Antiulcer Potential of Morin in Acetic acid-Induced Gastric Ulcer via Modulation of Endogenous Biomarkers in Laboratory Animals

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ABSTRACT

Background: Peptic ulcer disease is a result of an imbalance between aggressive and defensive factors. Morin, a bioflavonoid exhibits many biological activities such as antioxidant and anti-inflammatory properties. Objective: The present study was conducted to unravel the therapeutic potential of morin in acetic acid-induced gastric ulcer. Materials and Methods: Gastric ulcer was induced in male Wistar rats (180-220 g) by applying glacial acetic acid (10 M, 100 μL) to serosa of the stomach. Morin (10, 30 and 100 mg kg⁻¹, p.o.) was administered for 15 days after the induction of ulcer. After end of treatment gastric specimens were collected for biochemical and histological evaluation. Results: There was a significant (p<0.01 and p<0.05) reduction in the ulcer area and ulcer index by morin (30 and 100 mg kg⁻¹) treatment. It also significantly (p<0.01 and p<0.05) decreased the level of malondialdehyde (MDA) and increased levels of superoxide dismutase (SOD) as well as reduced glutathione (GSH). Histological aberration induced by acetic acid also reduced by morin administration. Conclusion: The present findings elucidate the antiulcer potential of morin in the acetic acid induced gastric ulcer by virtue of its antioxidant property.

Key words: Antiulcer, morin, oxidative stress, acetic acid induced gastric ulcer

INTRODUCTION

Human beings undergo the pathological condition of peptic ulcer disorder when an imbalance between aggressive and defensive factors for the same dominates, leading to loss of integrity and hence the functioning of gastric mucosal barrier and simultaneously its repair mechanisms. The aggressive factors include Helicobacter pylori infection and Non steroidal anti-inflammatory drugs that erode the protective gastric mucosa, pepsin, bile salts, impaired motility and consumption of alcohol. However, the protective factors (enhanced mucus secretion, production of bicarbonates, mucoprotective prostaglandin synthesis, microcirculation of sub epithelial components) are capable enough to combat the physiologically undesirable pre posteriorly and irrational aggressive factors. Therefore, research has inspired a big deal of thirst to the researcher to discover biocompatible agents that may aid in surge of the gastrodefensive factors and hence suppression of the aggressive ones.

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Present market scenario provide drugs such as H2-receptor blockers (like Ranitidine, Cimetidine, Famotidine, etc.) proton pump inhibitors (such as omeprazole, lansoprazole, pantoprazole, rabeprazole, etc.) and cytoprotecants (such as carbenoxolone, deglycyrrhizised liquorice, sucralfate, misoprostol and bismuth chelate, etc.) for treatment of peptic ulcer. However, most of them provides symptomatic relief in fraction of patient and are of a also associated with one or more side effect as well as drug-drug interactions. Mother nature from the pre-medieval period has a rich source of agents having least undesirable side effects and this led to finding of a novel therapeutic entities for treatment of gastric ulcer.

Animals models have played vital role in development of new therapeutic moieties for treatment of various disease. The serosal application of acetic acid is a simple and accurate animal model that mimics all the clinico pathological condition that resembles to humans of gastric ulcer. Advantages of acetic acid induced gastric ulcer model is having small coefficient of variation with low rate of perforation. Other acetic acid induced gastric ulcer models includes injection of acetic...
acid into gastric mucosa however, it causes persisting ischemia of upper and lower gastric mucosal parts due to embolism of blood vessels of submucosa. Other acute ischemia of upper and lower gastric mucosal parts due to distension of blood vessels of submucosa and stress induced ulcers are inappropriate since they penetrate the glandular portion’s muscle layers and at times relapse post healing. Hence, directing this natural treatment to the applied model is a perfect combination from both the sides.

