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Gastroprotective effect of garlic in indomethacin induced gastric ulcer in rats

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ABSTRACT

Objective: Garlic, in its natural plant state, has a great history in ancient medicine as a remedy for many diseases. In our study, the gastroprotective effect of aged garlic extract (AGE) and the possible underlying mechanisms were investigated in an experimental model of indomethacin-induced gastric ulcer.

Methods: Male Wistar rats were divided into four groups: (normal control, n = 20), ulcer control (indomethacin group, n = 20), (omeprazole group, n = 30) and (garlic group, n = 20). Each dose of garlic and omeprazole was given to rats orally daily for 10 consecutive days before induction of ulcer by indomethacin. Indomethacin was given as a single oral dose (100 mg/kg). Four hours later after indomethacin treatment, the rats were sacrificed and gastric tissue was obtained for histopathological examination, calculation of ulcer index and measurement of oxidative stress markers as well as gastroprotective mediators.

Results: The results showed that indomethacin induced gastric ulcer (ulcer index = 2900), was associated with a significant increase of tumor necrosis factor-alpha and malondialdehyde, and significant decrease of the gastroprotective mediators prostaglandin E2, glutathione (GSH) and nitric oxide (NO) compared with normal control. Pretreatment with AGE produced comparable results with those obtained in the omeprazole group; the preventive index in the AGE group was 83.4% compared with 94.5% in the omeprazole group. The prophylactic role of AGE in indomethacin-induced ulcer was, in part, mediated by decreasing oxidative stress and increasing gastric level of PGE2, GSH, and NO.

Conclusion: AGE corrected the histopathological abnormalities in gastric tissue and proved a promising gastroprotective role in gastric ulcer.

Introduction

Complementary and alternative medicine (CAM) comprise medicinal products that aren't used as part of conventional medicine. Safety and easy intake are the most attractive advantages regarding CAM. Interestingly, natural products are gaining a worldwide attention especially in the developing populations [1].

Garlic (*Allium Sativum*), belongs to family *Liliaceae* [2]. Since ancient times garlic has been considered folk medicine (CAM) [3], long cultivated in different cultures for food and spice [1]. It has been reported that garlic was used in the treatment of many diseases, like hypertension, and has antihypercholesterolemia, antiinflammatory, and antioxidant activity [4,5].

Aged garlic extract (AGE) is considered an important source of phytochemicals that possess antioxidant activity. These include lipid soluble organosulfur compounds, water soluble organosulfur compounds (e.g., S-allylcysteine (SAC) and S-allylmercaptocysteine), flavonoids, and phenolic compounds, which play an important role in scavenging free radicals [5].

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Non-steroidal antiinflammatory drugs (NSAIDs) are the most commonly used drugs throughout the world. The gastric damage caused by these drugs is known to be their most common and dangerous side effect [6]. Indomethacin is a well-known NSAID, with a high potential to cause gastric ulcer [7]. Gastric ulcer is induced by the disturbance of the normal balance between defensive and aggressive factors in favor of the aggressive ones [8]. Defensive factors involve mucus secretion, blood flow, cells renewal, prostaglandins (PGs), and nitric oxide (NO), while aggressive factors include NSAIDs, acid, oxidative stress, and ethanol [8,9].

In the present study, the gastroprotective effect of aged garlic extract and the possible underlying mechanisms were investigated in an experimental model of indomethacin induced gastric ulcer.

Materials and methods

Drugs

Indomethacin was obtained as a gift from Kahira Pharmaceuticals & Chemical Industries Co. (Cairo, Egypt). Omeprazole was purchased from Carbosynth LLC (San Diego, USA). Aged garlic extract (AGE) was purchased from Hunan 3 W Botanical Extract Inc. (Hunan, China).

Animal groups

The study was performed in accordance with the guidelines for the care and use of laboratory animals approved by Research Ethical Committee, Faculty of Pharmacy, Tanta University, Egypt. Male Wistar rats weighing 180 to 200 g (12-week-of-age) were obtained from the National Research Center, Giza (Egypt). The rats were housed in wire cages for 2 wk for acclimatization and allowed free access to water and standard pellet diet. The starting weight of animals was 230–250 g.

Rats were randomly divided into 4 groups: group 1 (control group, n = 20), group 2 (ulcer group, n = 20), group 3 (omeprazole group, n = 30) and group 4 (garlic group, n = 20). The number of rats indicated here was larger than that used in statistics (i.e., n = 8) as the tissue from a single stomach was not enough for chemical measures and so pooling was required. In addition, the number of rats in group 3 was higher (relative to that in other groups) as omeprazole decreased gastric secretion to some extent and so the number of rats was increased to accommodate this effect.

For rats in the pretreatment groups, omeprazole (5 mg/kg) [10] or AGE 200 mg/kg [5] was given orally (gavage) daily for 10 consecutive days; on the final day, these rats were given indomethacin, by single gavage of 100 mg/kg [11] for ulcer induction, 1 h after the omeprazole or garlic dosing. Rats in groups 1 and 2 (that had been dosed with vehicle in place of either drug for the 10 days) were dosed with, respectively, vehicle or indomethacin in parallel (in the final day).

All rats were fasted 24 h before indomethacin oral treatment; during this period, the rats were kept in wide wire mesh-bottom cages to avoid coprophagia; in addition, water access was prevented for 2 h before the indomethacin dosing. Four hours after the indomethacin/vehicle gavage, all rats were euthanized by ether and their stomachs excised.

