ORIGINAL ARTICLE

The ameliorative effect of fisetin, a bioflavonoid, on ethanol-induced and pylorus ligation-induced gastric ulcer in rats

Amit D. Kandhare, Kiran S. Raygude, Pinaki Ghosh, Subhash L. Bodhankar
Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Pune, Maharashtra, India

Fisetin, a tetrahydroxy flavone, exhibits many biological activities such as antioxidant, antibacterial and anti-inflammatory. The aim of present study was to unravel the therapeutic potential of fisetin at a dose of 10, 20, 30 mg/kg, per oral (p.o.), in ethanol-induced gastric ulcer and pylorus ligation-induced gastric ulcer. Omeprazole (20 mg/kg) was used as a standard drug. In ethanol-induced ulcer, after the pretreatment period of 1 hr gastric ulcer was induced with absolute ethanol at a dose of 8 ml/kg (p.o.), where as in pylorus ligation-induced gastric ulcer; after the pretreatment period of 1 hr ulcer was induced by tight ligation of pylorus portion of stomach. In the pylorus ligation-induced ulcer model there was a significant reduction in the ulcer area as well as the total volume, free acidity and total acidity and increase in the pH of gastric content along with the mucous production were found. There was a significant decrease in ulcer area and significant increase in the mucosal production in the ethanol-induced gastric ulcer model. Fisetin significantly lowered the level of lipid peroxidase, neutrophil infiltration along with gastric mucosal nitrite in both models of the gastric ulcer. The present findings elucidate the therapeutic value of fisetin in the prevention of experimental gastric ulcer by virtue of its antioxidant mechanism.

Key words: Antioxidant, antiulcer, fisetin, lipid peroxidation, nitric oxide

INTRODUCTION

Peptic ulcer is a gastrointestinal pathobiological condition culminating due to imbalance between corrosive (acid, pepsin, smokingand *Helicobacter pylori*) and restorative factors [mucin, prostaglandin (PG), bicarbonate, nitric oxide (NO) and growth factors].[1]

Ethanol promotes gastric denudation.[2] Ethanol is widely used to induce experimental gastric ulcer in animals.[3] Ethanol promotes oxygen derived free radicals, primarily superoxide anions, hydroxyl radicals and lipid peroxides leading to experimental gastric lesions.[4,5] It causes enhancement of mucosal permeability and release of vasoactive products, lead to vascular damage and gastric cell necrosis which, in turn, leads to ulcer formation.[6,7]

Pyloric ligation enhances mucosal damage because it interferes with gastric mucosal resistance and modulates the level of cytoprotective PGs, cytokines, membrane lipid peroxidation (TBARS) and endogenous glutathione.[8] The inhibition of gastric acid secretion and increasing the gastric mucosal production are the two ways for the healing of the peptic ulcer.[9] Pylorus ligation- and ethanol-induced ulcer occurs due to free radical generation.[10-12] Hence, the antioxidant drugs with multiple mechanism of protective action may reduce the tissue injury in the human disease.[13]

Treatment of peptic ulcers is aimed at either modulating corrosive factors (acid, pepsin, active oxidants, platelet aggravating factor "PAF", leukotrienes, endothelins, bile or exogenous factors including NSAIDs) or propagating the mucosal defensive factors [mucus, bicarbonate, normal blood flow, PGs, NO].[14]

Synthetic chemical moieties which promote ulcer healing belong to following therapeutic classes viz., proton pump inhibitors, histamine receptor blockers, drugs affecting the mucosal barrier and PG analog.[15] In the wake of prominent adverse effects (arrythmias, impotence, gynaecomastia, and haematopoeitic changes) of modern medicine,[16] herbal drugs provide a safe therapeutic option.

