The Defensive Effect of Quercetin on Indomethacin Induced Gastric Damage in Rats

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Abstract: The present study aimed to evaluate the effect of quercetin on indomethacin induced gastric damage in experimental rats. Animals were induced for gastric ulcer with indomethacin (200-250 kg bodyweight) in water, orally and treated orally with QUE (50 mg/kg bodyweight). The effective dose; this dose elicited a maximum reduction in lesion index. The gastroprotective effect of QUE was assessed from the levels of volume of gastric juice, pH, pepsin, acid output in gastric juice. The levels of DNA, protein bound carbohydrate complexes-hexose, hexoseamine, sialic acid, fucose in gastric juice the levels of RNA in gastric juice were assessed. A significant reduction in lesion index was observed in ulcer induced animals treated with quercetin compared to ulcerated rats (IND). A significant increase was observed in pH, protein bound carbohydrate complexes, nucleic acids with a significant decrease in volume of gastric juice, free and total acidity, pepsin concentration, acid output and the gastroprotective activity of quercetin. From the data presented in this study it could be concluded that quercetin acts as a gastroprotective agent probably due to its cytoprotective nature.

Key words: Indomethacin · Quercetin, protein bound carbohydrate · Gastric juice · Lipid peroxidation

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are therapeutically useful as analgesic and anti-inflammatory agents. They are the most frequently prescribed drugs worldwide. Aspirin is used increasingly for the treatment of arthritis, cerebrovascular disease and coronary artery disease. Indomethacin (IM) is another, most popular NSAID in India and abroad, prescribed extensively for rheumatoid arthritis, osteoarthritis, cervical spondylitis, ankylosing spondylitis and acute musculoskeletal disorders and infective inflammation.

A long-term use of NSAIDs among patients is associated with a range of oesophagho-gastro-duodenal changes with a very high morbidity and mortality [1]. Demonstration from observational and clinical studies indicated that NSAIDs may induced adverse upper gastrointestinal hemorrhage, peptic ulcer disease, liver and bone marrow toxicity [2, 3]. NSAIDs are also reported to cause small intestinal and kidney damage [4, 5].

Gastric ulcer is a common disorder whereas discontinuity in the gastric mucosa is observed [6]. It is caused by many factors like stress, drugs, alcohol, etc. [7] and is reported to be due to an imbalance between offensive acid-pepsin secretion and defensive mucosal factors like mucin secretion and cell shedding [8]. NSAIDs are widely used for a long term in the treatment of rheumatoid and osteoarthritis to relieve pain and inflammation [9]. However, NSAIDs also produce a broad range of toxic effects, frequently causing gastrointestinal (GI) toxicity that result in ulceration, bleeding and perforation of stomach [10].

The toxicity of NSAIDs is mainly attributed to inhibition of prostaglandin synthase activity that inhibits...
prostaglandin production in the GI tract resulting in accumulation of intracellular arachidonic acid [11]; NSAIDs also causes induction of mitochondrial injury in hepatocytes through uncoupling of oxidative phosphorylation [12]; and production of reactive metabolites that covalently bind to critical cellular proteins [13]. Chronic administrations of NSAIDs cause gastro duodenal mucosal erosions in approximately 35-60% of patients, gastric or duodenal ulceration in 10-25% of patients and severe complications, such as gastrointestinal hemorrhage or perforation in 1% of patients [14].

Based on the facts available in literature, the present study was directed towards an assessment of gastroprotective efficacy of quercetin, against NSAID induced gastric ulcer from the analysis of lesion index, total protein, acid output, pH, pepsin and protein bound carbohydrate complexes-hexose, hexosamine, sialic acid and fucose, nucleic acids (DNA, RNA).

MATERIALS AND METHODS

Chemicals: Ethanol, NaOH, Orcinol reagent, Sulphuric acid, indomethacin, HCl, Acetyl acetone reagent, Periodic acid, Sodium Meta arsenate, Thiobarbituric acid, Butanol, Sulphuric acid, TCA, Diphenylamine reagent, were obtained from Sigma Chemical. All other chemicals were of analytical grade.

Animals: Adult male albino rats of Wistar strain weighing 200-250 g were obtained from the Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalainagar, India. They were individually housed in polypropylene cages under a 12 h light dark cycle. The animals were maintained on a commercial diet manufactured by the Hindustan Lever Ltd. Mumbai, India. Food and water were provided ad libitum

Pyloric Ligation: Pyloric ligation was done 18 h after quercetin administration [15]. Briefly, the abdomen of the animals was opened under light anesthesia by a small midline incision below the xiphoid process, pyloric portion of the stomach was slightly lifted out and ligated avoiding damage to its blood supply. The stomach was replaced and the abdominal wall closed by interrupted sutures.

