



Regular Articles

Rutin, a natural flavonoid, protects against gastric mucosal damage in experimental animals

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Abstract

The effect of the natural flavonoid, rutin, was assessed in different acute and chronic gastric ulcer models in rats. Rutin, 50–200 mg/kg, was administered orally, twice daily for 5 days, and showed a dose-dependent ulcer protective effect on pylorus ligation (22.22 %–53.47 % protection, $P<0.05$), aspirin (27.95 %–58.06 % protection, $P<0.05$), cold restraint stress (25.51 %–77.77 % protection, $P<0.001$), and acetic acid (20.0 %–84.37 % protection, $P<0.05$ – 0.001)–induced acute and chronic ulcers. In addition, rutin offered protection (28.11 %–76.03 %) against ethanol-induced depletion of gastric wall mucus and it also reduced the ulcer index with a significant decrease in plasma corticosterone (25.80 % and 39.09 % protection, $P<0.05$), LPO (20.41 % and 53.06 % protection, $P<0.01$ and $P<0.001$), SOD (15.92 % and 26.51 % protection, $P<0.05$ and $P<0.001$) and increased catalase (25.95 % and 69.73 % protection, $P<0.05$ and $P<0.001$) activity. These results show that rutin has a significant ulcer protective activity by scavenging the reactive oxygen species produced by gastric damage.

Key words: Rutin; antiulcer; antioxidants; flavonoid

Introduction

Flavonoids are a ubiquitous group of polyphenolic substances that are widely distributed in the plant kingdom and have been reported to act in the gastrointestinal tract as either antiulcer, antispasmodic, antisecretory or antidiarrhoeal agents^[1, 2]. Flavonoids are known to inhibit the enzyme activity of histidine decarboxylase and, thus, reduce the formation of

histamine in the gastric mucosa^[3]. They also stimulate the mucosal content of prostaglandins and mucus in gastric mucosa resulting in cytoprotective effects. Several of them prevent gastric mucosal lesions produced by various models of experimental ulcer, and protect the gastric mucosa against different necrotic agents^[4, 5]. Oxygen-derived free radical have been implicated in the pathogenesis of a wide variety of clinical disorders, resulting in the production of gastric damage by physical, chemical and psychological factors that cause gastric ulceration in humans and experimental animals. Flavonoids are known for their significant oxygen radical-scavenging properties *in vivo* and *in vitro*, affecting various steps in the arachidonate cascade via cyclo-oxygenase or lipoxygenase^[6, 7].

Rutin (Fig. 1) is a flavonol glycoside composed

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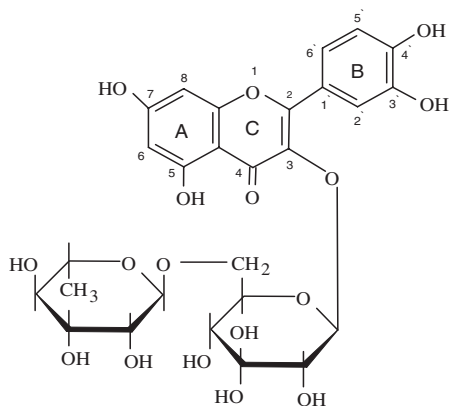


Fig. 1. Structure of rutin

of quercetin and the disaccharide rutinose. It is present in large amounts in the diet, and it is used empirically to treat a variety of diseases. Rutin is found in many plants, for example, black tea and apple skin peels. The richest source of rutin is the buckwheat plant *Fagopyrum esculentum* Moench, the flour of which is used to make pancakes. Rutin has been reported to have anti-inflammatory and vasoactive properties [8, 9] and prevent gastric mucosal ulceration in a number of animal models including restraint stress, reserpine [10] and ethanol [11]. Rutin is an important antioxidant and it has been reported to be a potent scavenger of hydroxyl and superoxide radicals [12, 13] and prevent lipid peroxidation [14]. The present study was designed to demonstrate the role of rutin on physical and chemical factors that induce gastric ulceration, as well as its free radical scavenging ability and effect on plasma corticosterone in a cold restraint stress model that produces gastric ulceration in rats.