Flavonoids are polyphenols obtained from many herbs having many biological activities. Morin (2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxychro-men-4-one) is a kind of flavonoid/a group of flavanols, obtained from guava leaves, onion, apple and other Moraceae families. Morin possesses an array of pharmacological properties like antioxidant, anti-inflammatory, anti-tumour and cytoprotective. It also showed protective effect against airway hyper responsiveness in allergic asthma via modulation of immune-inflammatory biomarkers. However, its antiulcer profile in acetic acid ulcer has been inadequately explored. Hence, the aim of present investigation was to evaluate the antiulcer potential of morin in acetic acid induced gastric ulcer in laboratory rats.

MATERIALS AND METHODS

Chemicals: Morin (Sigma Aldrich, USA), Omeprazole (Symed Pharmaceutical Pvt. Ltd., Hyderabad). Bovine serum albumin, tris buffer, sucrose, copper sulphate, sodium potassium tartrate, ethylene diamine tetra acetic acid disodium salt, Folin’s phenol reagent, sodium hydroxide, sodium bicarbonate, potassium chloride, hydrochloric acid and conc. sulphuric acid were purchased from S.D. Fine Chemicals, Mumbai, India.

Animals: Healthy adult male wistar rats (180-200 g) were obtained from the National Institute of Biosciences, Pune (India). The animals were housed in groups of 6 in solid bottom polypropylene cages. They were maintained at 24±1°C, with relative humidity of 45-55% and 12:12 h dark light cycle. The animals were acclimatized for a period of two weeks and were kept under pathogen free conditions. The animals had free access to standard pellet chow (Pranav Agro industries Ltd., Sangli, India) throughout the experimental protocol. The animals had access to filtered water. All experiments were carried out between 09:00 and 17:00 h. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune (CPCSEA/PCL/39/2014-15) and performed in accordance with the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India on animal experimentation.

Induction of acetic acid induced gastric ulcer: Rats (n = 6/group) were treated with acetic acid according to the modified method as described previously. Briefly, rats were anesthetized by thiopental sodium (35 mg kg⁻¹, i.p.) and subjected to coeliotomy to expose the stomach. Glacial acetic acid (10 M, 100 μL) was applied to the surface of the serosa at the junction of the gastric fundus and antrum for 1 min through a plastic tube and then immediately washed with sterile saline. Sham control animals also underwent surgery however, they received equal volume of saline instead of glacial acetic acid. After 1 h of recovery, the rats were subjected to their respective treatments group as follows:

- **Sham control:** (S): Rats received distilled water (10 mg kg⁻¹, p.o.) for 15 days
- **Acetic acid control:** (AAC): Rats received distilled water (10 mg kg⁻¹, p.o.) for 15 days
- **Omeprazole (20):** O (20): Rats received Omeprazole (20 mg kg⁻¹, p.o.) treatment for 15 days
- **Morin (10):** M (10): Rats received Morin (10 mg kg⁻¹, p.o.) treatment for 15 days
- **Morin (30):** M (30): Rats received Morin (30 mg kg⁻¹, p.o.) treatment for 15 days
- **Morin (100):** M (100): Rats received Morin (100 mg kg⁻¹, p.o.) treatment for 15 days

The morin was freshly prepared in three different dosages (10, 30 and 100 mg kg⁻¹) and administered to the animals. After the completion of treatment period, the animals were sacrificed with overdose of anesthetic ether and the stomach was removed. For determination of ulcer area, each stomach was incised along the greater curvature and washed with normal saline and was scanned using CCD scanner at a magnification of 2400 dpi. The images were processed using image J software and Adobe Photoshop to determine ulcer area. The ulcer index and % inhibition was determined according to method described previously.

Determination of total acidity: The entire gastric content was transferred into centrifuge tubes. It was used for estimation of total acidity. The tubes were centrifuged at 1000 rpm for 10 min and the gastric volume was directly read from the graduation on the tubes. The supernatant was then collected and total acidity was determined by titrating 1.0 mL of gastric
Biochemical estimation: The 500 mg tissue from the glandular portion of stomach was excised, washed, chopped and homogenized at 3000 rpm in chilled Tris buffer (10 mM, pH 7.4) at a concentration of 10% w/v. The homogenates were centrifuged at 10,000 g at 0°C for 20 min and employed to estimate various biochemical parameters viz., total protein, superoxide dismutase (SOD) contents, glutathione (GSH) content and lipid peroxidation [malondialdehyde (MDA)] content according to methods described previously.20-25

Histopathological studies: Freshly excised stomach of one animal from each group was washed with saline and preserved in 10% formaldehyde solution for histopathological studies. It was processed for 12 h using isopropyl alcohol, xylene and paraffin embedded for light microscopic study (Nikon E200). Paraffin embedded tissue section cut at 5 μm thickness were prepared and stained after deparaffination using hematoxyline and eosin stain (H and E) to verify morphological assessment of stomach damage. Photomicrographs were captured at a magnification of 40 X.