Protein and Nucleic Acids: Protein was estimated by the method of Lowry et al. [16] using alkaline copper reagent and Folin’s reagent. The colour developed was read at 640nm. The level of protein was expressed as mg/g tissue. DNA was estimated according to the method of Burton [17]. The nucleic acid extract was treated with diphenylamine reagent and the blue color developed was read at 640 nm. Values were expressed as mg/g tissue. RNA was determined by the method of Rawal et al. [18]. The nucleic acid extract was treated with Orcinol and the green color developed was read at 675 nm. Values were expressed as mg/g tissue.

Glycoprotein Components in Gastric Juice: Glycoproteins in gastric juice were precipitated, hydrolysed and the protein-bound hexose, hexosamine, fucose and sialic acid were estimated [19]. The known amount of defatted tissue was hydrolysed with 1.0 ml of 2N HCl and 1% phosphotungstic acid at 100 C for 4 h to liberate the protein-bound compounds.

The hydrolysate was neutralized with 4N sodium hydroxide and was used for the estimation. Estimation of hexose was done by the method of Neibes [20] using Orcinol reagent. The colour developed was read at 540 nm. The values were expressed as mg/g tissue. Hexosamine was estimated by the method of Wagner [21] using acetyl acetone reagent and Ehrlich’s reagent and read at 540 nm. The content of hexosamine was expressed as mg/g tissue. Analysis of sialic acid was carried out by the method of Warren [22]. Periodate solution and thiobarbituric acid was added and the absorbance was read at 540 nm. The levels of sialic acid were expressed as mg/g tissue. Fucose was estimated by the method of Diche and Shettles [23] using H2SO4 reagent and cysteine-HCl. The absorbance was read at 396 nm. The fucose content was expressed as mg/g tissue.

Statistical Analysis: Results are expressed as Mean ±S.D. for six animals in each group. The data were statistically evaluated by SPSS/10 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by Dennett’s T3 multiple comparison test. The significance levels were analyzed at \( p < 0.001 \), \( p < 0.01 \), \( p < 0.05 \).

RESULTS

Table 1 shows the dose dependent changes in lesion index of experimental group of rats. Rats administered with indomethacin showed marked increase in lesion index. A significant reduction (\( p < 0.001 \)) in the incidence of lesions was observed in rats treated with quercetin compared to indomethacin induced rats.
Table 1: Effect of pretreatment with quercetin (50mg/kg) on the volume of gastric secretion, total acidity, pepsin activity and protein content in indomethacin-treated rats

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>CON</th>
<th>INDO</th>
<th>IND+QUA</th>
<th>CON+QUA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>3.28±0.16</td>
<td>5.13±1.9#</td>
<td>4.00±1.77#</td>
<td>4.8±0.23NS</td>
</tr>
<tr>
<td>Total acidity (mEq/l/4 h)</td>
<td>121.0±10</td>
<td>74.4±15.5#</td>
<td>60.43±6.8#</td>
<td>68.5±4.8NS</td>
</tr>
<tr>
<td>Pepsin activity (mg tyrosine liberated/ mg protein/h)</td>
<td>1.56±0.2</td>
<td>2.56±0.6#</td>
<td>1.64±0.04#</td>
<td>1.65±0.24NS</td>
</tr>
<tr>
<td>Protein (mg/ml)</td>
<td>0.97±0.2</td>
<td>3.78±0.05#</td>
<td>2.35±0.07#</td>
<td>2.38±0.73NS</td>
</tr>
<tr>
<td>Ulcer score</td>
<td>0</td>
<td>25.8±0.75#</td>
<td>11.6±0.75#</td>
<td>9.14±1.27NS</td>
</tr>
<tr>
<td>PH</td>
<td>4.4±0.4</td>
<td>1.75±0.19#</td>
<td>4.05±0.21#</td>
<td>4.53±0.36NS</td>
</tr>
</tbody>
</table>

Values are expressed as mean±S.D. for six animals in each group. Groups are compared as follows: Control vs. IND, IND vs. IND + QUE and Control vs. QUE only given. Significance represented as #p < 0.001, NS: non-significant.