Materials and methods

Animals

Sprague-Dawley rats (150-180 g) were obtained from the animal house of the Central Drug Research Institute, Lucknow, India. They were kept in the

departmental animal house at 27 ± 2 °C and relative humidity 44 %-56 %, under light and dark cycles of 10 and 14 h, respectively, for 1 week before and during the experiment. The animals were provided with standard rodent pellet diet (Amrut, India) and the food was withdrawn 18-24 h before the experiment, although water was allowed *ad libitum*. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg. No. 222/2000/CPCSEA).

Experimental procedure

The rats were divided into groups of six animals each. Rutin at doses of 50, 100 and 200 mg/kg and the H_2 receptor blocker, ranitidine, at a dose of 50 mg/kg, were administered orally twice daily at 10:00 and 16:00 h, respectively, for 5 days for the acute study and up to 5 or 10 days for the chronic ulcer protective study. A control group of animals received a suspension of 1 % carboxymethyl cellulose in distilled water (10 ml/kg).

Aspirin (ASP)-induced ulcers

ASP, at a dose of 200 mg/kg (20 mg/ml), was administered to the animals on the day of the experiment and ulcers were scored after 4 hr [15]. The animals were sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5.0 ml 0.9 % NaCl and then the ulcers were scored using the glandular portion of the stomach by a person unaware of the experimental protocol. The ulcer index was calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach. The total severity of the ulcers was determined by recording the severity of each ulcer after histological confirmation as follows : 0, no ulcer; +, pin point ulcer and histological changes limited to superficial layers of mucosa and no congestion; ++, ulcer size less than 1 mm and half of the mucosal



thickness showed necrotic changes; +++, ulcer size 1-2 mm with more than two-thirds of the mucosal thickness destroyed with marked necrosis and congestion, muscularis remaining unaffected; +++++, ulcer either more than 2 mm in size or perforated with complete destruction of the mucosa with necrosis and haemorrhage, muscularis still remaining unaffected. The pooled group ulcer score was then calculated according to the method of Sanyal [16]. Calculation of the percentage protection is given below:

$$\text{Percentage protection} = \frac{(\text{Control} - \text{treated})}{\text{Control}} \times 100$$

Cold-restraint stress (CRS)-induced ulcers

Rats were deprived of food but not water for about 18 hr before the experiment. On the sixth day, the experimental rats were immobilized by strapping the fore and hind limbs on a wooden plank and kept in that position for 2 hr, at a temperature of 4-6 °C [17]. Two hours later, the animals were sacrificed by cervical dislocation and ulcers were examined on the dissected stomachs as described above.

Pylorus ligation (PL)-induced ulcers

Drugs were administered for a period of 5 days as described above and the rats were kept for 18 hr fasting and care was taken to avoid coprophagy. Animals were anaesthetized using pentobarbitone (35 mg/kg, i.p.), the abdomen was opened and pylorus ligation was performed without causing any damage to the blood supply. The stomach was replaced carefully and the abdominal wall was closed in two layers with interrupted sutures. The animals were deprived of water during the post-operative period [18]. After 4 hr, stomachs were removed and cut open along the greater curvature and a person scored the ulcers unaware of the experimental protocol scored the ulcers in the glandular portion of the stomach as described for the aspirin-induced ulcers.

Ethanol (EtOH)-induced ulcers

The gastric ulcers were produced in rats by administering 100 % EtOH (1 ml/200 g, 1 hr) and the animals were sacrificed by cervical dislocation, the stomach was incised along the greater curvature and then examined for ulcers [19]. The ulcer index was scored as described above.

