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Gastroprotective effect of crocin in ethanol-induced gastric injury in rats

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ABSTRACT

The present study investigated the gastroprotective effect of crocin in ethanol-induced gastric injury in rats. Rats were allocated into a normal group, an ulcer group, a crocin-treated group, an ulcer group pretreated with crocin, and an ulcer group pretreated with omeprazole as a reference anti-ulcer drug. Rats were sacrificed 3 h after ethanol administration. Prophylactic administration of crocin (50 mg/kg/day, i.p.) for 3 consecutive days before the administration of 70% ethanol (10 ml/kg, orally) resulted in significant gastroprotection compared to ethanol-ulcerated rats as manifested by significant reduction in the gastric ulcer index. Crocin pretreatment increased ethanol-lowered levels of gastric juice mucin and mucosal prostaglandin E_2 (PGE_2) and interleukin-6 (IL-6). Moreover, crocin significantly decreased ethanol-elevated tumor necrosis factor-alpha (TNF-α) level, myeloperoxidase activity and heat shock protein 70 mRNA and protein levels. It also restored ethanol-altered mucosal levels of glutathione, malondialdehyde and superoxide dismutase activity. Furthermore, crocin-pretreatment alleviated ethanol-induced mucosal apoptosis as revealed by significant down-regulation of cytochrome c and caspase-3 mRNA expression, significant decrease in caspase-3 activity and mitigated DNA fragmentation as indicated by significant decrements in comet parameters. The protective efficacy of crocin was further supported by histological assessment. No significant difference was observed between crocin and omeprazole (20 mg/kg orally 1 h before ethanol administration) regarding their mucin-secretagogue and antioxidant effects, as well as their effects on TNF-α, IL-6 and cytochrome c. On the other hand, omeprazole was superior in enhancing PGE_2 level and in alleviating neutrophil infiltration, caspase-3 activation and DNA fragmentation. Conclusively, crocin protects rat gastric mucosa against ethanol-induced injury via anti-inflammatory, anti-oxidative, anti-apoptotic and mucin-secretagogue mechanisms that are probably mediated by enhanced PGE_2 release.

1. Introduction

Peptic ulcer is one of the most common gastrointestinal diseases with 4–5% prevalence in the human society [1]. Major etiologic factors of peptic ulcer include *Helicobacter pylori* infection, excessive use of non-steroidal anti-inflammatory drugs (NSAIDs), alcohol consumption, smoking, psychological and physiological stress [2]. Especially, NSAIDs and alcohol consumption increase the risk for major upper gastrointestinal bleeding [3]. Alcohol is one of the most commonly abused drugs [4] and, therefore, alcohol-induced disorders of the gastrointestinal tract are very common [3].

The basic pathophysiology of gastric ulcers results from an imbalance between some aggressive and defensive factors. The gastric mucosa is continuously challenged by a variety of endogenous aggressive factors including excessive secretion of hydrochloric acid and pepsin, refluxed bile, leukotrienes, stress and reactive oxygen species (ROS) [5,6]. A major source of ROS in ethanol-injured gastric tissue is infiltrating neutrophils. Neutrophil adherence to the vascular endothelium and the subsequent release of oxygen-derived free radicals and proteolytic enzymes have been implicated as critical events in the pathogenesis of various forms of gastrointestinal ulceration including the gastric damage associated with intragastric ethanol administration [6,7]. To protect the gastric mucosa from such aggressive factors, a complex defense system has evolved, which includes the production of surface mucus and bicarbonate, surface active phospholipids, the

Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; γ-GCS, gamma-glutamylcysteinyl synthase; GUI, gastric ulcer index; GSH, reduced glutathione; HSP70, heat shock protein 70; H&E, hematoxylin and eosin; IL-6, interleukin-6; MDA, malondialdehyde; MPO, myeloperoxidase; NF-κB, nuclear factor kappa B; NSAIDs, non-steroidal anti-inflammatory drugs; 8-OH-dG, 8-hydroxy-2′-deoxyguanosine; PC-12 cells, pheochromocytoma cells; PGE_2, prostaglandin E_2; pNA, p-nitroanilide; ROS, reactive oxygen species; qPCR, quantitative polymerase chain reaction; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-alpha.

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regulation of gastric mucosal blood flow, non-enzymatic and enzymatic antioxidants, the acceleration of epithelial regeneration and the preservation of epithelial homeostasis. Prostaglandin, in particular prostaglandin E_2 (PGE_2), enhances these protective mechanisms and is, therefore, believed to comprise a major gastric mucosal defensive factor [5,6]. Heat shock proteins proved to be another key protective mechanism [8]. They are involved in the regulation of inflammation and in the maintenance of mucosal integrity. Their altered expression may be a marker of mucosal inflammation and may contribute to tissue injury. Heat shock protein 70 (HSP70) is considered to be the most universally stress inducible heat shock protein [9]. It is, therefore, considered a stress biomarker. It has been suggested to exert its cytoprotective action by protecting the mitochondria and by interfering with the stress-induced apoptotic program [10]. The pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) are also key mediators in the pathophysiology of gastric inflammation and are important in regulating the severity of gastric ulcers [11]. Ethanol, among other gastric irritants such as hydrogen peroxide and acids, damages the gastric mucosa by inducing not only necrosis but also apoptosis in gastric mucosal cells [12]. These gastric irritants induce apoptotic DNA fragmentation, chromatin condensation and caspase activation in gastric mucosal cells through a common pathway in which mitochondrial dysfunction plays an important role [13,14].