Flavonoids are phenolic moieties which have been evaluated for an array of conditions like antiviral, antithrombotic, anti-ischaemic, anti-inflammatory, antihistaminic, free-radical scavenging abilities as well as

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Address for correspondence: Dr. Subhash L. Bodhankar, Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Erandwane, Pune - 411 038, Maharashtra, India. E-mail: sbodh@yahoo.com
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being inhibitors of several enzymes such as phospholipase A_2, cyclooxygenase, lipoxygenase, glutathione reductase and xanthine oxidase.[17-21]

Fisetin (3, 3', 4', 7 tetrahydroxy flavone) is a major plant flavonoid and it has been evaluated for down regulation of *in vitro* glycogenolysis and gluconeogenesis.[22] It also evaluated for the disease conditions like cancer, allergic reaction, hyperthyroidism, diabetis and as *in vitro* antioxidant.[23-27] Plant-derived flavonoids have better potential to promote gastric ulcer healing properties. However, lack of systematic studies with respect to fisetin on experimental animal models of gastric ulcer laid the basis of this investigation.

The aim of investigation was to assess the gastroprotective activity of Fisetin in rat model of experimentally ethanol-induced and pyloric ligation-induced gastric ulcers.

MATERIALS AND METHODS

Animals

Healthy adult male Swiss albino mice (20-30 g) and male Wistar rats (180-200 g) were obtained from the National Institute of Biosciences, Pune (India). The animals were housed in solid bottom polypropylene cages. They were maintained at 24°C±1°C, with relative humidity of 45-55% and 12:12 h dark/light cycle. The animals were acclimatized for a period of 2 weeks and were kept under pathogen-free conditions. The animals had free access to standard pellet chow (Chakan Oil Mills, Sangli) throughout the experimental protocol, with the exception of overnight fasting before induction of the ulcer. The animals were provided with filtered water. The pharmacological and acute toxicity protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune (CPCSEA/08/2010).

Drugs and Chemicals

Fisetin was purchased from Sigma-Aldrich, India. Omeprazole was obtained as a gift sample from Symed Pharmaceutical Pvt. Ltd., Hyderabad. Acetic acid, anaesthetic ether, ethanol, formalin, tris buffer, sucrose, trichloroacetic acid, citric acid monohydrate, sodium nitrate, copper sulphate, sodium potassium tartarate, ethylene diamine tetra acetic acid disodium salt, folin's phenol reagent, sodium hydroxide were purchased from S.D. Fine Chemicals, Mumbai, India. Topfer's reagent was purchased from Hi-Media Pvt. Ltd., Mumbai, India. Sulphanilamide, naphthalaminediamine HCl and phosphoric acid were obtained from LobaChemi Pvt. Ltd., Mumbai, India.

Acute Toxicity Testing

The acute oral toxicity study was carried out in Swiss albino mice as per the OECD guideline. Graded doses of the fisetin were dissolved in 1% DMSO were administered orally and the animals were observed for 2 weeks following administration. Body weight, food consumption, fluid intake and psychomotor activities were recorded daily.

Experimental Procedure

Ethanol-induced gastric ulcer

The animals were divided into six groups, each group consist of six rats.

- Group I - Normal group (1% DMSO)
- Group II - Ethanol control (EC) group: Absolute ethanol (8 ml/ kg) + 1% DMSO
- Group III - Absolute ethanol (8 ml/kg) + omeprazole 20 mg/kg, per oral (p.o.)
- Group IV - Absolute ethanol (8 ml/kg) + Fisetin 10 mg/ kg, p.o.
- Group V - Absolute ethanol (8 ml/kg) + Fisetin 20 mg/ kg, p.o.
- Group VI - Absolute ethanol (8 ml/kg) + Fisetin 30 mg/kg, p.o.

Pylorus ligation-induced gastric ulcer

The animals were divided into six groups, each group consist of six rats.

- Group I - Normal group (0.5 ml 1% DMSO)
- Group II - Pylorus ligation control (PLC) group (0.5 ml 1% DMSO)
- Group III - Pylorus ligation + 0.5 ml omeprazole (20 mg/kg, p.o.)
- Group IV - Pylorus ligation + 0.5 ml Fisetin (10 mg/ kg, p.o.)
- Group V - Pylorus ligation + 0.5 ml Fisetin (20 mg/kg, p.o.)
- Group VI - Pylorus ligation + 0.5 ml Fisetin (30 mg/ kg, p.o.)

