Quercetin attenuates, indomethacin-induced acute gastric ulcer in rats

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Background: Peptic ulcer diseases are common and are induced by many factors, including stress, smoking, and ingestion of non-steroidal anti-inflammatory drugs. Quercetin is considered to be an anti-oxidant with healing effects on many experimental toxic injuries. The present study aimed to explore the possible effect of quercetin on acute gastric ulcer induced by indomethacin in rats.

Materials and methods: Three groups received indomethacin (30 mg/kg body weight) orally by orogastric gavage on two consecutive days. The rats received famotidine (50 mg/kg body weight), quercetin (50 mg/kg body weight), or vehicle alone for 15 consecutive days by oral gavage. The control group received no indomethacin but received vehicle for 15 days by oral gavage. The ulcer index, volume, and pH of gastric juice were measured, and the stomachs were examined by routine light microscopy.

Results: Compared with the control group, the indomethacin-treated rats showed a marked damage of the gastric mucosal surface and a high ulcer index. In the famotidine- and quercetin-treated groups, significantly increased antioxidant enzyme activities were observed. The congestion, erosions, and necrosis were reduced with mild inflammatory cell infiltration while no major damage of endothelial cells was observed in the treated rats.

Conclusions: The findings of the study show that quercetin had antioxidant effect and can protect gastric mucosa against indomethacin-induced gastric ulceration than famotidine. (Folia Morphol 2017; 76, 2: 252–261)

Key words: flavonoids, ulceration, nonsteroidal anti-inflammatory drugs, pH value, gastric juice

INTRODUCTION

An inflammation, irritation or erosion of the mucosal surface of the stomach is termed as gastritis, and it can range from a mild, asymptomatic form to severe ulceration [67]. When a gastric ulcer occurs in the lining of the stomach, it is known as a peptic ulcer, which refers to an open sore in the lining of the stomach or the duodenum [51]. Peptic ulcers are associated with high morbidity and mortality and related to many complications including bleeding, perforation, penetration and gastric outlet obstruction [6, 42, 51]. Moreover, many otherwise beneficial therapies, including low-dose aspirins, dual anti-platelet therapy and nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin, have been shown to be important risk factors for recurrent ulcers and related complications [61]. It has been reported that about 25% of gastric ulcers are caused by NSAIDs [11]. NSAIDs are widely used for pain control and prevention of malignancies, strokes, pre-eclampsia,
Alzheimer's disease, etc. [60, 73]. Furthermore, studies have reported that various non-pharmaceutical factors including stress, hunger and Helicobacter pylori invasion also cause gastric ulcers [63]. Hence, the prevention of gastrointestinal disorders, including gastric ulcers, is of utmost importance for both medical professionals and researchers.

Many synthetic anti-ulcer drugs (e.g., misoprostol) have been developed that can be specifically used for preventing or treating NSAID-induced gastric ulcers [44]. However, these drugs have many side-effects such as diarrhoea, itching, skin rash, and dizziness [20]. Therefore, there is a renewed interest in nontoxic, natural anti-ulcer formulations from medicinal plants [52]. Flavonoids are a naturally-occurring group of compounds with anti-inflammatory, anti-allergic, anti-viral, anti-bacterial and anti-tumour activities [14, 72]. One of these flavonoids, quercetin (3, 5, 7, 3', 4'-pentahydroxyflavone), has been reported to have the ability to prevent oxidant injury and cell death by scavenging free oxygen radicals [43]. Quercetin is widely distributed in fruits and vegetables such as onions, apples, berries, and tea [48]. Studies have shown that daily intake of quercetin may decrease the risks of cardiovascular diseases [39], tumour development, strokes and neurodegenerative diseases [53] by protection against oxidative DNA damage [21], lipid peroxidation [38] and inhibition of the release of inflammatory mediators [41]. Presently, quercetin is marketed in the United States primarily as a dietary supplement [69], with a recommended daily dose of 200–1200 mg (probably due to variable absorption from the digestive tract) [19].

Experimental studies have shown that quercetin can prevent ethanol-induced gastric ulcers [31]. Therefore, the present study was undertaken to extend this finding by examining the effects of quercetin on indomethacin-induced gastric lesions in rats.