Acetic acid-induced chronic ulcers

Induction of chronic gastric lesions was studied using the methods of Sairam [20]. A solution of 0.06 ml 50 % acetic acid was instilled into a glass tube 6 mm in diameter and allowed to remain for 60 seconds on the anterior serosal surface of the glandular portion of the stomach 1 cm away from the pyloric end under anesthesia. After removal of the acid solution, the abdomen was closed in two layers and animals were caged and fed normally. Rutin was given at a dose of 100 and 200 mg/kg on day one, orally, then twice daily, 4 h after the application of acetic acid and continued either up to 5 or 10 days after ulcer induction. The animals were then sacrificed 18 h after the last dose of drug either on the 6th or 11th day of the experiment to assess the ulcer size and healing. The ulcer index was calculated based upon the product of the length and width (mm²/rat) of the ulcers.

Determination of gastric wall mucus

Gastric wall mucus was determined according to the method of Corne [21]. The glandular segments from stomachs were removed, weighed and incubated in tubes containing 1 % alcian blue solution (0.16 M sucrose in 0.05 M sodium acetate, pH 5.8) for 2 h. The alcian blue bound extract was centrifuged at 3000 rpm for 10 min and the absorbance of the supernatant was measured at 498 nm. The quantity of alcian blue extracted (g/g of glandular tissue) was then calculated.

Estimation of lipid peroxidation (LPO)

The fundus of the cold restraint stress (CRS)-induced ulcer stomach was homogenized (5 %) in



ice-cold 0.9 % NaCl with a Potter-Elvehjem glass homogenizer for 30 seconds. The homogenate was then centrifuged at $800\times g$ for 10 min and the supernatant was again centrifuged at $12,000\times g$ for 15 min and the obtained mitochondrial fraction was used for subsequent determinations. A volume of the homogenate (0.20 ml) was transferred to a vial and mixed with 0.2 ml 8.1 % (w/v) sodium dodecyl sulfate solution, 1.50 ml 20 % acetic acid solution (adjusted to pH 3.5 with NaOH) and 1.50 ml 0.8 % (w/v) solution of thiobarbituric acid (TBA) and the final volume was adjusted to 4.0 ml with distilled water. Each vial was tightly capped and heated in a boiling water bath for 60 min. The vials were then cooled under running water. Equal volumes of tissue blank or test samples and 10 % trichloroacetic acid were transferred into centrifuge tubes and centrifuged at $1000\times g$ for 10 min. The absorbance of the supernatant fractions was measured at 532 nm (Beckman DU 650 spectrometer). A control experiment was carried out using the same experimental procedure except that the TBA solution was replaced with distilled water [22]. 1, 1, 3, 3-Tetraethoxypropan was used as a standard for the calibration curve and expressed as per milligram protein.

Assay of antioxidant enzymes

The fundus of the stomach was homogenized (5 %) and a mitochondrial fraction was prepared as described above. Decomposition of H_2O_2 in presence of catalase (CAT) was followed at 240 nm [23]. One unit of (U) catalase was defined as the amount of enzyme required to decompose 1 μmol of H_2O_2 per min, at 25 °C and pH 7.0. Results are expressed as units (U) of CAT activity/mg protein. Superoxide dismutase (SOD) activity was estimated by the inhibition of the nicotinamide adenine dinucleotide (reduced)-phenazine methosulphate-nitrobluetetrazolium reaction system as described by Nishikimi and modified by Kakkar [24, 25]. One unit of the enzyme is equivalent to 50 % inhibition of formazan

formation in 1 min at room temperature (25 ± 2 °C) and the results are expressed as units (U) of SOD activity/mg protein.

Estimation of plasma corticosterone (PC)

The animals were lightly anesthetized with ether and blood was collected from the supraorbital plexus using the microcapillary technique and the cold restraint stress (CRS)-induced ulcer model. Then 300 μl isooctane was added to 100 μl plasma. After mixing and centrifugation, the isooctane was discarded. Following this, 600 μl chloroform was added to each tube and, after extraction, 400 μl chloroform was transferred to another stoppered tube. To this, 800 μl acid-alcohol (50 %) solution (2:1) was added. After 1 h, the acid layer fluorescence was measured at 462 nm (excitation) and 354 nm (emission) using a spectrofluorimeter and expressed as $\mu\text{g/dl}$ [26].