Induction of Gastric Ulcer in Animal Models

Ethanol-induced gastric ulcer

Rats were fasted for 24 h. Fisetin, omeprazole or vehicle (1% DMSO) was administered orally to the animals. After the pretreatment period of 1 hr, gastric ulcer was induced with absolute ethanol at a dose of 8 ml/kg (p.o.)[12] administered to all groups by orally. The animals were scarified after 6 hours; stomachs were isolated and incised along the greater curvature and then observed for area of ulcer and total area of glandular region. The ulcer index and % inhibition was determined as per the method described by Dengiz *et al*.[28]

Pylorus ligation-induced gastric ulcer

The method of Shay rat ulcer was adopted.[29] The rats were fasted for 24 h. The drug, fisetin or omeprazole or vehicle (1% DMSO) was administered to the animals. During the

course of the experiment, food was withdrawn. After the pretreatment period of 1 h, the animals were anaesthetised with anaesthetic ether. The abdomen was opened by a small midline incision below the xiphoid process; pylorus portion of stomach was slightly lifted out and ligated. Precaution was taken to avoid traction to the pylorus or damage to its blood supply. The stomach was placed carefully in the abdomen and the wound was sutured by interrupted sutures. The animals were allowed for free access to water. Nineteen hours after pylorus ligation the rats were sacrificed 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 as per the method described by Dengiz *et al*.[28]

Determination of Gastric Volume, pH, Free Acidity and Total Acidity

The entire gastric content was transferred into centrifuge tubes. It was used for estimation of gastric volume, pH and 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 pH was determined using a digital pH meter. Free acidity was determined by titrating 1.0 ml of gastric juice against N/10 NaOH to pH 7 using 3-4 drops of Topfer's reagent as the indicator whereas total acidity was determined by titrating 1.0 ml of gastric juice against N/10 NaOH to pH 7 using phenolphthalein as the indicator and were expressed in terms as mEq/L.[29]

Determination of Gastric Mucus Production

Gastric mucus production was measured as described by Tan *et al*.[30] Briefly the rats which were subjected to absolute ethanol and pylorus ligation-induced gastric ulcer, after estimating the ulcer area, the gastric mucosa of each rat was gently scraped using a glass slide and the mucus obtained was weighed using a precision electronic balance.

Biochemical Estimation

Five hundred milligrams 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, to obtain supernatant volume of 4.5 ml. It was divided into aliquot to determine lipid peroxidation (malondialdehyde (MDA) content) (2.0 ml), myeloperoxidase (MPO content) (2.0 ml), total protein estimation (0.1 ml) and NO content (0.5 ml). Lipid peroxidation or MDA formation was estimated by the method of Slater and Sawyer.[31] The myeloperoxidase assay was determined according to the method described by Krawisz *et al*.[32] Total protein was determined by the method of Lowry *et al*.[33] Gastric NO level was estimated as nitrite according to the method described by Miranda *et al*.[34]

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 processed for 12 hr using isopropyl alcohol, xylene and paraffin embedded for light microscopic study (Nikon E200). Paraffin-embedded tissue section cut in 5 μm thickness were prepared and stained after deparaffination using hematoxylene and eosin stain (H and E) to verify morphological assessment of stomach damage. Photomicrographs were captured at a magnification of ×40.

Statistical Analysis

All the results were expressed as mean±S.E.M. Statistical comparisons were made between drug-treated groups and ulcer control groups. The data was statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple range tests using GraphPad Prism 5.0 software (GraphPad, San Diego, USA). $P<0.05$ was considered to be statistically significant.

RESULTS

Acute Toxicity Study

The animal did not show any signs and symptoms such as restlessness, respiratory distress, diarrhoea, convulsions and coma during the acute toxicity studies when fisetin was administered orally in graded doses up to the dosage of 50 mg/kg body weight.

Effect of fisetinon ethanol-induced gastric ulcer in rats

Intragastric administration of absolute ethanol (8 mg/kg) produced superficial or deep erosions, bleeding, and antral ulcers. However, pretreatment with fisetin reduced severity of ethanol-induced gastric ulcer [Figure 1].

The gastroprotective effects of 10, 20 and 30 mg/kg doses of fisetin on the ethanol-induced gastric ulcer in various gastric parameters are shown in Table 1. There were remarkable changes in the gastric parameters of fisetin-treated group as compared with the vehicle-treated animals.