Table 2: Level of protein bound carbohydrate complexes in the gastric juice of control and experimental group of rats

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>CON</th>
<th>INDO</th>
<th>IND+TAU</th>
<th>CON+TAU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total hexose</td>
<td>412.33 ± 12.11</td>
<td>294.02±19.18</td>
<td>384.92 ±14.64#</td>
<td>423.8 ± 9.94NS</td>
</tr>
<tr>
<td>Hexoseamine</td>
<td>205.55 ± 16.27</td>
<td>127.63 ± 6.94#</td>
<td>176.08 ± 13.8#</td>
<td>214.58 ± 1.83NS</td>
</tr>
<tr>
<td>Sialic acid</td>
<td>34.97 ± 3.87</td>
<td>20.47 ± 2.82#</td>
<td>31.67 ± 2.66#</td>
<td>35.3 ± 3.38NS</td>
</tr>
<tr>
<td>Fucose</td>
<td>46.22 ± 2.9</td>
<td>32.95 ± 1.83#</td>
<td>45.98 ± 4.0#</td>
<td>46.4 ± 3.06NS</td>
</tr>
<tr>
<td>TC</td>
<td>699.07 ± 30.34</td>
<td>475.07 ± 27.95#</td>
<td>638.65 ± 12.32#</td>
<td>720.08 ± 4.79NS</td>
</tr>
<tr>
<td>Protein</td>
<td>262.83 ± 9.15</td>
<td>340.28 ± 15.19#</td>
<td>279.95 ± 16.4#</td>
<td>267.83 ± 19.67NS</td>
</tr>
<tr>
<td>TC: P ratio</td>
<td>2.67 ± 0.2 1</td>
<td>41 ± 0.07#</td>
<td>2.29 ± 0.19#</td>
<td>2.7 ± 0.24NS</td>
</tr>
<tr>
<td>DNA</td>
<td>127.98 ± 9.97</td>
<td>215.1 ± 17.9#</td>
<td>156.13 ± 14.09#</td>
<td>124.33 ± 16.34NS</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. for six animals in each group. Groups are compared as follows: CONTROL vs. IND, IND vs. IND + QUE and CONTROL vs. QUE only given. Significance represented as #p < 0.001, NS: non-significant.

Levels of acid secretory parameters in gastric juice of control and experimental group of rats were shown in table 1. The volume of gastric juice, free and total acidity, pepsin concentration and acid output of gastric juice were increased significantly (p < 0.001) in indomethacin toxicity induced rats with a significant decrease (p < 0.001) in pH compared to control rats. Indomethacin toxicity induced + Quercetin treated rats showed a significant decrease (p < 0.001) in volume of gastric juice, free and total acidity, pepsin concentration and acid output with a significant increase (p < 0.001) in pH compared to indomethacin toxicity induced rats. No significant changes were observed in rats treated with Quercetin alone. Table 2 depicts the levels of protein bound carbohydrate complexes and DNA in the gastric juice of control and experimental group of rats. IND caused a significant decrease (p < 0.001) in TC:P with a concomitant increase (p < 0.001) in protein and DNA. In IND+ QUE rats, a significant increase (p < 0.001) in the carbohydrate concentration in terms of TC:P ratio and significantly lowered (p < 0.001) protein concentration in gastric juice was observed. QUE tended to increase mucus activity in IND + QUE group. The concentration of DNA in gastric juice was significantly decreased (p < 0.001) in IND+QUE. Quercetin only administered rats registered no significant changes.

**DISCUSSION**

The gastro protective effectiveness of the quercetin was evident from significant reduction in the lesion index in QUE treatment rats as against ulcerated rats. The wide range of biochemical and pharmacological activities in vitro including antioxidant, antitumour, antiviral and antimicrobial, enzyme inhibiting and radical scavenging properties. Their presence in fairly high concentrations has commonly been used to explain the claimed curative and palliative efficacy of a variety of traditional herbal medicines and the reports of profound health beneficial effects of certain foodstuffs [24]. Quercetin may prevent ulcer development due to their protein precipitating and vasoconstricting effects [25] their severe action helps in precipitating microproteins on the ulcer site, thereby forming an impervious layer over the lining that hinders gut secretions and protects the underlying mucosa from toxins and other irritants [26]. Flavonoids are reported to possess the property of preventing the formation of lesions by various necrotic agents [27]. Antioxidants prevent the lesion formation caused by various ulcerogens [28]. Quercetin in accelerating wound healing has been explained by several mechanisms, such as stimulating the contraction of the wound and increasing the formation of epithelisation[29].
The gastro protective action of quercetin. Acid is considered as an important factor in the development of acute and chronic gastric mucosal lesions. Suppression of gastric acid by surgical and a variety of pharmacological means [30] provides effective and rapid healing of ulcer [31]. Gastric acid has been shown to play an important permissive role in NSAID associated mucosal injury [32]. Acid reducing property is discussed with several anti-ulcer drugs.

In the present study, increase in pH and decrease in acidity, acid output and pepsin concentration were evidenced in ulcerated animals treated with QUE, which is highly desirable for gastroprotection and antiulcer effect. Ulcerated rats showed an alteration in the peptic activity which is in accordance with previous report [33]. The modification in pepsin concentration on QUE treatment depicts the efficacy of QUE on gastric secretions. The gastroprotective effect of QUE may be due to the direct action on the acid producing cells.