For decades, ulceration of the gastric mucosa was regarded as being caused by excessive gastric acid secretion. Therefore, the control of gastric acid secretion became the basis upon which ulcer therapy was designed: from vagotomy to anti-cholinergic drugs, histamine H_2 receptor antagonists, antacids, and, more recently, proton pump inhibitors. It was certainly recognized that increased acid secretion alone was unlikely to produce ulcers, because many patients with ulcers secreted acid at normal rates [6]. Indeed, an alcohol concentration of higher than 5% has no effect on gastric acid secretion [15]. Therefore, most of the drugs currently available in the market show limited efficacy against gastric ulcer diseases and their long-term use is associated with disturbing side effects [1]. Thus, there is an urgent need to introduce more effective and safe anti-ulcer agents.

Crocin, crocetin digentiobiose ester, is a water-soluble carotenoid pigment found in the stigma of *Crocus sativus* L. [16] and the fruit of *Gardenia jasminoides* [17]. A number of pharmacological studies demonstrated that crocin has a wide range of activities including antioxidant [18], antihyperlipidemic [17], anti-inflammatory [19], cardioprotective [20], hepatoprotective [21] and anticancer [22] effects. Crocin also exhibited anti-apoptotic effects in a number of the aforementioned models [20,21].

Kianbakht and Mozaffari [23] demonstrated the antioxidant effect of crocin in indomethacin-induced gastric injury in rats. Yet, its effect on specific gastric defensive factors and on other important ulcerogenic mechanisms, as inflammation and apoptosis, has not yet been explored in any ulcer model. Also, the protective potential of crocin on ethanol-induced gastric ulcer has not yet been examined.

Therefore, the aim of the current study was to investigate the gastroprotective effect of crocin against ethanol-induced gastric injury in rats in an attempt to introduce a relatively safe alternative for the conventional anti-ulcer products.

2. Materials and methods

2.1. Animals

Male Wistar rats (180–220 g, 6–7 weeks old) obtained from the laboratory animals' farm of the Egyptian Organization for Biological Products and Vaccines, Cairo, Egypt, were used. The animals were housed in a 12 h light/dark cycle, at a humidity of 60 ± 10% and a temperature of 25 ± 2.0 °C. Access to food and water throughout the experimental period was allowed *ad libitum*. All the animal protocols complied with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and were approved by the Ethical Committee for Animal Experimentation at Faculty of Pharmacy, Cairo University.

2.2. Chemicals

Ethanol, crocin, omeprazole, orcinol, 5,5′-dithiobis-2-nitrobenzoic acid, thiobarbituric acid, pyrogallol, hexadecyltrimethylammonium bromide and *O*-dianisidine hydrochloride were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Other chemicals were of Analar grade or of the purest grade available.

2.3. Experimental design

The detailed experimental design is depicted in Fig. 1. Rats were randomly allocated into 5 groups (14 rats each). The first, third and fourth groups received daily i.p. injections of appropriate volumes of saline for 3 days. The animals in the second and fifth groups received daily i.p. injections of crocin (50 mg/kg) dissolved in saline for 3 days [20]. On the third day of the experiment, all the animals were fasted for 24 h but had free access to water. On the fourth day, 70% ethanol was given orally to each animal in groups III, IV and V at 10 ml/kg to induce gastric ulceration [24]. The animals in the fourth group were administered omeprazole, a proton pump inhibitor, orally 1 h before ethanol administration at a dose of 20 mg/kg [25]. Omeprazole was used as a reference anti-ulcer drug. Three hours after ethanol administration, animals were sacrificed by decapitation.

2.4. Collection of gastric juice

Six rats from each group were subjected to pyloric ligation according to Shay et al. [26], modifying its duration to 3 h, for the collection of gastric juice. This was carried out 3 h before sacrifice in groups I and II, and just before ethanol administration in groups III, IV and V. Immediately after decapitation, stomachs were removed following ligature of the oesophocardiac junction. The gastric juice was drained, centrifuged at 600*g* and the supernatant was used for the determination of mucin content.

2.5. Determination of gastric mucosal injury index

Following decapitation of the other rats not subjected to pyloric ligation, the stomachs were opened along the greater curvature, washed with cold saline, blotted dry between filter paper and pinned flat on a cardboard to be subjected to gross lesions evaluation. The gastric ulcer index (GUI) was determined according to the Guth standard [27]: spot erosion was recorded as 1 point, erosion length <1 mm was recorded as 2 points, 1–2 mm was recorded as 3 points, 2–3 mm was recorded as 4 points, and >3 mm was recorded as 5 points. The score was doubled if the erosion width was >1 mm.

2.6. Preparation of subcellular fractions of stomach

After the evaluation of the GUI, the gastric mucosae were scraped off, weighed and homogenized in 200 mM potassium phosphate buffer, pH 7.4, to obtain 10% aqueous homogenates. Aliquots of the resulting homogenate were used to determine the levels of reduced glutathione (GSH) and malondialdehyde (MDA) as an index of lipid peroxidation. Another aliquot of the mucosal