Effect of fisetin on ulcer area and ulcer index is shown in Table 1. The results indicated that fisetin (20 and 30 mg/kg) produced significant ($P<0.001$) low ulcer area (52.05±5.61 and 32.49±2.95 mm^2, respectively) and ulcer index (7.31±0.79 and 4.47±0.40, respectively) as compared with the ethanol control group (105.3±4.26 and 14.54±0.70). Fisetin showed a dose-dependent inhibition against ethanol-induced ulcers in

rats. Maximum inhibition was observed in the omeprazole-treated group.

Mucus production significantly changed from 16.87±2.06 mg to 32.51±2.33 mg as the dose of the fisetin was increased from 10 to 30 mg/kg as compared with 12.83±1.09 mg for the ethanol control rats [Table 1].

The effect of fisetin on the ulceration process in ethanol-induced ulcer was evaluated by the oxidative stress marker level, MPO activity and gastric NO [Table 2].

The concentration of MPO in the ethanol control rat was significantly higher (4.09±0.21 U/mg) as compared to normal rat (1.65±0.25 U/mg) whereas this increase in the concentration of MPO was reduced in a dose-dependent manner in fisetin-pretreated rats with 20 mg/kg (3.00±0.20, $P<0.05$) and 30 mg/kg (1.92±0.24, $P<0.001$).

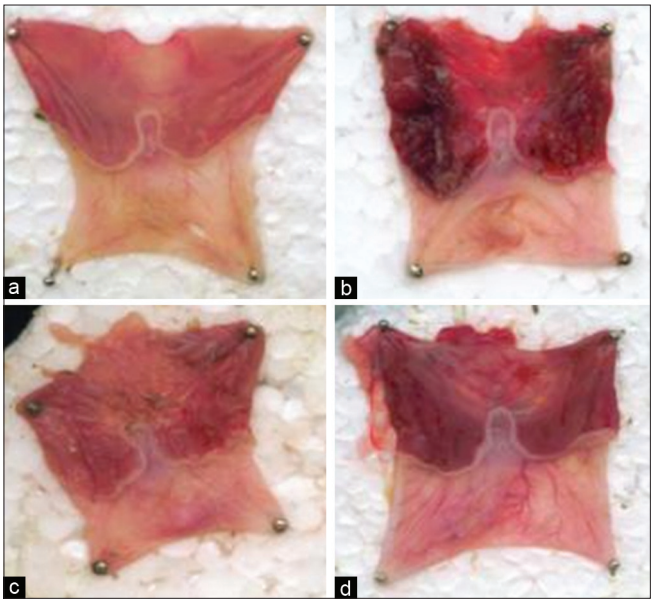

Figure 1: Representative stomachs of rats after ethanol-induced gastric ulcer (a) normal rat (b) ethanol control rat (c) rat pretreated with omeprazole (20 mg/kg) (d) rat pretreated with fisetin (30 mg/kg)

Induction of ulcer produced a significant increase in MDA concentration (6.55±0.48 nmoles/mg of protein) as compared with the normal group (3.16±0.35 nmoles/mg of protein). Pretreatment with fisetin (30 mg/kg, p.o.) significantly decreased MDA concentration (3.63±0.64 nmoles/mg of protein) as compared with ethanol control group ($P<0.001$ respectively).

In ethanol control rat the nitrite level was significantly lowered (24.40±2.18 µg/mg) as compared to normal rats (56.02±3.61µg/mg). Fisetin treatment 20 and 30 mg/kg (38.96±2.18 and 45.98±1.91 µg/mg, respectively, $P<0.001$) significantly inhibited this reduction in nitrite levels [Table 2].

Histological observation of ethanol-induced gastric lesions in ethanol control group had severe disruption to the surface epithelium and edema of the submucosal layer with leucocytes infiltration [Figure 2a]. Rats that received pretreatment with fisetin had reduced ulcer area, with mild disruption to the surface epithelium with mild edema and leucocytes infiltration of the submucosal layer [Figure 2b]. Omeprazole-treated rat had mild infiltration and haemorrhages [Figure 2c]. In case of normal rat stomach there was no inflammatory cell and no oedema, the epithelium was found intact [Figure 2d].