**MATERIALS AND METHODS**

**Animal grouping**

Forty adult male albino rats (150–250 g body weight) were obtained from the animal house, Faculty of Medicine, Umm Al-Qura University. All animal procedures were performed according to the approved protocols and in accordance with the recommendations for the proper care and use of laboratory animals. The animals were categorised into four groups (10 in each):

- Group 1 (control) received 1 mL of 1% sodium carboxymethyl cellulose (CMC) by oral gavage for 15 consecutive days;
- Group 2 received indomethacin (30 mg/kg body weight) dissolved in 1% CMC for 2 days, followed by 1% CMC for 15 consecutive days;
- Group 3 received indomethacin in 1% CMC as in Group 2 and additionally received famotidine (50 mg/kg) dissolved in 1% CMC for 15 consecutive days;
- Group 4 received indomethacin in 1% CMC as in Group 2 and additionally received a suspension of quercetin (50 mg/kg body weight) in 1% CMC for 15 consecutive days.

**Chemicals**

Indomethacin (Sigma, Aldrich) was dissolved in 1% sodium CMC to prepare a solution. After the animals had been fasted for 24 h but with free access to tap water, they were administered 1 mL of this solution orally (30 mg/kg body weight) for 2 consecutive days to induce acute gastric ulcer [3].

Famotidine tablets (Famotidine was purchased from Medical Union Pharmaceutical [MUP] Company, Abou Sultan — Ismailia, Egypt.) were crushed and dissolved in a small volume of CMC. Then 1 mL of this solution was administered to the animals by orogastric gavage (Famotidine was 50 mg/kg administered as suspension 1% sodium CMC [2 mL, oral] once daily for 15 consecutive days.), starting from 6 h after the final indomethacin administration [36, 58].

Quercetin (Sigma, Aldrich, Germany) was suspended in 1% sodium CMC immediately before its administration by orogastric gavage. A dose of 50 mg/kg body weight was administered to the animals daily for 15 consecutive days, starting from 6 h after indomethacin administration [45].

**Measurement of gastric juice volume and acidity**

On the last day of the experimental period, all the rats were fasted for 12–14 h and were allowed to have access to only drinking water. The abdomen was incised after scarification by ether anaesthesia, and both the stomach and duodenum were exposed. A fistula made by a poly-ethane tube was inserted into the stomach via a small incision made in the duodenum and held in place by a ligature around the pylorus. The oesophagus was also clamped to prevent reflux and loss of gastric mucosa. The collected fluid was placed in tubes and centrifuged for 5 min at 5000 rpm, and the
volume of the supernatant was expressed as mL/100 g body weight. The acid output was determined by titrating with 0.01 NNaOH using phenolphthalein as an indicator and was expressed as μEq/6 h as output. The volume of gastric juice was measured by a graduated cylinder and expressed in mL [55].

Macroscopic and ulcer index study

The stomach of the rats was opened longitudinally, spread out, pinned, and washed with saline. The lesions in the mucosa were examined under a 10× magnifying glass to assess the ulcer formation, and the number and severity of the ulcers per stomach (see below). Digital images were taken for the whole stomach of each animal to serve as a permanent record. Lesions of the mucosa ranging from hyperaemia at one extreme, through haemorrhagic lesions, and up to full erosions at the other extreme were recorded. The ulcer index of each animal was calculated by using the following scoring system [45]:

- 0 = normal coloured stomach;
- 0.5 = red coloration;
- 1 = spot ulceration;
- 1.5 = haemorrhagic streaks;
- 2 = ulcers between 3 and 5 mm in greatest dimension;
- 3 = ulcers greater than 5 mm in greatest dimension;
- 4 = perforations.

The mean ulcer score for each animal was expressed as the ulcer index. The effectiveness of the two treatments (famotidine and quercetin) was then calculated as a curative ratio using the following equation:

\[
\text{Curative ratio (CR)} = \left( \frac{LC - LT}{LC} \right) \times 100
\]

where LC is the ulcer index in rats treated solely with indomethacin and LT is the ulcer index in rats treated with indomethacin along with a therapeutic treatment (either famotidine or quercetin) [55].

Measurement of gastric enzyme activity

The activities of the enzymes, i.e., gastric-reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT), in rat stomach tissues were determined. For each biochemical estimation, 0.5 g of the whole gastric tissue sample was taken and ground with liquid nitrogen in a mortar. The ground tissues were then treated with 4.5 mL of the appropriate buffer (such as Tris-HCl buffer or phosphate buffer). The mixtures were homogenised on ice using an Ultra-Turrax homogeniser for 15 min. The homogenates were then used for determining the enzymatic activities. All assays were carried out at the room temperature in triplicates [25].

