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Ameliorative effect of the sea cucumber *Holothuria arenicola* extract against gastric ulcer in rats

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Abstract *Holothuria arenicola* is the most important and abundant sea cucumber species in the Mediterranean Sea on the Egyptian coast. This work aimed to investigate the prophylactic and the curative effects of *H. arenicola* extract HaE (200 mg/kg) on gastric mucosal damage following indomethacin and cold stress in healthy rats. Sixty-four rats were randomly divided into four main groups. Rats of the first group (8 rats/group) were administered distilled water orally (control group), rats of the second group (8 rats/group) were administered single oral dose of indomethacin (150 mg/kg) and exposed to cold stress (4 ± 1 °C) for 30 min to induce gastric ulcer (GU) model (ulcer group), rats of the third group, prophylactic group (24 rats/group) were treated with HaE and/or ranitidine (RAN) and then exposed to GU and rats of the fourth group, curative group (24 rats/group) were exposed firstly to GU and then treated with HaE and/or RAN. The results clearly indicate that pre-treatment with HaE and/or ranitidine significantly decreases the ulcer index, showing 72.50%, 53.11% and 80.56% ulceration inhibition, respectively. However, post-treatment with HaE and/or ranitidine significantly decreases the ulcer index, showing 51.66%, 62.41% and 67.78% ulceration inhibition, respectively. The results also showed that pre and post-treatment with HaE and/or RAN significantly decreased gastric malondialdehyde (MDA) level and enhanced reduced glutathione (GSH), catalase (CAT), glutathione-S-transferase (GST) and superoxide dismutase (SOD) levels. The results clearly indicate that pre-treatment with HaE is preferable.

Introduction

Gastric ulcer disease (GUD) is a common problem of the gastro-intestinal tract with increasing incidence and prevalence attributed to loss of balance between aggressive and protective factors. Gastric ulcer develops because of several endogenous aggressive factors, including hydrochloric acid, pepsin, refluxed bile, leukotrienes and reactive oxygen species (ROS), and several exogenous factors including non-steroidal anti-inflammatory drugs (NSAIDs), stress, alcohol and *Helicobacter pylori* infection are major causative agents for gastric mucosal damage and ulceration (Rang et al., 2003; Nartey et al., 2012). NSAIDs including indomethacin are effective in the treatment of a variety of acute and chronic pain conditions. Their use has been associated with gastric mucosal damage as a side effect

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(Fukushima et al., 2014). Indomethacin is a synthetic NSAIDs that is known to induce severe gastric mucosal lesions (Abbas and Sakr, 2013). It is a potent inhibitor of prostaglandins' synthesis, which are important mediators of the inflammatory response (Robbin et al., 2009).

In rats, indomethacin, has been shown to cause significant gastrointestinal damage (Takeuchi et al., 2002) and oxidative stress (Okayama et al., 2009).

Oxidative stress, which is a state of unbalanced levels of reactive oxygen species (ROS), causes a variety of conditions that stimulate either additional ROS production or a decline in antioxidant defenses. Reactive oxygen species (ROS) and free radicals play an important role in the pathogenesis of several human diseases, including GUD (Gulcin, 2012; Mei et al., 2013). Indomethacin, a well-known NSAID, induces erosions and lesions in gastroduodenal tract through multifaceted processes, such as inhibition of cyclooxygenase (COX)-mediated prostaglandin synthesis, over expression of interleukin-1 (IL-1), polymorphonuclear leukocyte infiltration of generation of ROS and induction of apoptosis (Ansari and Lal, 2009). Several phenotypes of gastrointestinal diseases, such as peptic ulcer disease and gastroparesis, are known to be related to antioxidant property dysfunction (Suzuki et al., 2012). Stress has become an integral part of human life and organisms are constantly subjected to stressful stimuli that affect numerous physiological processes. It has been demonstrated that restraint and cold (4 °C), as well as indomethacin (Kleiman-Wexler et al., 1989), can induce ulceration.