Statistical analysis: All the results were expressed as Mean±SEM. Statistical comparisons were made between drug-treated groups and acetic acid control groups. The data was statistically analyzed by one way analysis of variance (ANOVA) followed by Dunnett’s test, *p<0.05, **p<0.01, ***p<0.001 as compared to acetic acid control group, S: Sham control, AAC: Acetic acid control, O (20): Omeprazole (20 mg kg−1) treated, M (10): Morin (10 mg kg−1) treated, M (30): Morin (30 mg kg−1) treated and M (100): Morin (100 mg kg−1) treated

Table 1: Effect of Morin on various gastric parameters of acetic acid induced ulcer in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer area (mm²)</th>
<th>Ulcer index</th>
<th>Total acidity (mEq L⁻¹)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AAC</td>
<td>3.60±0.60</td>
<td>5.22±0.90</td>
<td>37.50±11.09</td>
<td>-</td>
</tr>
<tr>
<td>O (20 mg kg⁻¹)</td>
<td>0.73±0.20***</td>
<td>0.57±0.12***</td>
<td>45.30±17.08</td>
<td>86.90</td>
</tr>
<tr>
<td>M (10 mg kg⁻¹)</td>
<td>2.66±0.11*</td>
<td>2.39±0.10</td>
<td>45.55±15.00</td>
<td>50.04</td>
</tr>
<tr>
<td>M (30 mg kg⁻¹)</td>
<td>1.49±0.10***</td>
<td>1.42±0.17***</td>
<td>40.07±8.16</td>
<td>71.19</td>
</tr>
<tr>
<td>M (100 mg kg⁻¹)</td>
<td>2.14±0.10**</td>
<td>2.06±0.15**</td>
<td>40.41±5.77</td>
<td>57.31</td>
</tr>
</tbody>
</table>

Data is expressed as Mean±SEM (n = 5) and analyzed by one way ANOVA followed by Dunnett’s test, *p<0.05, **p<0.01, ***p<0.001 as compared to acetic acid control group, S: Sham control, AAC: Acetic acid control, O (20): Omeprazole (20 mg kg⁻¹) treated, M (10): Morin (10 mg kg⁻¹) treated, M (30): Morin (30 mg kg⁻¹) treated and M (100): Morin (100 mg kg⁻¹) treated

RESULTS

Effect of Morin on ulcer area, ulcer index, total acidity and percentage of inhibition in acetic acid induced ulcer in rats: Exposure of gastric serosa to acetic acid resulted in formation of ulceration in gastric mucosa. However, there was a significant (p<0.001) decrease in acetic acid induced ulcer by omeprazole (20 mg kg⁻¹) treatment as compared to acetic acid control group. Administration of morin (10, 30 and 100 mg kg⁻¹) significantly (p<0.05, p<0.001 and p<0.01, resp.) decreased ulcer area as compared to the acetic acid control group (Table 1). The ulcer index was significantly (p<0.001) reduced by omeprazole (20 mg kg⁻¹) treatment as compared to acetic acid control group. Administration of morin (30 and 100 mg kg⁻¹) significantly (p<0.001 and p<0.01) reduced ulcer index as compared to acetic acid control group (Table 1). Administration of omeprazole (20 mg kg⁻¹) showed 86.90% inhibition against acetic acid induced gastric ulcer, whereas morin (10, 30, 100 mg kg⁻¹) showed 50.04, 71.19 and 57.31% inhibition against acetic acid induced ulcer in rats (Table 1). Exposure of gastric serosa to acetic acid did not produced significant alteration in total acidity in acetic acid control rats as well as omeprazole (20 mg kg⁻¹) and morin (10, 30, 100 mg kg⁻¹) treated rats (Table 1).