Effect of fisetinon pylorus ligation-induced gastric ulcer in rats

Pylorus ligation of the stomach for 19 hours resulted in the accumulation of gastric acid which in turn leads to

Table 1: Effect of fisetin on various gastric parameters of ethanol-induced gastric ulcer

Groups	Ulcer area (mm²)	Ulcer index	% Inhibition	Mucous production (mg)
Normal	–	–	–	–
Ethanol control	105.3±4.26	14.54±0.70	–	12.83±1.09
Omeprazole (20 mg/kg)	19.81±2.85***	2.75±0.42***	81.02	38.01±1.44***
Fisetin (10 mg/kg)	89.97±8.20	12.75± 1.16	13.49	16.87±2.06
Fisetin (20 mg/kg)	52.05±5.61***	7.31±0.79***	49.70	24.98±1.62***
Fisetin (30 mg/kg)	32.49±2.95***	4.47±0.40***	69.22	32.51±2.33***

Data are expressed as mean±S.E.M. from five rats and analyzed by one-way ANOVA followed by Dunnett's test. *$P<0.05$, **$P<0.01$, ***$P<0.001$ as compared to ethanol control group ($n = 5$)

Table 2: Effect of fisetin on various biochemical parameters in stomach of ethanol-treated rats

Parameters	Normal	Ethanol control	Omeprazole (20 mg/kg)	Fisetin		
				10 mg/kg	20 mg/kg	30 mg/kg
MPO (U/mg)	1.65±0.25	4.09±0.21	1.81±0.24***	3.93±0.24	3.00±0.20*	1.92±0.24***
Lipid peroxidation (nmoles of MDA/mg protein)	3.16±0.35	6.55±0.48	3.50±0.50***	5.85±0.35	5.30±0.44	3.63±0.64***
NO (µg/mg)	56.02±3.61	24.40±2.18	48.85±1.85***	23.38±1.54	38.96±2.18***	45.98±1.91***

Data are expressed as mean±S.E.M. from five rats and analyzed by one-way ANOVA followed by Dunnett's test. *$P<0.05$, **$P<0.01$, ***$P<0.001$ as compared to ethanol control group ($n = 5$); MPO – Myeloperoxidase; MDA - Malondialdehyde; NO – Nitric oxide

autodigestion of gastric mucosa and formation of pointed gastric lesions [Figure 3].

Discernable changes were found in the gastric parameters of fisetin-treated group as compared with the pylorus ligation (PL) control animals [Table 3].

Fisetin-treated (20 and 30 mg/kg) groups showed a dose-dependent significant (P<0.01 and P<0.001) reduction in ulcer area (57.17±2.58 and 31.36±3.95 mm^2) as compared to control group (72.68±3.10 mm^2). Omeprazole also exhibited significant (P<0.001) ulcer area reduction (23.12±2.18 mm^2) when compared to control. Fisetin showed a dose-dependent inhibition in pylorus ligation-induced ulcers in rats.

Gastric secretion was accompanied by a highly significant increase in mucus production from 21.17±1.81 mg in the PL control to 39.69±3.67 mg and 42.82±3.24 mg for the dose of 20 and 30 mg/kg (P<0.001). Omeprazole (20 mg/kg) similarly produced a complete inhibition of lesion formation which was accompanied by a highly significant reduction in gastric acid levels [Table 3]. The PL control rats showed significantly higher gastric juice volume as compared (11.13±0.48 ml) to normal rats. Rats pretreated with fisetin (10, 20 and 30 mg/kg) showed a significantly lowered volume of gastric juice (9.08±0.43, 7.93±0.39 and 5.83±0.54 ml) (P<0.01 and P<0.001) as compared to the PL control group. The pH of gastric fluid was significantly lower (P<0.001) in animals pretreated with fisetin (20 and 30 mg/kg) (3.80±0.32 and 4.46±0.23) as compared to PL control rats (2.12±0.29) [Table 4].

