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# Echinochrome pigment as novel therapeutic agent against experimentally - induced gastric ulcer in rats

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ABSTRACT

*Objective:* evaluate the antiulcer healing effect of echinochrome pigment (Ech 5 and 10 mg/kg) extracted from sea urchin *(Paracentrotus lividus)*.

*Methods:* Severe gastric ulceration induced in rats by administration of indomethacin in combination with cold stress (IND + CS) for 2 h. The antiulcer effect of Ech was indicated by determination of gastric juice volume, gastric juice acidity, and ulcer index as well as determination of gastric malondialdhyde (MDA), glutathione (GSH), catalase (CAT), glutathione–S-transferase (GST), superoxide dismutase (SOD), nitric oxide (NO) activities. Moreover, macroscopic and microscopic evaluations of the stomachs were determined.

*Results:* The anti-ulcer healing effect of Ech against IND + CS induced oxidative tissue injury was investigated by a significant decrease in MDA level, GST and CAT activities and a significant increase of GSH content, NO level and SOD activity. In addition, Ech treatments (5 mg/kg and 10 mg/kg) reduce gastric lesion area to (73.51% and 75.50%, respectively) with great amelioration of the gastric juice volume and acidity. Also, affirmed by maintaining macroscopic and microscopic gastric mucosal integrity.

*Conclusions:* Ech pigments had an insightful effect against peptic ulcer-induced oxidative stress in rats, as it alleviates the alterations in gastric acidity and ulcer index as well as the oxidative stress markers.

## 1. Introduction

Peptic ulcers affect many people around the world and defined as a disruption in the mucosa of the stomach or duodenum [1]. Peptic ulcers occur when there is an imbalance between the protective and aggressive factors [1]. Nonsteroidal anti-inflammatory drugs (NSAIDs) including indomethacin are the major detrimental effects on gastric mucosa [2]. Indomethacin, has been proved to cause considerable gastrointestinal injury and oxidative stress [3,4]. The development of the gastric mucosal lesions induced by indomethacin is mainly mediated through the generation of oxygen free radicals and lipid peroxidation [5].

Currently available treatments for peptic ulcers include systemic and non-systemic antacids and groups of drugs which reduce acid secretion [6]. Generally, synthetic drugs are often encountered with some drawbacks due to the limited efficacy and adverse effects [7,8]. 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 [9]. Currently, 80% of the world population depending on natural products which considered as an alternative approach to control the disease.

Sea urchins (*Paracentrotus lividus*) are small, spiny, globular animals which, with their close kin, such as sand dollars, constitute the class Echinoidea of the echinoderm phylum [10]. Moreover, the use of sea urchin shells confers certain beneficial advantages, including antioxidant and pharmaceutical effects [11,12]. It was reported that, sea urchin contains polyhydroxylated naphthoquinone (PHNQ) pigments [13,14], that may be echinochromes and spinochromes, according to their main sources [15]. PHNQ pigments have been found to have ideal antimicrobial, antialgal, cardioprotective and antioxidant activity [13]. Echinochrome, 7(2)-ethyl-2, 3, 5, 6, 8-pentahydroxy 1, 4-naphthoquinone, is one of several spinochromes that occur as pigments in the shells and gonads of sea urchins [15,16]. It has been used as a human dietary supplement in Russia with no reported serious side effects [17]. The quinone pigments of sea urchins, specifically echinochrome and spinochromes, are known for their effective antioxidant [18].

Scavenging reactive oxygen species (ROS) is a new strategy to prevent and treat chronic and degenerative diseases such as peptic ulcer [11,19,20]. Concerns about adverse effects of synthetic antioxidants have increased the demand for natural antioxidant agents [13]. Moreover, gastric ulcer is essentially a deep wound in the stomach wall that involves tissue damage. Recently, Cai et al. [21] proved that nature

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naphthoquinone shikonin promote wound healing by reducing reactive oxygen species and inflammation. Thereby, the present study looks forward to investigate the anti-ulcerogentic efficacy of Echinochrome A pigment as an antioxidant and wound healing agent.

## 2. Materials and methods

### 2.1. Animals

Thirty male albino Wistar rats (*Rattus norvegicus*) weighing between 180–200 g were used for this study. The animals were obtained from the animal house of National Research Center, Cairo, Egypt. Animals were grouped and housed randomly into five wires-meshed cages (six rats each), where they acclimatized to laboratory conditions for one week before the commencement of the experiment. The animals were housed under standard conditions of temperature (23 ± 2 °C), humidity (55 ± 15%), and 12-hour light (7:00 am - 7:00 pm). They were fed with standard commercial diet and allowed water *ad libitum*.

#### 2.1.1. Ethical consideration

Experimental protocols and procedures used in this study were approved by the Cairo University, Faculty of Science Institutional Animal Care and Use Committee (IACUC) (Egypt), (CUFS/PHY/02/15). All the experimental procedures were carried out in accordance with international guidelines for the care and use of laboratory animals.