Effect of Morin on acetic acid induced alteration in gastric mucosal total protein and MDA level in rats: There was a significant (p<0.001) increase in the level of gastric mucosal MDA and total protein in acetic acid control rats as compared to sham control group. However, elevated gastric mucosal MDA and total protein level was significantly (p<0.01) decreased by omeprazole (20 mg kg⁻¹) treatment as compared to acetic acid control group. Whereas, administration of morin (30 and 100 mg kg⁻¹) also significantly (p<0.01 and p<0.05) reduced the level of MDA and total protein in gastric mucosa (Fig. 1a, d).

Effect of Morin on acetic acid induced alteration in gastric mucosal SOD and GSH level in rats: There was a significant (p<0.001) decrease in the level of gastric mucosal SOD and GSH in acetic acid control rats as compared to sham control group. Whereas gastric mucosal SOD and GSH level was significantly (p<0.001) increased in omeprazole (20 mg kg⁻¹) treated group as compared to sham control group. Whereas gastric mucosal SOD and GSH level was significantly (p<0.001) increased in omeprazole (20 mg kg⁻¹) treated group.
Fig. 1(a-d): Effect of Morin on acetic acid induced alteration in (a) Total protein level, (b) SOD level, (c) GSH level and (d) MDA level in gastric tissue. Data are expressed as Mean± SEM (n = 5) and analyzed by one way ANOVA followed by Dunnett’s test. *p<0.05, **p<0.01, ***p<0.001 as compared to acetic acid control group. S: Sham control, AAC: Acetic acid control, O (20): Omeprazole (20 mg kg⁻¹) treated, M (10): Morin (10 mg kg⁻¹) treated, M (30): Morin (30 mg kg⁻¹) treated and M (100): Morin (100 mg kg⁻¹) treated.

Table 2: Effect of Morin on histopathological changes of rat stomach in acetic acid induced ulcer

<table>
<thead>
<tr>
<th>Groups</th>
<th>Necrosis</th>
<th>Haemorrhage</th>
<th>Inflammation</th>
<th>Congestion</th>
<th>Oedema</th>
<th>Cellular infiltration</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AAC</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>O (20 mg kg⁻¹)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M (10 mg kg⁻¹)</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>M (30 mg kg⁻¹)</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M (100 mg kg⁻¹)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

S: Sham control, AAC: Acetic acid control, O (20): Omeprazole (20 mg kg⁻¹) treated, M (10): Morin (10 mg kg⁻¹) treated, M (30): Morin (30 mg kg⁻¹) treated and M (100): Morin (100 mg kg⁻¹) treated. -: No abnormality detected, +: Damage/active changes up to less than 25%, ++: Damage/active changes up to less than 50%, +++: Damage/active changes up to less than 75%, ++++: Damage/active changes up to more than 75%.

Effect of Morin on histopathological changes of rat stomach in acetic acid induced ulcer: Administration of morin (30 and 100 mg kg⁻¹) significantly (p<0.01 and p<0.05) increased the level of SOD and GSH in gastric mucosa as compared to the acetic acid control group (Fig. 1b, c). Rats that received treatment with morin (10 mg kg⁻¹) showed disruption to the surface epithelium with presence of oedema, hemorrhage and increased haemorrhage along with inflammatory infiltration (Fig. 2b). Omeprazole (20 mg kg⁻¹) treatment reduced the histological aberration induced in gastric tissue after acetic acid exposure, however it showed presence of mild inflammatory infiltration and oedema (Fig. 2c). Rats that received treatment with morin (10 mg kg⁻¹) showed disruption to the surface epithelium with presence of oedema and infiltration of inflammatory cells in submucosal layer (Fig. 2d). However, morin (30 and 100 mg kg⁻¹) treated groups showed regeneration and decreased haemorrhage and oedema with presence of moderate inflammatory infiltration (Fig. 2c, f) (Table 2).
DISCUSSION