Histological evaluation

Ulcerated portions from stomachs of the ethanol-induced ulcer group were removed with a scalpel and fixed for 4 hr in 4 % buffered paraformaldehyde, then dehydrated gradually in ethanol and embedded in paraffin using xylene as the intermediate solvent. Serial sections were obtained by cutting the block in a plane perpendicular to the mucosal surface with a microtome. Coded gastric sections were stained with haematoxylin and eosin before light microscope evaluation.

Statistical analysis

Values were represented as mean \pm SEM for six rats. The analysis of variance (ANOVA) test was followed by individual comparisons by the Newman Keuls test for the determination of the level of significance. A value of $P<0.05$ was considered statistically significant.

Results



Rutin, a natural anti-oxidant flavonoid, given (50-200 mg/kg) twice a day for 5 days, prevented the formation of acute gastric ulcers in a dose-related manner. The ranges of the percentage protection were PL 22.22 %-53.47 % ($P < 0.05$), ASP 27.95 %-58.06 % ($P < 0.05$) and CRS 25.51 %-77.77 % ($P < 0.05$ - $P < 0.001$), respectively. The percentage protection of ranitidine ranged from 58.54 %-79.42 % ($P < 0.05$ - $P < 0.001$), in the different gastric ulcer models (Fig. 2). When compared with rutin and ranitidine 50 mg/kg was

not found statistically significant. Secretion of mucus and bicarbonate by the surface epithelium constitutes a mucus-bicarbonate barrier, which is regarded as the first line of defence against potential ulcerogens. The gastric wall mucus was significantly [52.48 %-76.03 % ($P < 0.05$ - $P < 0.01$)] enhanced by rutin and this is regarded as a first line of defence against EtOH-induced gastric ulcers demonstrating a cytoprotective action (Table 1). The gastric wall mucus was not statistically significantly when compared with rutin

Table 1. Effect of rutin (twice daily for 5 days) on ethanol (EtOH)-induced gastric ulcers and gastric wall mucus in rats

Treatment and dose (mg/kg)	Ulcer Index (mm ² /rat)	Protection (%)	Gastric wall mucus (g/g wet glandular tissue)
Normal control	0.0 ± 0.0	-	269.3 ± 15.3
EtOH 1 ml/200 g	24.2 ± 4.9	-	170.1 ± 12.7 †
Rutin 50	17.3 ± 5.2	28.11	187.3 ± 12.8
Rutin 100	11.5 ± 2.9	52.48	207.6 ± 13.8
Rutin 200	5.8 ± 1.1**	76.03	280.3 ± 14.8**
Ranitidine 50	6.6 ± 1.6*#	72.73	211.5 ± 9.8*#

Statistical analysis was carried out using a one way ANOVA test followed by individual comparison by the Newman Keuls test. Values are expressed as mean ± SEM for six rats.

Not significant when compared with rutin 50 mg/kg vs ranitidine 50 mg/kg

† $P < 0.001$ compared with the respective control group.

* $P < 0.05$, ** $P < 0.001$ compared with the respective EtOH (disease control) group.

