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Quercetin, A Bioflavonoid, Protects Against Oxidative Stress-related Gastric Mucosal Damage in Rats

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Abstract – Quercetin and its sugar conjugates are the most abundantly distributed bioflavonoids and represent the largest proportion of flavonols in the plant kingdom. The present study was undertaken to demonstrate the effect of quercetin on the role of reactive oxygen species (ROS) in the development of gastric ulcers in rats. Administration of quercetin in doses of 50, 100 and 200 mg kg^{-1} twice daily for 5 days, showed dose dependent significant protection against ethanol (EtOH), aspirin (ASP), cold-restraint stress (CRS) and pylorus ligation (PL) -induced gastric ulcer models and the results were comparable with those elicited by sucralfate. The thiobarbituric acid reactive substances in the stomach mucosa, an index of lipid peroxidation and regulation of plasma corticosterone were significantly increased in CRS-induced gastric ulceration. The quercetin (100 mg kg^{-1}) and reduced glutathione effectively inhibited gastric lesions induced by CRS with a significant decrease in the lipid peroxidation and plasma corticosterone. These results indicate that quercetin a bioflavonoid exerts its antiulcer effect in light of free radical scavenging and plasma corticosterone in cold restraint stress ulcers.
Key words: Quercetin, ulcer, antioxidant, corticosterone, ROS

Introduction

Flavonoids are a group of low molecular weight polyphenolic compounds that are ubiquitous in nature. Quercetin and its sugar conjugates are the most abundantly distributed bioflavonoids and represent the largest proportion of flavonols in the plant kingdom and more than 70 flavonoid derivatives have been isolated and characterized (Havsteen, 1983; Alcaraz and Ferrandiz, 1987). The extraction, purification and identification of the flavonoids including quercetin have been reported in ethanolic extract of *Bidens pilosa* L (Alvarez *et al.*, 1999) and *Maytenus aquifolium* Martius leaves (Vilegas *et al*, 1999). Fig 1 illustrates the chemical structure of quercetin. Phytogenic agents have traditionally been used by herbalists and indigenous healers for the prevention and treatment of peptic ulcers (Borrelli and Izzo, 2000).

Gastric hyperacidity and ulcer are very common causing human suffering today. One of the most common diseases is caused by an imbalance between damaging factors within the lumen and protective mechanisms within the gastroduodenal mucosa. Although prolonged anxiety, emotional stress, hemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritation, the mechanism is still very poorly understood (Flemstrom and Turnberg, 1984). Oxygen derived free radicals have been implicated in the pathogenesis of a wide variety of clinical disorders, resulting gastric damage induced by physical, chemical and psychological factors that cause gastric ulceration in human and experimental animals (Rao *et al*, 1999). Most of the available drugs are thought to act on the offensive factors like antacids which neutralize acid secretion and H_2 receptor blockers like ranitidine, famotidine, anticholinergics like pirenzepin, telezipine, proton pump blockers like omeprazole etc., which interfere with acid secretion. Sucralfate and reduced glutathione are reported

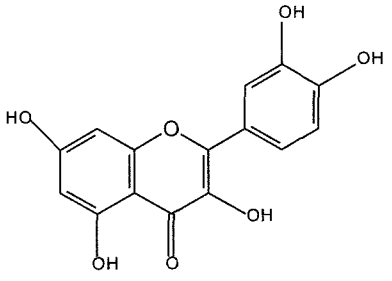

Fig. 1. Quercetin.

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to have antiulcer and antioxidant activity (Sairam *et al*, 2001).

Biological stress, defined as "the nonspecific response of the body to any demand", is a complex neuroendocrine reaction (Robert and Szabo, 1983). Complex neurochemical mechanisms are involved in the organisms biological response to noxious stimuli like stress. The pathologic alterations occur with the 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 (Henke, 1979). The antiulcerogenic and antioxidant effect of quercetin was reported on HCl plus ethanol (Suzuki *et al*, 1998) and 50% ethanol induced mucosal injury in rats (Martin *et al*, 1998). Recently the involvement of neural mechanism in the regulation of stress responsiveness and complex neurotransmitter interactions were reported causing gastric ulceration (Puri *et al*, 1994). The present study was designed to demonstrate the role of quercetin on physical and chemical factors that induced gastric ulceration and the status of free radical scavenging and plasma corticosterone activity in cold restraint stress that induced gastric ulceration in rats.