**Determination of GSH.** The mucosal surface of the stomach was collected by scraping, and then weighed and homogenised in 2 mL of 50 mM Tris-HCl buffer containing 20 mM EDTA and 0.2 mM sucrose (pH 7.5). Then the homogenate was immediately precipitated with 0.1 mL of ice-cold 25% trichloroacetic acid, centrifuged at 4000 rpm for 40 min at 4°C. The supernatant was used for the determination of GSH levels. Absorption was measured at 412 nm using a spectrophotometer. The GSH levels in the gastric mucosa were expressed as nmol/mg tissue [18].

**Assay of SOD.** The SOD was estimated based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with nitro blue tetrazolium to form Formosan dye. The SOD activity was then measured at 560 nm by the degree of inhibition of this reaction and was expressed as mmol/min/mg tissue [12].

**Assay of CAT.** The decomposition of \( H_2O_2 \) was carried out in the presence of CAT at 240 nm. The CAT activity was defined as the amount of enzyme required to decompose 1 nmol of \( H_2O_2 \) per minute, at 25°C and pH 7.8. The stomach tissues were homogenised in a potassium phosphate buffer solution (0.05 M; pH 7.4) and then centrifuged at 3215 rpm for 15 min. A hydrogen peroxide solution (0.05 M) was prepared with potassium phosphate buffer, and the substrate solution for the assay. A volume of 0.1 mL of the supernatant was mixed with 1.9 mL of phosphate buffer solution, and the absorbance decline was measured at 240 nm. The results were expressed as mmol/min/mg tissue [50].

Histological study

The specimens from the stomachs of all rats were fixed in 10% formalin and were then processed to form paraffin blocks. Serial sections of 5 μm in thickness were prepared and subjected to routine haematoxylin and eosin (H&E) staining [7].

Statistical analysis

All the results were expressed as mean ± standard error of the mean (SEM). The t-test for comparison between groups and one-way analysis of variance (ANOVA) for multi-sample groups at \( p < 0.05 \) were used to assess statistical significance in various groups of animals by using SPSS program version 2.01 (SPSS, 2008, UK).
Biological activities

Antioxidant. A diet rich in flavonoids reduces the risk for oxidative-stress related chronic diseases such as asthma [35], diabetes [2], coronary heart disease [28], lung cancer [71], and stroke [49]. Antioxidation could be a result of metal chelation [4], scavenging of radicals [68], enzyme inhibition [56], and/or induction of the expression of protective enzymes [46].

Within the flavonoid family, quercetin is the most potent scavenger for reactive oxygen species (ROS), including O$_2^-$ [15], and reactive nitrogen species (RNS) like nitric oxide [8], and ONOO [29]. Quercetin has been shown to be an excellent in vitro antioxidant activity [62].

Anti-inflammatory. Cyclooxygenase and lipoxygenase play an important role as inflammatory mediators. They are involved in the release of arachidonic acid, which is a starting point for a general inflammatory response. Quercetin is known to possess strong anti-inflammatory capacities [27].

Quercetin’s anti-inflammatory activity appears to be due to its antioxidant and inhibitory effects on inflammation-producing enzymes (cyclooxygenase, and lipoxygenase) and the subsequent inhibition of inflammatory mediators, including leukotrienes and prostaglandins [9].

Antiulcer effects. Quercetin has been shown to have antulcer and gastroprotective effects [16]. An interesting aspect of quercetin’s anti-ulcer effect is that it has been shown to inhibit growth of H. pylori in a dose-dependent manner in vitro [59].

It was reported that quercetin inhibits oxidative damage in ethanol-induced gastric lesions of rats [31].

It was found that the oral use of quercetin before and after administration of diclofenac sodium has been shown to be associated with fewer ulcers in test animals [74].

With oral administration to mice and rats, quercetin consistently did not induce any significant changes in several mutagenicity/genotoxicity endpoints (i.e., micronuclei, chromosomal aberrations, sister chromatid exchange, unscheduled DNA synthesis, and alkali-labile DNA damage) in somatic cells in comparison to untreated controls. Quercetin has been studied for its potential carcinogenicity in numerous long-term experimental animal studies, the majority of which indicated no evidence of significantly increased incidences of neoplasm formation related to its oral administration. Human studies have failed to show any adverse effects associated with the oral administration of quercetin in a single dose of up to 4 g or after 1 month of 500 mg twice daily [54].

Organs weight analysis hearts, lungs, livers, spleens, and kidneys of the sacrificed rats were carefully removed, washed with saline solution, dried with filter paper, and weighed independently. The relative organs weight was calculated as previously described (Chapman et al., 1959) using the following equation: Relative organ’s weight: Organ weight = Total body weight/100 [10].