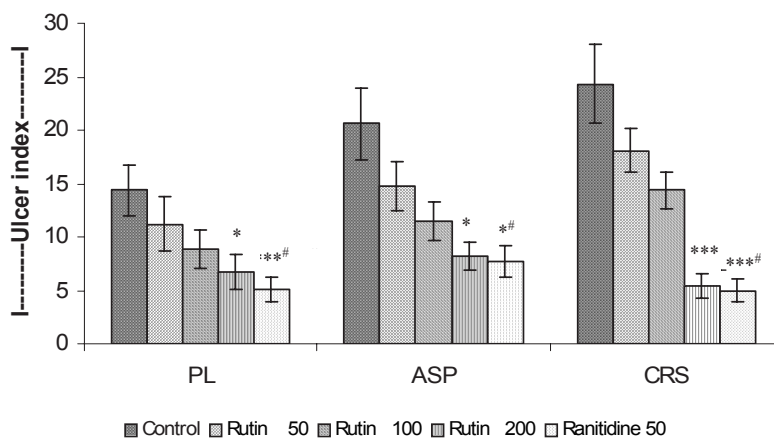


Fig. 2. Effect of rutin (twice daily for 5 days) on pylorus ligation (PL), aspirin (ASP) and cold restraint stress (CRS)-induced gastric ulcers in rats. Values are expressed as mean ± SEM for six rats. # Not significant when compared with rutin 50 mg/kg vs ranitidine 50 mg/kg. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared with the respective control (disease control) group.



and ranitidine 50 mg/kg. In the case of chronic ulcers induced by 50 % acetic acid, rutin reduced the ulcer index significantly after 5 ($P < 0.01$) and 10 days ($P < 0.05 - P < 0.001$) of treatment (Fig. 3).

A summary table (Table 2) is presented to show the severity of the ulcer index as well as the enzyme activities. While studying the role played by the reactive oxygen species on CRS-induced gastric damage, lipid peroxidation and SOD were increased significantly in the ulcerated stomachs ($P < 0.001$). Pretreatment with rutin and a general antioxidant significantly reduced the ulcer index, LPO ($P < 0.01$ and $P < 0.001$), and SOD levels ($P < 0.05$ and $P < 0.001$) and increased the CAT activity ($P < 0.05$ and $P < 0.001$) in comparison with the CRS ulcers. Rutin almost completely protected against gastric ulceration by scavenging the free radicals and this was associated with the plasma corticosterone concentration ($P < 0.05$).

The results of the histological evaluation showed that pretreatment with rutin prevented hemorrhage, edema, necrosis, erosions and deep ulceration induced by ethanol (Fig. 4).

Discussion and conclusion

The present study confirms the protective effect of rutin (a naturally occurring flavone) due

to its gastroprotective activity as demonstrated by its significant inhibition of the formation of ulcers induced by various physical and chemical agents. Pylorus ligation-induced ulcers are due to autodigestion of the gastric mucosa and break down of the gastric mucosal barrier [27]. Synthetic NSAIDs like aspirin cause mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and back diffusion of H^+ ions [28]. The ethanol-induced ulcers are found mainly in the glandular part of the stomach are reported to potentiate the formation of leukotriene C4 (LTC4), mast cell secretory products [29] and reactive oxygen species [30] resulting in damage to the rat gastric mucosa [31]. Ethanol-induced depletion of gastric wall mucus is prevented by rutin. This implies that a concomitant increase in prostaglandins [32] or sulfhydryl compounds [33] helps protect the stomach from ethanol injury. A copious amount of gastric mucus is secreted during superficial mucosal damage and provides a favorable microenvironment for repair by restitution. An increase in gastric motility, vagal overactivity [34], mast cell degranulation [35], free radical generation, decreased gastric mucosal blood flow [36] and decreased prostaglandin synthesis [6] are involved in the production of stress-induced ulcers. Complex neurochemical mechanisms are involved in the

Table 2. Effect of rutin (twice daily for 5 days) on plasma corticosterone (PC), lipid peroxidation (LPO), catalase (CAT), and superoxide dismutase (SOD) activities on cold restraint stress (CRS)-induced ulcers.