Materials and Methods

Animals – Sprague-Dawley rats (150-180 g) were purchased from the animal house of the Central Drug Research Institute, Lucknow. They were kept in the departmental animal house at 27±2°C and relative humidity 44-56%, light and dark cycles of 10 and 14 h respectively for one week before and during the experiments. Animals were provided with standard rodent pellet diet (Amrut, India) and the food was with drawn 18-24 h before the experiment though water was allowed *ad libitum*. All experiments were performed in the morning according to current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmerman, 1983).

Chemicals – Quercetin, reduced glutathione and 1,1,3,3-tetraethoxypropane were purchased from Sigma chemical Co., St-Louis, MO. All other reagents were of analytical grade and obtained from the S.D fine chemicals Ltd., India.

Treatment – Quercetin in doses of 50, 100 and 200 mg kg^{-1} and cytoprotective drug sucralfate in the dose of 250 mg kg^{-1} were administered orally twice daily at 10:00 and 16:00 hours respectively for five days before gastric ulcers were induced. The drug samples were prepared in 1% carboxymethyl cellulose (CMC). Control group of animals received suspension of CMC (10 ml kg^{-1}).

Ethanol (EtOH)-induced ulcers – The gastric ulcers were induced in rats by administrating EtOH (1 ml/200 g, 1 h) (Hollander *et al*, 1985) and the animals were sacrificed by cervical dislocation and stomach was incised along the greater curvature and examined for ulcers. The ulcer index was scored, based upon the product of length and width of the ulcers present in the glandular portion of the stomach (mm^2/rat).

Aspirin (ASP)-induced ulcers – ASP in 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 h (Goel *et al*, 1985). The animals were sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5.0 ml of 0.9% NaCl and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of the stomach.

Cold-restraint stress (CRS) ulcers – One hour after the last dose of the treatment on day five the animals were fasted for 18 h and subjected to immobilization by strapping the fore and hind limbs on a wooden plank and kept for 2 h, at temperature of 4-6°C (Gupta *et al*, 1985). Reduced glutathione 150 mg kg^{-1} was injected intraperitoneally twice: once before 20 h and another 1 h prior to subjecting the animals to cold restraint stress (Das and Banerjee, 1993). Two hours later, the animals were sacrificed by cervical dislocation and ulcers were examined in the dissected stomachs.

Pylorus ligated (PL) ulcers – Rats were deprived of food, but not water, for 18 h before the experiment and care was taken to avoid coprophagy. Animals were anaesthetized using pentobarbital (35 mg kg^{-1}, i.p.), the abdomen was opened by a midline incision and a ligature was placed at the pyloric end of the stomach taking care not to exclude any blood vessels. The stomach was replaced carefully and the abdomen wall was closed in two layers with interrupted sutures. The animals were deprived of water during the post-operative period. After 4 h, stomachs were dissected out and cut open along the greater curvature and ulcers present were scored as described below (Shay *et al*, 1945).

Determination of gastric ulcer index – The stomach of each rat was excised and cut along the greater curvature, washed carefully with 5.0 ml of 0.9% NaCl and inflated on cork. The ulcers were examined with the help of a magnifying glass (10x) and scored by an observer unaware of the experimental protocol in the glandular portion of the stomach. Ulcer index was expressed by adding the total number of ulcers per stomach and the total severity of ulcers. The total severity of the ulcers was determined by recording the severity of each ulcer in pluses (+) after histological confirmation. The curative ratio (% C) was

determined as follows: % C = 100 (Ulcer index $_{treated}$ X 100/Ulcer index$_{control}$) (Sanyal *et al*, 1982; Sairam *et al*, 2001).