Methods

Measurement of gastric pH and ulcer index

The stomach obtained was opened along the greater curvature then the gastric content was drained into a centrifuge tube. The gastric juice was centrifuged at 1000 rpm for 10 min (4°C), the clear supernatant was recovered and the pH measured [12] using a pH 211 meter.

For ulcer index measurement, each stomach was washed by saline after drainage of the juice and examined by a magnifying lens. Ulcer score was calculated as the mean of ulcers in each group (total number of ulcers divided by rats number, n = 8), ulcer index (UI) was then calculated by multiplying ulcer score ×100 [13]. The preventive index was calculated according to the method described by Hano et al., preventive index: (UI of ulcerated group − UI of treated group × 100)/UI of ulcerated group [14].

Determination of gastric glutathione (GSH) and malondialdehyde (MDA)

To prepare tissue for assaying stomach levels of glutathione (GSH) and malondialdehyde (MDA) assays, 250 mg tissue was homogenized in 2.5 mL potassium phosphate buffer (pH 7.5) using a Polytron homogenizer (PT 3100, Kinematica, Luzern, Switzerland) and then centrifuged at 4000 rpm for 15 min at 4°C.

The concentration of reduced GSH in the stomach tissue homogenate was determined colorimetrically with the method described by Beutler et al. [15], using a kit from Biodiagnostics, (Giza, Egypt) according to manufacturer instructions. Reduction of 5,5′-dithiobis 2-nitrobenzoic acid (DTNB) by reduced GSH to give yellow product that was measured at 405 nm in a Unico 2100 spectrophotometer (Unico, Dayton, NJ, USA). Levels were then calculated using a kit-provided formula and presented as mg/g tissue.

The concentration of MDA in stomach tissue homogenate was determined colorimetrically using a kit from Biodiagnostics, Egypt, and following a method described by Satoh [16] and Ohkawa et al. [17]. In the protocol, thiobarbituric acid (TBA) reacts with MDA present in the sample [in acidic medium, at 95°C for 30 min] to form TBA-reactive products (TBARS). The absorbance of these pink products was then measured at 534 nm in the spectrophotometer. Levels were then calculated using a kit-provided formula and presented as nmol/g tissue.

Determination of stomach nitric oxide (NO)

For tissues used for assaying stomach nitric oxide (NO) levels, 250 mg tissue was homogenized in 2.5 mL ice-cold normal (0.9%) saline. Thereafter, 1 mL absolute ethanol was added to 0.5 mL homogenate to precipitate the proteins and the samples were then centrifuged at 3000 rpm for 10 min at 4°C.

The gastric nitric oxide was determined by measuring its nitrite (an indicator of original NO present). This method depends on reduction of nitrate to nitrite by vanadium trichloride (VCl_3) followed by addition of Griess reagent [18]. In brief, a sample of homogenate supernatant (500 μL) was mixed with an equal volume of VCl_3 and of Griess reagent (0.1% N-(1-naphthyl)-ethylenediamine in distilled water and 2% sulphanilamide in 5% hydrochloric acid). After incubation at 37°C for 30 min, the absorbance of the mixture was measured at 540 nm in the spectrophotometer [18]. Sodium nitrite standards assessed in parallel, values were compared with it, and the nitrite concentration in each sample was calculated and presented as nmol NO/g tissue.

Determination PGE_2 and TNF-α

Enzyme-linked immunosorbent assay (ELISA) kits were utilized for measurement of gastric (stomach tissue homogenate supernatant) content of prostaglandin E2 (PGE2) and tumor necrosis factor-alpha (TNF-α) following the protocol provided by the manufacturer. The kits were obtained from CUSABIO Biotech Co., Ltd (Wuhan, People's Republic of China) and Glory Science Co., Ltd (Zhejiang, People's Republic of China), respectively.

Histopathology

Stomach was fixed in formalin (10%) for 24 h, embedded in paraffin to form blocks, which were serially sectioned (5 μm thick) using a Leica RM2135 microtome, mounted on glass slides, then stained by hematoxylin and eosin solution then evaluated under light microscope by certified pathologist (in blinded manner). The pathologist is Prof. Dr. Mona A. Yehia at Medical Research Center, Alexandria, Egypt.

Statistical analysis

Analysis of data was performed by Statistical Package for Social Science (SPSS, Armonk, NY, USA) software version 17.0. Data are presented as mean ± SD. Statistical comparison among groups was performed by one-way analysis of variance (ANOVA). Statistical significance was set at $P < 0.05$.

Results

Effect on gastric acidity, ulcer index, and preventive index

Administration of indomethacin showed a significant decrease in gastric pH (↓41.06%) when compared with normal control group. Pretreatment with AGE significantly raised the gastric pH to 3.38 ± 0.09, whereas pretreatment with omeprazole significantly increased the gastric pH to 5.13 ± 0.14, which was above the control value (Table 1).

The ulcerated indomethacin group showed ulcer score of 29 ± 4.8 and UI of 2900. Rat groups treated with AGE or omeprazole before indomethacin showed a significant decrease of ulcer score, which was 4.8 ± 1.4 and 1.6 ± 0.51, respectively. Omeprazole showed a greater gastroprotective effect with UI of 160 and preventive index 94.5%, while AGE showed UI of 480 and a preventive index of 83.4% (Table 1).