Treatment and dose (mg/kg)	PC	LPO	CAT	SOD
Normal control	22.6 ± 3.5	0.37 ± 0.02	33.4 ± 2.1	98.8 ± 10.1
CRS	37.6 ± 4.6 †	0.49 ± 0.02 ††	18.5 ± 1.3 ††	215.4 ± 11.4 ††
Rutin 100	27.9 ± 3.6	0.39 ± 0.03 **	23.3 ± 1.4 *	181.1 ± 5.8 *
Rutin 200	22.9 ± 2.8 *	0.23 ± 0.01 ***	31.4 ± 1.8 ***	158.3 ± 4.7 ***
Ranitidine 50	21.6 ± 1.1 **	0.27 ± 0.01 ***	31.1 ± 1.3 ***	149.4 ± 4.8 ***

Statistical analysis was carried out using a one way ANOVA test followed by individual comparison by the Newman Keuls test. Values are expressed as mean±SEM for six rats.

† $P < 0.05$, †† $P < 0.001$ compared with the respective control group.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the respective CRS (diseased control) group.

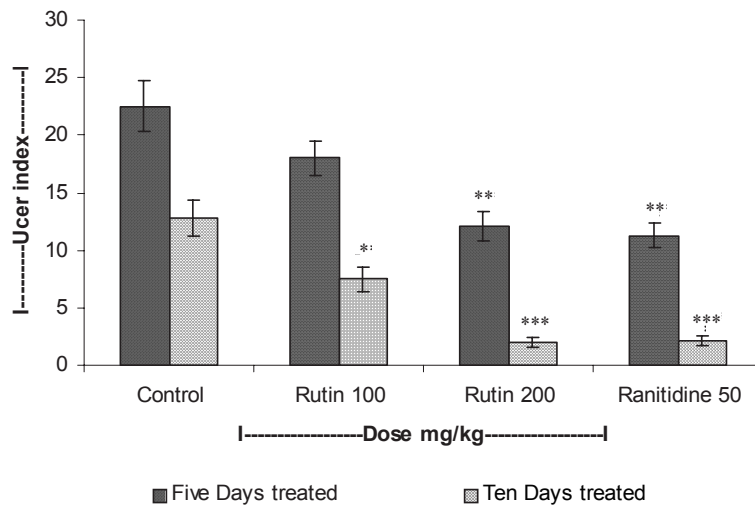


Fig. 3. Effect of rutin (twice daily for 5 and 10 days) on 50 % acetic acid-induced chronic ulcers in rats. Values are expressed as mean \pm SEM for six rats. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the respective control (disease control) group.