Estimation of Plasma corticosterone – The animals were lightly anesthetized with ether and blood was collected from the supraorbital plexus using the microcapillary technique. Three hundred microlitres of isooctane was added to 100 µl of plasma. After mixing and centrifugation, the isooctane was discarded. Six hundred microliters of chloroform was added to each tube and after extraction 400 µl of chloroform was transferred to another stoppered tube. To this 800 µl of sulphuric acid-alcohol (50%) solution (2:1) was added. After 1 h, acid layer fluorescence was measured at 462 nm (excitation) and (emission) using a spectrofluorimeter and expressed as µg/dl (Glick *et al*, 1964).

Estimation of lipid peroxidation – The fundic part of the stomach was homogenized (5% concentration) in ice-cold 0.9% NaCl with a Potter-Elvehjem glass homogenizer for 30 seconds. The homogenate was centrifuged at 800×g for 10 min followed by centrifugation of the supernatant at 12,000×g for 15 min to obtain the mitochondrial fraction (Das and Banerjee, 1993). Lipid peroxidation product, malondialdehyde, was estimated using 1,1,3,3-tetraethoxy-propane as the standard and is expressed as nmoles mg^{-1} protein (Jamall and Smith, 1985).

Statistical analysis: All the data were presented as mean ±SEM and analysed by Wilcoxon Sum Rank Test (Padmanabha pillai *et al.,* 1982) followed by unpaired Students t-test for the possible significant interrelation between the various groups. A value of $P<0.05$ was considered statistically significant .

Results and Discussion

Quercetin, a natural antioxidant bioflavonoid was given (50-200 mg kg^{-1}), twice daily for five days showed dose dependent antiulcer activity, and significant effect was seen with of 100 and 200 mg kg^{-1}, against various validated gastric ulcer models induced by ethanol, aspirin, and cold restraint stress ulcers. The incidence of ethanol-induced ulcers which is predominant in the glandular part of stomach was reported to stimulate the formation of leukotriene C_4 (LTC_4) resulting in the damage of rat gastric mucosa (Peskar *et al*, 1986). Similarly the synthetic aspirin is an established ulcerogen in an empty stomach particularly in the incidence of ulceration observed on the glandular part of the stomach (Ferreira and Vane, 1974). Evidence was established that prostaglandins normally were protective in stomach function by maintaining gastric micro circulation (Menguy and Desbaillets, 1967) and caused gastric secretion of bicarbonate (Vane, 1971) and mucus (Flemstrom and Turnberg, 1984). Quercetin with 200 mg kg^{-1} dose significantly reduced the severity of the ulcer index in pylorus ligation induced ulcers. The effect was comparable to the cytoprotective drug sucralfate. The percent protection ranged from 29.41% to 83.48% in all the models (Table 1 and Table 2). In addition, pylorus ligation induced ulcers reduced the mucosal blood flow, auto-digestion of mucosa and break down of the gastric mucosal barrier. The results

Table 1. Effect of quercetin on ethanol and aspirin-induced changes in gastric ulcers in rats

Treatment (mg kg^{-1})	Ethanol induced ulcers		Aspirin induced ulcers	
	Ulcer Index	% Curative ratio	Ulcer Index	% Curative ratio
Control	21.8 ± 5.6	–	22.6 ± 3.4	–
Quercetin 50	13.2 ± 3.9	39.45	14.3 ± 2.3	36.72
Quercetin 100	8.3 ± 2.2[a]	61.93	8.6 ± 2.8[b]	61.94
Quercetin 200	3.6 ± 1.1[b]	83.48	4.2 ± 1.3[c]	81.41
Sucralfate 250	3.2 ± 1.2[b]	85.30	5.6 ± 2.9[b]	75.22

Values are mean±SEM for six rats.
P: [a]<0.05, [b]<0.01 and [c]<0.001 compared to respective control group.