Fisetin shows gastroprotective effect on the PL-induced gastric damage in rats. The gastroprotective effect of 10, 20 and 30 mg/kg doses of Fisetin on total and free acidity are shown in Table 4. Fisetin-treated groups showed discernable changes in the above parameters as compared to the PL control animals. Fisetin had significant (P<0.001) gastroprotective effect at a dose of 20 and 30 mg/kg, since it decreased the free acidity (63.83±3.39 and 38.89±2.67 mEq/l resp.) and reduced the total acidity (78.30±4.66 and 53.58±3.86 mEq/l respectively)as compared to PL control rat.

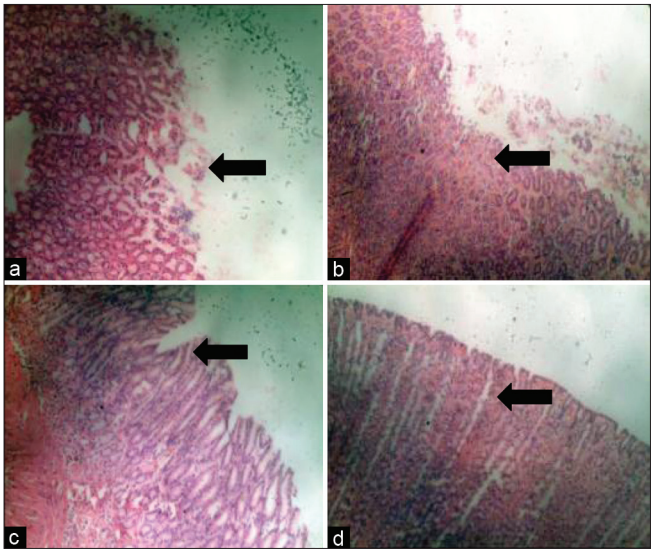

Figure 2: Photomicrographs of sections of stomach from ethanol-treated rats stained with H and E stomach microscopic image of (a) Normal rat (b) Ethanol-induced ulcer rat (c) Omeprazole (20 mg/kg) treated rat (d) Fisetin (30 mg/kg) treated rat. Images (×40) are typical and representative of each study group

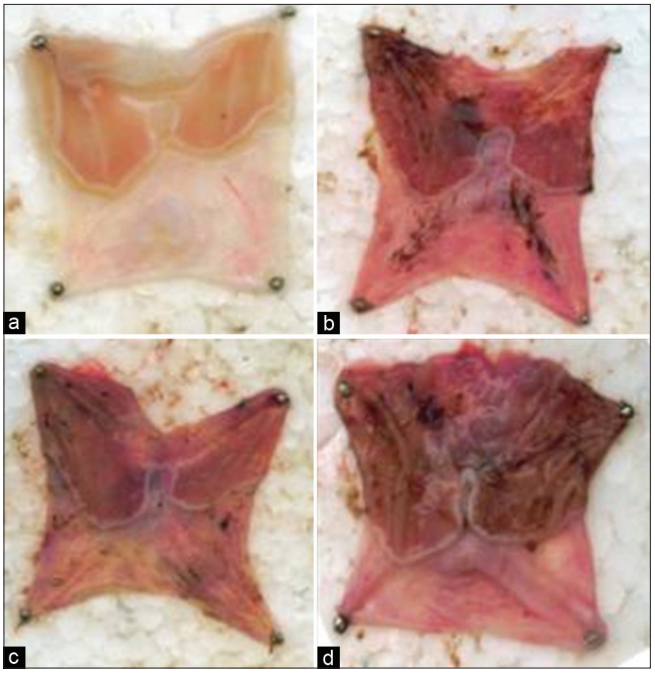

Figure 3: Representative stomachs of rats after pylorus ligation-induced gastric ulcer (a) Normal rat (b) Pylorus ligated control rat (c) Rat pretreated with omeprazole (20 mg/kg) (d) Rat pretreated with fisetin (30 mg/kg)

Table 3: Effect of fisetinon various gastric parameters in pylorus-ligated rats

Groups	Ulcer area (mm^2)	Ulcer index	% Inhibition	Mucous production (mg)
Normal	-	-	-	-
PL control	72.68±3.10	12.03±0.64	-	21.17±1.81
Omeprazole (20 mg/kg)	23.12±2.18***	3.87±0.42***	69.11	50.33±1.53***
Fisetin (10 mg/kg)	68.08±4.57	11.43±0.81	5.02	29.60±0.92
Fisetin (20 mg/kg)	57.17±2.58**	9.68±0.53*	19.51	39.69±3.67***
Fisetin (30 mg/kg)	31.36±3.95***	5.36±0.70***	55.45	42.82±3.24***