Many synthetic compounds such as ranitidine and omeprazole, which block acid secretion, are now used as antiulcer drugs. Since synthetic drugs are often encountered with some adverse effects, treatment with natural products is now considered as an alternative approach to control the disease. Natural products have long been recognized as a rich source of potent therapeutics, but further development is often limited by high structural complexity and high molecular weight (Ononye et al., 2013). Sea cucumbers are marine invertebrates of the phylum of Echinodermata, sometimes referred to as marine ginseng, produce numerous compounds with diverse functions and are potential sources of active ingredients for agricultural, nutraceutical and pharmaceutical products (Bahrami et al., 2014). They are also remarkably rich in vitamins, trace elements, and polysaccharides (chondroitin sulfate), which reduce arthritis pain and inhibit viral activities, and saponin glycosides that inhibit cancer activities (Hamel and Mercier, 2004). *Holothuria arenicola* is the most important and abundant sea cucumber species in the Mediterranean Sea on the Egyptian coast (Kilada et al., 2000). In addition to their flavor, sea cucumbers are commonly used to treat wounds, eczema, arthritis, hypertension, and impotence (Sugawara et al., 2006; Ridzwan, 2007; Subramaniam et al., 2013). Gastric ulcer is essentially a deep wound in the stomach wall that involves epithelium, endothelium, connective tissue, and smooth muscle. Therefore, healing of a gastric ulcer means a restoration of those tissues that have been damaged during ulceration. So, the present study aims to assess the prophylactic and the curative effect of the sea Cucumbers *H. arenicola* extract (HaE) in comparison with ranitidine in a model of indomethacin and cold stress-induced gastric ulcer in male albino rats and to investigate its underlying anti-oxidative mechanisms. The present study also conducts to assess the employment of (HaE) as alternative or complementary to the established antiulcer drug ranitidine.

Materials and methods

Chemicals and reagents

Indomethacin was obtained from Sigma Chemical Co. (St. Louis, MO, USA). 2,2-diphenyl-1-picrylhydrazyl (DPPH). Ranitidine (50 mg/kg) was obtained from Hamdoun Pharmacy (Mohamed Roshdi St, Agoza, Egypt). Kits for all biochemical parameters and other chemicals and reagents were purchased from the Biodiagnostic Company (El Moror St, Dokki, Egypt).

H. arenicola sample collection

Samples were collected from the Abu-Qir Bay in the Egyptian Mediterranean coast of the eastern Alexandrian coast (May–June 2012). Samples were gently cleaned and washed to remove sediment and other small organisms attached to their bodies.

Samples were maintained in stock tank filled with filtered seawater at ambient temperature. The samples were packed immediately with ice prior to sending to the lab and kept at –80 °C until extracted.

Preparation of the H. arenicola extract (HaE)

The phosphate buffer extract was prepared according to the method of Yasumoto et al. (1967).

After being sliced open and its internal organs removed, the body wall of the sea cucumber *H. arenicola* was blended in phosphate buffer (in a volume = 4 × tissue weight) and extracted at room- temperature (25 °C) with pH 7.2 for 5 h. The filtered was collected immediately, concentrated and lyophilized using LABCONCO lyophilizer, shell freeze system, USA.

Free radical scavenging activity

The free radical scavenging activities of the extract and ascorbic acid were analyzed by the DPPH assay (Sanchez-Moreno et al., 1998). A 1.0 ml of the test extract, at gradient final concentrations of 10–80 mg/ml, was mixed with 2 ml of 0.3 mM DPPH solution in MeOH in a cuvette. The absorbance was taken at 517 nm after 20 min of incubation in the dark at room temperature. The experiment was done in triplicates. The percentage antioxidant activity was calculated as follows:

%Antioxidant Activity [AA] = $100 - [\{(Abs_{sample} - Abs_{blank}) \times 100\}/Abs_{control}]$. Where Abs_{sample} was the absorbance of sample solution (2.0 ml) + DPPH solution (1.0 ml, 0.3 mM), Abs_{blank} was the absorbance of Methanol (1.0 ml) + sample solution (2.0 ml), $Abs_{control}$ was the absorbance of DPPH solution (1.0 ml, 0.3 mM) + methanol (2.0 ml).