Table 2. Effect of quercetin on cold restraint stress (CRS) and pylorus ligation-induced changes in gastric ulcers in rats

Treatment (mg kg^{-1})	CRS induced ulcers		Pylorus ligation induced ulcers	
	Ulcer Index	% Curative ratio	Ulcer Index	% Curative ratio
Control	24.2 ± 3.6	–	13.6 ± 2.8	–
Quercetin 50	16.7 ± 2.1	30.99	9.6 ± 2.1	29.41
Quercetin 100	11.1 ± 1.7[b]	54.13	8.9 ± 1.6	34.56
Quercetin 200	4.3 ± 0.9[c]	82.23	6.1 ± 0.8[a]	55.15
Sucralfate 250	5.2 ± 1.1[c]	78.51	4.8 ± 1.6[a]	64.70

Values are mean ± SEM for six rats.
P: [a]<0.05, [b]<0.01 and [c]<0.001 compared to respective control group.

Table 3. Effect of quercetin on ulcer index, plasma corticosterone and lipid peroxidation against cold restraint stress induced gastric ulcers in rats

Treatment (mg kg^{-1})	Ulcer Index	Plasma corticosterone	Lipid peroxidation
Control	0.0 ± 0.0	21.3 ± 3.1	0.41 ± 0.01
Cold restraint stress	23.8 ± 3.2^{y}	38.2 ± 3.5^{x}	0.52 ± 0.02^{y}
Quercetin 100	10.3 ± 1.8^{b}	26.4 ± 2.3^{a}	0.35 ± 0.02^{c}
Reduced glutathione 150	4.4 ± 1.7^{c}	22.8 ± 1.2^{b}	0.26 ± 0.01^{c}

Values are mean ± SEM for six rats.
P: ^{x}<0.01 and ^{y}<0.001 compared to respective control group.
P: ^{a}<0.05, ^{b}<0.01 and ^{c}<0.001 compared to respective cold restraint stress group.

in the present study showed that quercetin inhibited the ethanol, aspirin and pylorus ligation- induced ulceration in rats. On the other hand, the mucosal protection induced by nonprostanoid compounds was perhaps mediated through the mobilization of endogenous prostaglandins (Konturek *et al*, 1987; Cho *et al*, 1983). Thus, it is possible that quercetin appears to be involved in close relationship between the predisposing factors.

Like wise several factors were reported to participate in the maintenance of the physiological milieu of the organism, and stress was an averse force which disrupted homeostasis and complex neurotransmitter interactions were involved (Mormede *et al*, 1988). Henke (1979) stated that the central nervous system played an important role in stress ulceration and regulation of plasma corticosterone. The experimental data stated that the cold restraint stress aggravated the ulcer severity, lipid peroxidation and plasma corticosterone as compared to unstressed rats. The higher lipid peroxidation level indicated increased production of O_2^- within the tissue as elevated O_2^- level was thought to increase the concentration of cellular radical level. These radicals functioned in concert to induce cell degeneration via peroxidation of membrane lipids, breaking of DNA strands and denaturing cellular proteins (Fridovich, 1986). Pretreatment of rats with quercetin and reduced glutathione attenuated both the ulcerogenic and corticosterone elevating effects (Table 3). However, the use of sucralfate is well documented in healing gastric ulcers and preventing the recurrence of peptic ulcers (Sairam *et al.*, 2001).

In support of this conclusion, a number of reports from different laboratories demonstrated that the generation of reactive oxygen species (ROS) perhaps was involved in the pathogenesis of CRS -induced gastric mucosal injury *in vivo* and the acid played no role in causing cold restraint stress ulcer (Halliwell and Gutteridge, 1985). This effect was significantly reversed by prior administration of quercetin a bioflovonid, providing a close relationship between free radical scavenging and the involvement of endocrinological (plasma corticosterone) responses. The antiulcerogenic and antioxidant effects of various flavonoids were documented (Martin *et al*, 1998). Evidence was provided that the reduced glutathione exerted its antioxidant defense mechanism by metabolizing lipid peroxides and scavenging endogenous H_2O_2 conforming the observation by Das and Banerjee (1993) and Hirota *et al* (1990).

