceptibility to oxidative stress-induced dysfunction of intestinal epithelial barrier in rats

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ABSTRACT

The protective effects of oregano essential oils (OEO) against oxidative stress in intestine of rats were investigated. The results showed that rats orally treated with 5 or 20 mg/kg BW with OEO abolished diquat-induced oxidative stress in jejunum, thereby maintaining jejunal architecture. The protective effect of OEO might cause modification of selected intestinal microbiota, inhibition of inflammatory cytokine expression and subsequent increased occludin expression in jejunum by enhancement of antioxidative capacity. These results revealed that OEO exerted a protective effect against diquat-induced oxidative injury in intestine of rats.

1. Introduction

In addition to nutrient digestion and absorption, gastrointestinal (GI) tract has been demonstrated to perform important immunologic, metabolic, and barrier functions. Intestinal barrier is physically composed of epithelial cells linked through tight junctions (TJs), regulated physiological and enzymatic barriers, and immunological barriers. Disruption of TJs and loss of barrier function are associated with a number of diseases, such as Crohn's disease, intestinal mucositis, celiac disease, fatty acid liver disease, food allergies and acute pancreatitis (DeMeo, Mutlu, Keshavarzian, & Tobin, 2002).

Accumulating evidence indicates that an important pathophysiological factor common to the previously mentioned states is oxidative stress, which increases intestinal permeability via disrupting of TJs and lipids of cell membranes (Niki, 1987). In most cases, oxidative stress is characterized by increased generation of reactive oxygen species (ROS) which overwhelm the antioxidant capacity, subsequently resulting in damage to

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Abbreviations: GSH-Px, glutathione peroxidase; IL-1β, interleukin 1β; IL-6, interleukin 6; OEO, oregano essential oils; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TNF-α, tumor necrosis factor-α; TJs, tight junctions; ZO, zonula occludens

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cellular macromolecules such as lipids, proteins, and DNA. Indeed, oxidative stress is frequently associated with elevated proinflammatory cytokines, such as IL-1β, TNF-α and IL-6 (Padgett, Broniowska, Hansen, Corbett, & Tse, 2013; Rada, Gardina, Myers, & Leto, 2011), which can undermine the integrity of the intestinal barrier largely by inducing the disappearance of zonula occludens (ZO-1) or occluding (Al-Sadi, Boivin, & Ma, 2009; Shigeshiro, Tanabe, & Suzuki, 2013; Suzuki, Yoshinaga, & Tanabe, 2011). Within the gastrointestinal tract, several kinds of commensal bacteria, especially *Enterococcus faecalis* and *Escherichia coli*, could produce extracellular ROS (Huycke & Moore, 2002), which may disrupt TJs of cell membranes.

Oregano (Origanum vulgare L.) is an aromatic plant with a wide distribution throughout the Mediterranean area and Asia (Vokou, Kokkini, & Bessiere, 1993). The oregano essential oils (OEO) comprises more than 20 ingredients, most of which are phenolic antioxidants (Vekiari, Oreopoulou, Tzia, & Thomopoulos, 1993) and have antimicrobial (Lambert, Skandamis, Coote, & Nychas, 2001; Sivropoulou et al., 1996) properties and potent *in vivo* antioxidant properties in rats experimentally challenged by carbon tetrachloride-induced oxidative stress (Botsoglou et al., 2008) and broiler chickens after iron induced oxidative stress (Giannenas, Florou-Paneri, Botsoglou, Christaki, & Spais, 2005; Papageorgiou et al., 2003). In addition, it has been shown that essential oil dietary supplementation containing carvacrol exerts a positive effect on intestinal microbiota with a concomitant enhancement in growth performance of broilers (Tiihonen et al., 2010).

In the current study, we investigated whether oral treatment of OEO reduced diquat-induced intestinal oxidative stress and dysfunction of intestinal epithelial barrier in rats. We evaluated the redox status by measuring the accumulation of ROS and thiobarbituric acid reactive substances (TBARS) and activation of antioxidant in jejunum. To evaluate the effect of OEO on ROS-producing involved microbiota, we determined populations of *Lactobacillus, Enterococcus faecalis* and *Escherichia coli* in jejunum. We also determined the morphology of jejunum, mRNA and protein expression of ZO-1 and occluding, as well as mRNA expression of *IL-1β*, *TNF-α* and *IL-6* in jejunum.