RESULTS

Macroscopic examination (Fig. 1; Table 1)

The gross examination of the stomach lining of the control rats showed that the mucosa had a whitish colour with prominent rugae. However, the examination of the mucosal lining in indomethacin-treated rats revealed haemorrhages or linear breaks/erosions to the mucosal surface. In addition, the ulcer index in the rats of this treated group was very high, with an average of 40.52 ± 0.73 mm. This confirms the effectiveness of the treatment in inducing peptic ulcers. However, indomethacin-treated rats who had additionally received either famotidine or quercetin showed fewer hyperaemic areas and linear brown lesions. The ulcer index for the rats treated with famotidine was 27.3 ± 0.66 mm, which is significantly lower than that of the group treated with indomethacin alone (p < 0.05). The rats treated with quercetin showed an ulcer index of only 19.55 ± 0.62 mm, which was significantly lower than all the other indomethacin-treated groups (p < 0.05). Thus, it is clear that the treatment of animals with quercetin produced more effective protection against gastric ulcers compared to the treatment with famotidine.

Biochemical studies

Gastric juice volume. The volume of gastric juice obtained from rats with indomethacin-induced gastric ulcer (control + ve) untreated ulcer group was substantially greater than that in normal control rats (0.8 ± 0.14 mL compared with 0.26 ± 0.05 mL) (Table 2). Comparatively, indomethacin-treated rats that also received either quercetin or famotidine did not exhibit any increase in the volume of gastric juice (0.22 ± 0.15 and 0.30 ± 0.42, respectively) compared to control animals.

Gastric juice pH. The pH of gastric juice was found to be reduced in indomethacin-treated rats (mean 3.07 ± 0.58) compared with that of the normal rats (4.57 ± 0.24) (Table 2). However, indomethacin-treated rats who also received quercetin had pH values similar to
that of the controls. The pH values of those rats who received famotidine were even less acidic (5.52 ± 0.21).

Levels of gastric enzyme activity. The antioxidant markers GSH, SOD and CAT were found to be significantly decreased in the indomethacin-treated group when compared with the control group. However, those indomethacin-treated rats that were subsequently treated with either famotidine or quercetin had levels of antioxidant markers closer to that of the normal group and significantly higher than those of rats treated with indomethacin alone (Table 3).
The gastric glands were found to be narrow, tightly packed, straight, and perpendicular to the surface epithelium, occupying the whole thickness of the lamina propria. The lamina propria contained blood vessels and smooth muscle fibres (Fig. 2). The glands contained mucous cells (columnar with pale cytoplasm), parietal cells (central rounded nuclei) and chief cells in the basal region (columnar with basal oval nuclei, basal basophilic cytoplasm, and pale apical region) (Fig. 3). The resulting mucosal lesions in indomethacin-treated rats showed superficial damage with a distorted shape of the gastric glands. The cells lining the damaged glandular area appeared to be shrunken with deeply acidophilic cytoplasm. The basal part of the gland showed loss of architecture and inflammatory cells in the lamina propria with dilated and congested capillaries (Fig. 4).

The H&E-stained sections in famotidine-treated rats showed deep eosinophilic cytoplasmic epithelial cells with vacuolisation of some cells and an increase in mucus-secreting cells with inflammatory cells in the lamina propria and widened gastric pits (Fig. 5).
In quercetin-treated rats, the gastric mucosa was found to be similar to the control group with some inflammatory cells and congested blood vessels in the lamina propria (Fig. 6).

DISCUSSION

Flavonoids are a group of naturally occurring compounds that are widely distributed as secondary metabolites in the plant kingdom. Quercetin, a flavonoid, prevents oxidant injury and cell death using several mechanisms, such as scavenging oxygen radicals [1]. The present study assessed the effects of quercetin on gastric ulceration induced by indomethacin in adult male albino rats. Like other NSAIDs, indomethacin is an analgesic that exhibits side effects such as ulcerogenic action on stomach [40].

Quercetin’s anti-inflammatory activity appears to be due to its antioxidant and inhibitory effects on inflammation-producing enzymes (cyclooxygenase, lipoxygenase) and the subsequent inhibition of inflammatory mediators, including leukotrienes and prostaglandins [34]. Inhibition of histamine release by mast cells and basophiles also contribute to quercetin’s anti-inflammatory activity [33].