Data are expressed as mean±S.E.M. from five rats and analyzed by one-way ANOVA followed by Dunnett's test. *P<0.05, **P<0.01, ***P<0.001 as compared to pylorus ligation control group (n = 5); PL – Pylorus ligation

The effects of fisetin on the ulceration process in gastric tissue were evaluated by the estimation of biochemical markers. These results are presented in Table 5. They showed that the ulcer caused by pylorus ligation was associated with an increase in MPO activity (4.25±0.21U/mg). In the groups pretreated with fisetin (30 mg/kg, p.o.) the MPO activity was significantly decreased (2.40±0.31 U/g respectively) (P<0.001), as compared with PL control group. At doses of 20 and 30 mg/kg fisetin significantly (P<0.05 and P<0.001 respectively) decreased the levels of MDA (7.93±0.91 and 6.62±0.74 nmoles/mg protein respectively) as compared to PL control rats (3.73±0.59 nmoles/mg protein).

In pylorus ligated rats showed significantly lower nitrite levels (26.57±1.77 µg/mg). Whereas pretreatments with fisetin (30 mg/kg) significantly (P<0.01) enhanced the nitrite content (42.86±2.60) [Table 5].

Macroscopic change of pylorus ligation induce ulcer was shown in Figure 4. Histopathological changes on pylorus ligation model showed degeneration, haemorrhage, edematous appearance of the gastric tissue [Figure 4a], whereas fisetin (30 mg/kg) [Figure 4b] and omeprazole-treated groups (20 mg/kg) [Figure 4c] showed regeneration and prevents the formation of hemorrhage and edema. Normal rat does not stomach any sign of inflammation in cells and edema as the epithelium remained intact [Figure 4d].

DISCUSSION

The antiulcer effect of Fisetin was examined against gastric lesions-induced by pylorus ligation and ethanol. Fisetin prevented the mucosal lesions-induced by ethanol and meliorated pylorus ligation-induced gastric damage.

Pylorus ligation produces the mucosal damage by altering the level of cytoprotective PGs and cytokines as well as interfering with the gastric mucosal resistance.[8]

The secretion and accumulation of gastric acid are two important factors responsible for the production of gastric ulcer by the pylorus ligation. At pH 2 pepsinogen is converted to pepsin, whereas its inactivation occurs at pH 6. In presence of active pepsin, acid gets accumulated and causes the gastric ulcer in the pylorus ligated rat. The

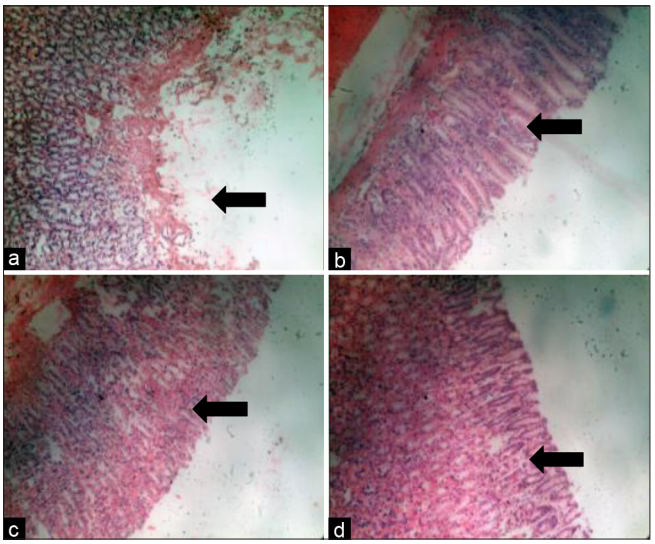

Figure 4: Photomicrographs of sections of stomach from pylorus ligated rats stained with H and E. Stomach microscopic image of (a) Normal rat (b) Pylorus ligation-induced ulcer rat (c) Omeprazole (20 mg/kg) treated rat (d) Fisetin (30 mg/kg) treated rat. Images (×100) are typical and representative of each study group