2. Materials and methods

2.1. Analysis of OEO composition

OEO was obtained from National University of Salta (Salta Province, Argentina). The composition of OEO were analyzed in the laboratory using Hewlett-Packard 6890 gas chromatograph equipped with a cross-linked 5% PH ME siloxane Hewlett-Packard-5MS capillary column (25 m × 0.25 mm ID, 0.25 μm film thickness), coupled to a Hewlett-Packard 5972A mass spectrometer (Hewlett Packard Ltd., Bracknell, UK). The essential oils were diluted with petroleum ether and were injected to this gas chromatography–mass spectrometry (GC-MS) system. The GC operating conditions were as follows: helium as carrier gas with a flow rate 2.0 ml/min; column temperature programming from 60 to 275 °C at 4 °C/min; injector and detector temperatures, 275 °C. The MS operating parameters were as follows: ionization potential, 70 ev; resolution, 1000; ion source temperature, 250 °C. Identification of components was based on GC retention indices and the fragmentation patterns of the mass spectra with those of authentic samples, as well as the NIST 98 and HPCH 2205 GC–MS libraries. Relative percentage amounts were obtained directly from GC peak areas. The absolute concentration of carvacrol was determined by gas chromatography using external standard method. The GC operating conditions were the same as above. The absolute concentrations of other ingredients were calculated based on the relative percentage amounts.

2.2. Animals

Males Wistar rats, obtained from Wuhan Administration Office of Laboratory Animal (Wuhan, Hubei, China) were used for the current study. All experimental protocols and housing arrangements were approved (number: SCXK20080004) by the China Department of Agricultural guidelines and the experimental protocol was approved by the Huazhong Agricultural University Animal Care and Use Committee. On arrival, rats were caged in a temperature (20 to 23 °C)-controlled colony room on a 12:12-h light:dark schedule. All animals were provided standardized pelleted feed and drinking water *ad libitum*.

2.3. Experimental groups

After a week of acclimatization, 50 rats were assigned to 5 treatments (n = 10/group) randomly. Rats in lower concentration of OEO treatment (LO) and higher concentration of OEO treatment (HO) were orally treated with OEO at 5 and 20 mg/kg bw, respectively. Rats in vitamin E treatment (VE) were orally treated with OEO at 20 mg/kg bw. Rats in the control treatment (CT) and negative control treatment (NC) were orally treated with equal volume of saline for 14 days. At day 15, all treatments, except CT, received 0.1 mmol/kg bw of diquat i.p. dissolved in saline. CT group received an equal dose of saline i.p.

2.4. Plasma collection and tissue preparation

All rats were anesthetized 6 h after the injection, blood was drawn from the posterior venaorbitalis and centrifuged at 3000 *g* for 10 min at 4 °C immediately to obtain plasma. All plasma samples were stored at –20 °C until analysis. The whole jejunum was harvested and then the digesta was collected and stored at –80 °C until analysis. The jejunum segment (15 cm proximal to the ileum) was removed and washed with ice-cold saline to remove its contents. Two consecutive pieces (5 cm long each) were then dissected. Mucosa from one piece of the tissue was scraped for the subsequent determination of proteins and mRNA expression and were immediately frozen in liquid nitrogen and stored at –80 °C until analysis. The other piece of the tissue (2 cm) was dissected, wrapped in surgical gauze, and then frozen by immersion in liquid nitrogen. The distal jejunum (3 cm to the jejunum) was cut off and fixed in 10% formaldehyde-phosphate buffer, and kept at 4 °C for morphological analysis.

2.5. Assay of antioxidant active and TBARS in jejunum

One piece of frozen jejunum was crushed in liquid nitrogen and then homogenized on ice in 4 mL of physiological saline