Induction of gastric ulcer after exposure to necrotic agents, such as acetic acid is associated with various mechanisms such as, decreased gastric blood flow and solubilization of mucus constituents in the stomach which causes elevated influx of Na⁺ and K⁺ ions along with loss of H⁺ ions that results in increased secretion of pepsin into gastric mucosa. Dysregulation of oxido-nitrosative stress of gastrointestinal mucosa resulted in necrosis leading to development of peptic ulcer. Thus, gastric ulcer is a consequence of an imbalance between aggressive factors and the maintenance of mucosal integrity through endogenous defense mechanisms.

There is an array of animal models developed for studying the healing process of peptic ulcer, however only a few of them are a “real” models of peptic ulcers and acetic acid induced ulcers is one of them which received wider attention. This model has very close resemblance to human ulcers because acetic acid-induced ulcers usually relapse and do not easily repair like human ulcers and bear many similarities with clinical ulcer.

It has been well documented that gastric mucosal integrity is regulated by balance between aggressive and defensive factors that controls cell apoptosis and proliferation. However, disturbance in this balance leads to ulcer. In acetic acid induced ulcer, there was up-regulation of aggressive factors (such as acid, pepsin) and down-regulation of defensive factors (such as mucin, prostaglandin, antioxidant, etc.) leading to ulcer formation. Acetic acid control rats showed formation of gastric ulcer reflected by formation of ulceration in gastric mucosa whereas administration of morin showed inhibition in ulcer formation which may be due to restoration of defensive factors.

Reactive Oxygen Species (ROS) played a vital role in the progression of various diseases. However, natural compounds along with potential antioxidant property are topics of high current interest for the treatment of such disease of ROS originated and gastric ulcer being one of them. Elevated level of oxygen derived free radical is a hallmark of gastric ulceration which play pivotal role in disruption of gastro protective mechanism. During the stressful conditions, SOD plays a vital role in maintenance of cellular oxidative balance. It has a central role in detoxification of ROS via scavenging superoxide anion, that form hydrogen peroxide. Therefore, decreased SOD activity reflected the toxic effects of ROS produced by acetic acid on gastric mucosa. However, it has been documented that...
antioxidants has an ability to replenish decreased SOD level in ulcerative condition. Administration of morin showed up regulation in SOD level by virtue of its antioxidant property. These findings are in accordance with the previous studies where administration of morin restored the decreased level of SOD in tissue.

GSH is an abundant tripeptide, non-enzymatic biological antioxidant, that plays a decisive role in free radical (hydrogen peroxide, superoxide and alkoxy radicals) detoxification and thus maintain cell metabolism and integrity. However, elevated ROS caused depletion of intracellular GSH, leading to cell damage in acetic acid induced ulcer. In the present study, acetic acid control rats showed depletion of GSH level in gastric mucosa whereas this depletion in GSH level was restored by morin treatment reflecting its free radical scavenging property.

Several studies concluded that polyunsaturated fatty acids are most vulnerable to free radical attacks and the initial products of lipid peroxidation are conjugated dienic hydroperoxide. It involves the formation and propagation of lipid radicals, the uptake of oxygen and rearrangement of double bonds in unsaturated lipids which eventually results in destruction of membrane lipids. Biological membranes are often rich in unsaturated fatty acids and bathed in oxygen-rich metal containing fluid. Therefore it is not surprising that membrane lipids are susceptible to peroxidative attack. In this study, elevated level of gastric mucosal MDA in acetic acid control rats suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms. However, administration of morin resulted in a significant decrease in MDA level which suggests its efficacy in preventing mucosal damage via its free radical quenching potential. In conclusion, morin showed protective effect against acetic acid induced gastric ulcer via enhancing antioxidant enzyme activity leading to an accelerated ulcer healing. Results of the present investigation suggest that morin may find immense therapeutic potential in clinical application in a variety of conditions where cellular damage is a consequence of oxidative stress. However, further study is in progress for elucidation of actual mechanism of action of morin at the molecular level.

DECLARATION OF INTEREST
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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