Table 4: Effect of fisetin on basal gastric secretion in pylorus-ligated rats

Groups	Volume of gastric fluid (ml)	pH of gastric fluid	Free acidity (mEq/l)	Total acidity (mEq/l)
Normal	-	-	-	-
Pylorus ligation control	11.13±0.48	2.12±0.29	88.67±3.19	119.4±5.36
Omeprazole (20 mg/kg)	4.96±0.37***	4.96±0.17***	27.10±1.92***	46.79±3.59***
Fisetin (10 mg/kg)	9.08±0.43**	2.67±0.33	78.21±5.71	107.5±5.44
Fisetin (20 mg/kg)	7.93±0.39***	3.80±0.32***	63.83±3.39***	78.30±4.66***
Fisetin (30 mg/kg)	5.83±0.54***	4.46±0.23***	38.89±2.67***	53.58±3.86***

Data are expressed as mean±S.E.M. from five rats and analyzed by one-way ANOVA followed by Dunnett's test. *P<0.05, **P<0.01, ***P<0.001 as compared to pylorus ligation control group (n = 5)

Table 5: Effect of fisetin on various biochemical parameters in stomach of pylorus-ligated rats

Parameters	Normal	PL control	Omeprazole (20 mg/kg)	Fisetin		
				10 mg/kg	20 mg/kg	30 mg/kg
MPO (U/mg)	1.73±0.28	4.25±0.21	2.03±0.21***	3.98±0.22	3.46±0.27	2.40±0.31***
Lipid peroxidation (nmoles of MDA/ mg protein)	3.73±0.59	11.40±0.84	5.20±0.65***	11.10±0.80	7.93±0.91*	6.62±0.74***
NO (µg/mg)	59.53±3.58	26.57±1.77	52.60±2.92***	26.05±2.51	30.98±2.94	42.86±2.60**

Data are expressed as mean±S.E.M. from five rats and analyzed by one-way ANOVA followed by Dunnett's test. *P<0.05, **P<0.01, ***P<0.001 as compared to pylorus ligation control group (n = 5); MDA – Malondialdehyde; MPO – Myeloperoxidase; PL – Pylorus ligation; NO – Nitric oxide

pH range from 4 to 6 causes inactivation of pepsin but it still remains stable.[35] Fisetin increases the pH of the gastric contents and thus exerts its gastroprotective effect by inactivation of pepsin. Fisetin was found to decrease the acid volume and total acidity of gastric fluid.

Free radicals generated due to induction of stress which results in formation of mucosal erosion and changes in antioxidant enzyme. [5,36] Results of the present investigation are in tune with the previous studies as major factors responsible for the pathogenesis of ethanol-induced[11] and pylorus ligation-induced[10] gastric mucosal injury is the formation of reactive oxygen species.

In various gastric injuries MPO acts as a marker of neutrophil infiltration. In the ulcerated tissue the elevated level of the neutrophil infiltration may be associated with the increase in MPO enzyme activity as well as H_2O_2.[37,38] This elevated level of MPO enzyme was significantly attenuated by fisetin by inhibiting neutrophil infiltration.

Membrane lipids are susceptible to peroxidative attack as it contains the high amount of unsaturated fatty acids and lies in oxygen-rich environment. Free radicals which are formed during the lipid peroxidation cause the rearrangement of the double bond in the unsaturated fatty acids of the membrane and result in the destruction of the lipid membrane.[39] Fisetin significantly attenuates the elevated lipid peroxidation (LPO) level in both the experimental models, which reveals its gastroprotective effect.

Nitrate and nitrite are hallmarks of endogenously produced potential antioxidant. NO is a potent chain-breaking antioxidant in free radical-mediated lipid Peroxidation.[40-42] Fisetin exerts its gastroprotective effect by attenuating the elevated LPO level and increasing in the level of nitrite in both the experimental models.

Hence, by virtue of its antioxidant potential and altering the level of NO along with the reduction in neutrophil infiltration, fisetin exerts its gastroprotective effects in ethanol and pylorus ligation-induced ulcers in laboratory animals.

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