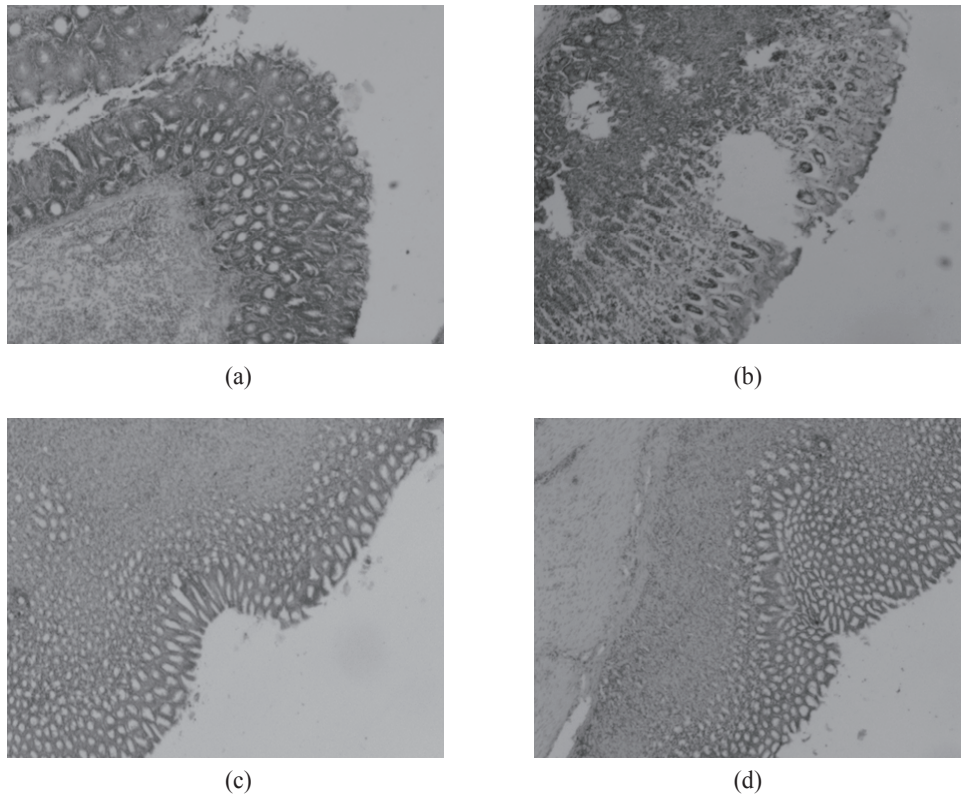


Fig. 4. (a) Histopathological evaluation of rutin on ethanol-induced gastric lesions in rats (Hematoxylin and eosin10x) showing a normal mucosal wall. (b) Section of ulcerated stomach of ethanol-induced ulcer rats (diseased control group) showing hemorrhagic lesion of the gastric mucosa. (c) Section of stomach wall of rutin (100 mg/kg)-pretreated rats showing regeneration of mucosal lesions. (d) Section of stomach wall of rutin (200 mg/kg)-pretreated rats showed near normal cytoarchitecture of the gastric mucosa.



biological response of the organism to noxious stimuli like stress. The pathologic alterations occur with changes in the synthesis, actions and degradation of hormones, neurotransmitters and neuromodulators. The central nervous system plays an important role in stress ulceration and regulation of plasma corticosterone [37]. As the aetiopathogenesis of these ulcer models is different, the mechanism of rutin action should include a number of predisposing factors. On the other hand, the mucosal protection induced by nonprostanoid compounds may perhaps be mediated via the mobilization of endogenous prostaglandins [38]. Therefore, it is conceivable that the observed gastric ulcer protection produced by rutin, provides general evidence for the close relationship between these factors.

Gastric ulceration is a common chronic condition which may persist for 10 to 20 years, characterized by repeated episodes of healing and re-exacerbation. The acetic acid-induced ulcer model closely resembles clinical ulcers in terms of location, chronicity and severity and is the most reliable model to study the healing process [39]. Rutin significantly healed the penetrating ulcers induced by acetic acid after five and ten days treatment.

Stress plays an important role in aetiopathology of gastroduodenal ulceration. Stress-induced ulcers are probably mediated by histamine release with enhancement of acid secretion, free radical generation and a reduction in mucous production [26, 27]. There is substantial evidence that oxygen-derived free radicals play an important role in the pathogenesis of the injury to various tissues, including the digestive system. Superoxide, hydrogen peroxide (H_2O_2) and hydroxyl radical ($\cdot OH$) are important ROS causing tissue damage and the lipid peroxide level is an indicator of the generation of ROS in tissues [40]. The experimental data show that cold restraint stress aggravated the ulcer severity, lipid peroxidation and plasma corticosterone compared with the values in unstressed rats. The higher lipid peroxidation and SOD levels

indicated increased production of $O_2\cdot^-$ within the tissues as an elevated $O_2\cdot^-$ level is thought to increase the concentration of cellular radicals. These radicals functioned in concert to induce cell degeneration via peroxidation of membrane lipids, breaking of DNA strands and denaturing cellular proteins [41]. This effect was significantly reversed by prior administration of rutin confirming a close relationship between free radical scavenging activity and the involvement of endocrinological (plasma corticosterone) responses. These findings imply that the oral administration of rutin has a protective effect against gastric ulcers. Rutin is an antioxidant and may quench the free radicals responsible for oxidative damage in rats. However, further detailed experimental and clinical studies are needed before rutin can be used in dietary supplementation for gastric disorders.

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