Thus, quercetin significantly reduced lipid peroxidation in gastric mucosa, perhaps due to maintenance of balance between enzymatic and non enzymatic antioxidants levels, effectively countering the free radical generation. In conclusion, quercetin almost completely protected acute gastric ulceration, at least in part by scavenging the free radicals that involved in the endocrinological mechanism.

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References

Alcaraz, M. J., and Ferrandiz, M. L., Modification of arachidonic acid metabolism by flavonoids. *J. Ethnopharmacol.* 21, 209-229 (1987).

Alvarez, A., Pomar. F., Sevilla., and Montero, M.J., Gastric antisecretory and antiulcer activities of an ethanolic extract of *Bidens pilosa* L. var. radiata Schult. *J. Ethanopharmacol.* **67**(3), 333-340 (1999).

Borrelli, F., and Izzo, A. A., The plant kingdom as a source of anti-ulcer remedies. *Phytother. Res.* **14**(8), 581-591 (2000).

Cho, Ch., Hua, M. F., Chou, C. K., and Ho, L. T., Protection of zinc sulphate against necrosis induced by ethanol in rats. *Procedings of Netherland Science Council and Royal Society.* **7**, 261-267 (1983).

Das, D., and Banerjee, R. K., Effect of stress on the antioxidant enzymes and gastric ulceration. *Mol. Cell. Biochem.* **1125**, 115-125 (1993).

Ferreira, S. J., and Vane, J. R., New aspects of the model of actions of NSAIDs. *Ann. Rev. Pharmacol.* **14**, 57-70 (1974).

Flemstrom, G., and Turnberg, L. A., Gastroduodenal defence

mechanisms. *Clin. Gastroenterol.* **13**, 327-354 (1984).

Fridovich, I., Biological effects of superoxide radical. *Arch. Biochem. Biophys.* 247, 1-11 (1986).

Glick, D., Redlich, D. V., and Levine, S., Fluorimetric determination of corticosterone and cortisol in 0.02-0.05 ml of plasma or submilligram samples of adrenal tissue. *Endicrinol.* **74**, 653-655 (1964).

Goel, R. K., Govinda, Das. D., and Sanyal, A. K., Effect of vegetable banana powder on changes induced by ulcerogenic agents on dissolved mucosubstances in gastric juice. *Indian. J. Gastroenterol.* **4**, 249-251 (1985).

Gupta, M. B., Nath, R., Gupta, G. P., and Bhargava, K. P., A study of the antiulcer activity of diazepam and other tranquillosedatives in albino rats. *Clin. Exp. Pharmacol.* **12**, 61-63 (1985).

Halliwell, B., Gutteridge, J. M. C., Oxygen free radicals and the nervous system. *Trends. Neurosci.* **8**, 22-26 (1985).

Havsteen, B., Flavonoids, a class of natural products of high pharmacological potency. *Biochem. Pharmacol.* **32**, 1141-1148 (1983).

Henke, P. G., The hypothalamus- anygdala axis and experiment gastric ulcers. *Neurosci. Biobehav. Rev.* **3**, 75-82 (1979).

Hirota, M., Inoue, M., Ando, Y., and Morivo, Y., Inhibition of stress-induced gastric mucosal injury by a long acting superoxide dismutase that circulates bound to albumin. *Arch. Biochem. Biophys.* **280**, 269-273 (1990).

Hollander, D., Taranawski, A., Krause, W. J., and Gergely, H., Protective effect of sucralfate against alcohol-induced gastric mucosal injury in the rat. *Gastroenterol.* **88**, 366-374 (1985).

Jamall, I. S., and Smith, J. C., Effects of cadmium on glutathione peroxidase, superoxidase dismutase and lipid peroxidation in the rat heart: a possible mechanism of cadmium cardiotoxicity. *Toxicol. Appl. Pharmacol.* **80**, 33-42 (1985).

Konturek, S. J., Redecki, T., Piastucki, I., Brzozowski, T., and Drozdowicz, D., Gastroprotection by colloidal bismuth subcitrate (De-nol) and sucralfate. Role of endogenous prostaglandins. *Gut.* **228**, 201-205 (1987).