In the present study, we observed various macroscopic changes in the gastric mucosa. Normal white gastric mucosal rugae with a reduction in ulcer index were observed in both the control group and indomethacin-treated rats who also received quercetin. Contrarily, severe redness of the mucosa with brown haemorrhagic patches and a very high ulcer index were observed in those rats treated with indomethacin alone. These findings are similar to previous reports [65] in which the redness was attributed to vasodilatation and congestion of blood vessels, and the brown colorations were due to the effect of HCl on the haemorrhagic lesions.

The biochemical results showed that the volume of gastric secretion and its acidity were increased and the gastric enzymes were significantly decreased in indomethacin-treated rats compared with the control group. These results are in agreement with the findings of Seckin et al. [57]. Moreover, we also observed the destruction of the mucosal epithelial cells as well as a highly eosinophilic cytoplasm and pyknotic nuclei along the length of fundic glands from the neck to the base. This is in agreement with the study of Drăghia [17], which reported a decrease in oxidative activity in the surface epithelium and vascular endothelium. NSAIDs, such as indomethacin, produce beneficial effects through their ability to block cyclooxygenase, thereby inhibiting prostaglandin production [23]. Decreased prostaglandin level contributes to the pathogenesis of gastroduodenal mucosal ulcerations [26]. In addition, mucosal lesions due to NSAID-induced gastrointestinal damage are associated with an increase in ROS such as superoxide radical anions and hydroxyl radicals [64]. These mechanisms, including those related to prostaglandin suppression, lead to microvessel occlusion and subsequent hyper-production of reactive oxygen metabolites. In particular, indomethacin causes gastric erosions with increased lipid peroxidation and decreased glutathione peroxidase activity [47]. The presence of superoxide anions (O2−) may be responsible for the gastric damage as well as other tissue damage. Therefore, many researchers have focused on oxygen-derived free radicals. This mechanism, in combination with ROS, damages membrane proteins by inducing lipid peroxidation and attacking unsaturated fatty acids [24]. Therefore, antioxidant defence systems and their components, including antioxidant enzymes, foods, and drugs, are considered to be important in preventing the toxic and disease-causing effects of oxygen-derived free radicals. Oxygen-handling cells have antioxidant enzymes that can protect them [66].

Both famotidine and quercetin treatments restored the gastric mucosa to near normality. However, the famotidine-treated rats had inflammatory cells infiltration with congested blood vessels in their lamina propria, whereas the quercetin-treated rats had an increase in surface mucous cells due to the increase in the apical cytoplasmic content of mucus granules. In addition, a widening of the gastric pits with increased mucus secretion was also observed. The histological results of the indomethacin group were confirmed by that of Fawcett’s [22] study, which reported an apparent increase in mucin in stomachs in which most of the surface mucous cells were filled with rounded mucoid granules. The role of gastric mucins has been widely explained as forming a barrier between the surface epithelial cells and gastric juices; mucus may have the ability to resist enzymatic hydrolysis due to its carbohydrate moieties. Acidic mucin may have a role in protecting the pit cells against the effect of HCl during its passage. The alternating layers of neutral and acidic mucins in the surface coat act as a safeguard against HCl and other digestive enzymes in the lumen [70].

Studies have shown that some of the altered cells lack the ability to recover [5]. In this study group,
the treatment with indomethacin led to the loss of architecture and vacuolation of cells while the quercetin treatment resulted in a normal mucosa except for an increase in mucus granules in surface mucus cells. Quercetin exerts antioxidant influences by several mechanisms; these include scavenging oxygen radicals, protecting against lipid peroxidation, and chelating metal ions [37]. The high antioxidant activity of quercetin may be attributed to its capacity to diffuse through the cell membrane at a high rate [32]. Quercetin induces a decrease in the proinflammatory cytokines [30]. Gastric mucosa can be repaired after mucosal injury by mucosal restitution and re-epithelisation by sloughing off the damaged cells. This is achieved by rapid migration of viable stem cells to the injured site to replace the dead cells during ulcer healing [13]. However, human studies have failed to show any adverse effects associated with the oral administration of quercetin in a single dose of up to 4 g or after 1 month of 500 mg twice daily [54].

One of the limitation of this study is that quercetin has been studied for its potential carcinogenicity in numerous long-term experimental animal studies, the majority of which indicated no evidence of significantly increased incidences of neoplasm formation related to its oral administration.

CONCLUSIONS

Quercetin has effective anti-ulcer activity against indomethacin-induced ulcerogenesis, and it can be used for therapy of ulcerogenesis and gastric mucosal injury. In particular, patients treated with NSAIDs may benefit from the consumption of a quercetin-rich diet.

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