### 2.2. Drugs and other chemicals

Indomethacin was obtained from Sigma Chemical Co. (St. Louis, MO. USA), Ranitidine (RAN 50 mg/kg) (Zantac ™) was obtained from Seif Pharmacy (Dokki St, Egypt). The kits for all biochemical parameters and other chemicals and reagents were purchased from Biodiagnostic Company (El Moror St., Dokki, Egypt).

### 2.3. Samples collection

Sea urchins (*Paracentrotus lividus*) were collected from the Mediterranean coast of Alexandria (Egypt) and transported to the laboratory packed in ice. The samples were thoroughly washed with sea water to remove sand and overgrowing organisms at the collection site. The collected specimens were identified by the standard literature of taxonomic guide by Clark and Rowe [22].

#### 2.3.1. Echinochrome (Ech) extraction

The echinochrome pigment from purple sea urchin shells were isolated by Amarowicz method with slight modifications [10,23]. Shells, spines and gonads were immediately removed and the shell was shade air-dried at4 °C for 2 days in the dark and then were grounded and coarsely powdered in a grinder. The gonads were also dried by the same method. The shells, spines and gonads powders were dissolved by gradually adding 6 M HCl. The pigments in the solution were extracted 3 times with the same volume of diethyl ether. The ether layer collected was ashed with 5% NaCl until the acid was almost removed. The ether solution including the pigments was dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure using a rotary evaporator at 40 °C. Ech powder of sea urchin was dissolved in 5% dimethyl sulfoxide (DMSO).

### 2.4. Induction of severe gastric ulcers

Ulcer was induced by a combination of indomethacin and cold stress (IND + CS) [24]. Indomethacin (IND) was administered as a single oral dose (150 mg/kg) [25] dissolved in 5% sodium bicarbonate [5]. The animals exposed to cold stress (CS) by keeping it 2 h at temperature of 3-5 °C [26].

### 2.5. Experimental design

Before the experiment, rats will deprived of food, but not water, for 20–24 h. The animals were divided into five groups of six rats each.

**Group 1:** Animals administered 5% DMSO. They served as the overall control group.

**Group 2:** Ulcer model group (IND + CS).

**Groups 3, 4 and 5:** rats exposed to (IND + CS) model for 2 h, then the animals were orally treated once with Ech at dosages of 5 and 10 mg/kg body weight [27] and RAN (50 mg/kg), respectively for one hour.

### 2.6. Animals handling

At the end of the experimental period, animals were euthanized under deep anesthesia with sodium pentobarbital. Stomach was removed and immediately blotted using filter paper to remove traces of blood, then the stomach was dissected out, incised along the greater curvature and the gastric juice was collected. Mucosa was rinsed with cold normal saline to remove blood contaminant. The hemorrhagic and ulcerative lesions of the stomach were counted, then the stomach of rats stored at –80 °C for biochemical analysis.

### 2.7. Macroscopic evaluation

The severity of macroscopic lesions formed was estimated using an ulcer index as previously reported by Kulkarni [28] using the following scale: the stomachs with no injuries received scores 0; 0.5 = red coloration; 1.0 = spot ulcers, 1.5 = hemorrhagic streaks; 2.0 = ulcers with area > 3 but ≤5mm$^2$; 3.0 = ulcers > 5mm$^2$, the ulcer index was calculated as

$$\text{Ulcer index (UI)} = \text{UN} + \text{US} + \text{UP} \times 10,$$

Where UI = ulcer index, UN = average number of ulcers per animal, US = average of severity score, and UP = percentage of animals with an ulcer.

The percentage of inhibition against ulceration was determined using the expressions:

$$\%\text{Ulcer inhibition} = [\text{UI}_{\text{IND+CS}} - \text{UI}_{\text{test}}] \times 100 / \text{UI}_{\text{IND+CS}}.$$

### 2.8. Analysis of gastric juice

#### 2.8.1. Determination of gastric juice volume

The gastric juice collected from each animal was centrifuged at 1000 g for 10 min to remove any solid debris and the volume of the supernatant was measured.

#### 2.8.2. Determination of gastric acidity

An aliquot of 1 ml of gastric juice diluted with 1 ml distilled water was taken in a conical flask and titrated against 0.01 N NaOH with phenolphthalein as an indicator till a permanent pink color is obtained [29]. The volume of NaOH was then noted. The total acidity, expressed as mEq/L was then calculated by the following equation:

$$\textbf{Acidity} = \text{Vol. of NaOH} \times \text{N} \times 100 / 0.1 \text{ mEq/L}$$

### 2.9. Determination of lipid peroxidation, enzymatic and non-enzymatic antioxidant activity

#### 2.9.1. Determination of lipid peroxide

The assay method of Ohkawa et al. [30] was adopted for malondialdehyde (MDA), a biochemical marker of lipid peroxidation. When MDA heated with thiobarbituric acid (TBA) under acid conditions forms a pink-colored product that has a maximum absorbance of