Martin, M. J., La-Casa, C., Alarcon-de-la-Lastra, C., Cabeza, J., Villegas, I., and Motilva, V., Antioxidant mechanisms involved in gastroprotective effects of quercetin. *Naturforsch[C]* **53**(1-2), 82-88 (1998).

Menguy, R., and Desbaillets, L., Role of inhibition of gastric mucous secretion in the phenomenon of gastric mucosal injury by indomethacin. *American. J. Digest. Dis.* **12**, 862-866 (1967).

Mormede, P., Dantzer, R., Michaauel, B., Kelley, K.W., and Le, Moal. M., Influence of stressor predictability and behavioral control on lymphocytes activity, antibody responses and neuroendocrine activity in rats. *Physiol. Behav.* **43**, 577-583 (1988).

Padmanabha pillai, N., Ramaswamy, S., Gopalakrishinan, V., and Ghosh, M. N., Effect of cholinergic drugs on acute and chronic morphine dependence. *Arch. Int. Pharmacodyn.* **257**, 147-154 (1982).

Peskar, B. M., Lange, K., Hoppe, U., and Peskar, B. A., Ethanol stimulates formation of leukotriene C_4 in rat gastric mucosa. *Prostaglandins.* **31**, 283-293 (1986).

Puri, S., Ray, A., Chakravarti, A. K., and Sen, P., Role of dopaminergic mechanisms in the regulation of stress responses in experimental animals. *Pharmacol. Biochem. Behav.* **48**(1), 53-56 (1994).

Radhakrishnan, K., Pandurangan A. G and Pushpangadan, P., *Utleria salicifolia*- A new Ethnobotanical record from Kerala, India. *Fitoterapia.* LXIX(5), 403-405 (1998).

Rao, Ch. V., Maiti, R.N., and Goel, R. K., Effect of mild irritant on gastric mucosal offensive and defensive factors. *Indian J. Physiol. Pharmacol.* **44**, 185-191 (1999).

Robert, A., and Szabo, S., *Selyes guide to stress research.* Selye H edn: Van Nostrand Reinhold Co, New York, 1983.

Sairam, K., Rao, Ch. V., and Goel, R. K., Effect of *Convolvulus pluricaulis* Chois on gastric ulceration and secretion in rats. *Indian J. Exp. Biol.* **39**, 350-354 (2001).

Sairam, K., Rao, Ch. V., Dora Babu, M., and Goel, R. K., Prophylactic and curative effects of *Bacopa monniera* in gastric ulcer models. *Phytomed.* **8**, 423-430 (2001).

Sanyal, A. K., Pandey, B. L., and Goel, R. K., The effect of a traditional preparation of copper, tamrabhasma, on experimental ulcers and gastric secretion. J Ethnopharmacol. **5**, 79-89 (1982).

Shay, H., Komarov, S. A., Fels, S. S., Meranze, D., Gruenstein, M., and Siplet, H., A simple method for the uniform production of gastric ulceration. *Gastroenterol.* **5**, 43-61 (1945).

Suzuki, Y., Ishihara. M., Segami, T., and Ito, M., Anti-ulcer effects of antioxidants, quercetin, alpha-tocopherol, nifedipine and tetracycline in rats. *Japn. J. Pharmacol.* **78**(4), 435-441 (1998).

Vane, J. R. Inhibition of prostaglandin synthesis as a mechanism of action of aspirin like drugs. *Nature. New. Biol.* **231**, 232-235 (1971).

Vilegas. W., Sanommiya, M., Rastrelli, L., and Pizza, C., Isolation and structure elucidation of two new flavonoid glycosides from the infusion of *Maytenus aquifolium* leaves. Evaluation of the antiulcer activity of the infusion. *J. Agric. Food. Chem.* **47**(2), 403-406 (1999).

Zimmerman, M., Ethical guidelines for investigations of experimental pain in conscious animals. *Pain.* **16**, 109-110 (1983).

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