Increase in mucosal resistance and decrease in aggressive factors mainly acid and pepsin are associated with gastric protection offered by prostaglandins. Prostaglandins E2 and I2 are the predominant prostaglandins synthesized by gastric mucosa and are known to inhibit the secretion of gastric acid and stimulate the secretion of mucus, bicarbonate and hydrophobic surfactant like phospholipids secretion in gastric epithelial cells [34]. The possible involvement of QUE on enhancing mucosal resistance could have offered gastroprotection. Literature reports that flavonoids can be related to the antiulcer activity, playing a major role in some mechanisms of action [35]. Not only flavonoids, but also other phenolic substances are known for their antioxidant activities, which can be related to some antiulcer mechanisms [36]. The potent antioxidant property of quercetin could have resulted in its ulcer healing activity. Moreover, one of the essential criteria to determine the status of mucus resistance/barrier is the state of mucus secretion. This mucus consists of mucin type of glycoproteins, which can be estimated by the ratio of total carbohydrates to protein. These high molecular weight glycoproteins are mainly responsible for viscous and gel forming characteristics of the mucus. Increased mucus secretion by the gastric mucosal cells can prevent gastric ulceration by several mechanisms, including lessening of stomach wall friction during peristalsis and gastric contractions, improving the buffering of acid gastric juice and by acting as an effective barrier to back diffusion of H+ ions [37]. Quercetin exhibited ulcer healing activity by increasing hexosamine and carbohydrate/protein ratio and adherent mucus content against indomethacn induced ulcer. This results in the increase in mucus secretion.

The importance of mucus secretion as a response to gastric mucosal trauma has long been recognized [38]. Mucosal barriers are the most significant factors for gastric protection [39]. More the production of mucous, the less was the degree of ulceration. Mucus also protects the mucosa and sub-mucosa from inflammatory reaction. The higher the mucin contents the lower is the free acidity. Mucosal defense agents are a new dimension in the treatment of gastro-duodenal diseases [40]. The strengthening of the mucin barrier further led to a decrease in DNA content of the gastric juice, indicating a decrease in cell shedding [41].

In the present study the DNA content in gastric juice increased upon quercetin treatment, which acts as a reliable index for cell exfoliation and represents the increase in life span of mucosal cells. Cell shedding is an indication of integrity of the gastric mucosa. The enhanced level of cell shedding denotes the loss of integrity and decrease life span of cells whereas decreased shedding would indicate enhanced life span of cells and promotion of defensive mechanism [42]. In the present study, the QUE administration reduced cell shedding, thereby indicates the integrity of mucosa and its defensive mechanism and the increase in DNA content in gastric mucosa indicates gastric mucosal renewal.

The decrease in the protein content of gastric juice suggests the decrease of leakage of plasma protein into gastric juice [43], the mechanism of action of QUE may be due to the coating property that has a protective property on the gastric mucosa. Repair of gastric mucosa is both by restitution and cell proliferation. Recently, some polyphenolics have been found to have a preventive action on gastric injury in rats. Some research has focused on the antiulcer activity of polyphenol [44]. This activity was mainly explained by strong antioxidant power and/or by some other factors, such as strong protein-binding capacity [45], modulation of leukocyte function [46] and mucus production and restoration [47]. The strong protein-binding capacity has been reported to be a characteristic of highly polymerized procyanidins [48]. It is believed that the antioxidant activity of polyphenols is an important factor because reactive oxygen and/or free radicals are related to the occurrence of ulcers. Some saponins [49] have been found to promote ulcer healing by forming protective mucus barrier on the gastric mucosa [24]. Since the quercetin contains triterpenoid saponins and flavonoids, the ulcer healing activity observed in the present study could be attributed to these constituents.
NSAIDs inhibit cyclooxygenase enzyme complex that convert free eicosapolyenoic acids like arachidonic acid to cyclic endoperoxides, the key intermediate in prostaglandin synthesis [49]. Gastric ulcerprotective effect of QUE might be mediated by stimulating the production of prostaglandin which adds further evidence to confirm the efficacy of quercetin against gastric ulcer induced by indomethacin.

The gastroprotective effect may be attributable to the cytoprotective, prostaglandin inducing property and antioxidant potential in quercetin. The antioxidant effect of the QUE could possibly be due to the presence of flavonoids and also due to the presence of other phytoconstituents characteristic of the QUE in a synergistic combination, although further studies are required to identify the exact mechanism underlying the inhibitory role of quercetin [50]. However, it is important to note that previous investigations have exhibited the anti-ulcer activity of quercetin. In conclusion, quercetin may represent an attractive therapeutic option for healing against NSAID induced gastric ulcers. Further studies are progressing in our laboratory, focusing the detailed mechanism of action of quercetin in gastric ulcer.

REFERENCES


