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ANTIOXIDANT MEDIATED ULCER HEALING POTENTIAL OF HYPERIN ISOLATED FROM FLOWERS OF *RHODODENDRON ARBOREUM* IN EXPERIMENTAL RAT

Neeraj Verma [*1], Anil Pratap Singh [2] and Ch. V. Rao [3]

Jan Kalyan Institute of Technical Excellence [1], UPSIDC, Near Umra Tiraha, Kursi Road, Lucknow - 226026, Uttar Pradesh, India.
Government Public Analyst Laboratories [2], Lucknow - 226020, Uttar Pradesh, India.
Department of Pharmacology, Pharmacognosy and Ethnopharmacology Division [3], National Botanical Research Institute (Council of Scientific and Industrial Research), Rana Pratap Marg, Lucknow - 226001, Uttar Pradesh, India.

Keywords:

Antiulcer activity, Antioxidant, Hyperin, *Rhododendron arboreum*

Correspondence to Author:
Neeraj Verma

Director,
J. K. Institute of Technical Excellence, Lucknow - 226026,
Uttar Pradesh, India.

E-mail: neerajcology@gmail.com

ABSTRACT: Hyperin, a flavonoid is an active phytochemical constituent gift in numerous plants as well as, *Hypericum perforatum*, *Drosera rotundifolia*, *Stachys byzantina*, *Prunella vulgaris*, *Rumex acetosella*, *Abelmoschus manihot* and *Rhododendron arboreum*. It's protecting result on cultured PC12 cells against toxicity induced by hydrogen peroxide and tert-butyl hydroperoxide. Hyperin, isoquercitrin and quercetin isolated from ethyl acetate fraction of the root of *A. chiisanensis* showed lipopolysaccharide-induced nitrite production in rat peritoneal macrophages. As a crucial bioactive compound, hyperoside (hyperin) has been documented to possess antihyperglycemic, antiviral activity, antinociceptive, medicament, cardioprotective, hepatoprotective, and gastric-mucosal protecting result. The current study evaluates the ulcer healing potential of hyperin against absolute ethanol- (necrotizing agent), aspirin- (non-steroidal medicament drug) and histamine- (gastric secretion stimulator *via* H_2 receptor) iatrogenic ulcers in rats. Hyperin (25 mg/kg and 50 mg/kg) was administered orally to the long fasted rats, one hour before the absolute ethanol/ aspirin/ histamine challenge. The ulcer index, gastroprotective potential, the status of the inhibitor enzymes enzyme (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) along with reduced glutathione (GSH), and lipid peroxidation were studied the models altogether. Pre-treatment with hyperin showed a dose-dependent decrease within the ulcer index altogether models. Hyperin at each dose, induced vital inhibitor activity by attenuating the ulcer elevated levels of MDA and fixed the ulcer-depleted levels of GSH, SOD, CAT, GPx, and GR. Hyperin possesses potent antiulcer activity, which can be attributed to an underlying antioxidant activity.

INTRODUCTION: A peptic ulcer is an abraded place of the gastric or duodenal mucosa resulting from the action of gastric juice.

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It is a continual and recurrent disorder and is the most predominant of the gastrointestinal disorders. Its miles usually identified that peptic ulcers are caused by a loss of equilibrium among aggressive gastric elements and mucosal protecting factors [1]. A gastric ulcer is a few of the maximum severe illnesses in the international.

The etiology of gastroduodenal ulcers is prompted by using various competitive factors, including acid, pepsin, bile acid, meals ingredients, bacterial

products, and capsules. Those agents boom gastric acid and pepsin secretion, decrease the gastric blood float, suppress the endogenous generation of prostaglandins, inhibit mucosal growth and cellular proliferation, and regulate the gastric motility [1, 2]. Then again, shielding mechanisms of the gastric mucosal consist particularly of practical, humoral and neural factors. Mucus as alkaline secretions, microcirculation and motility act as useful factors, prostaglandins (PGs) and nitric oxide (NO) as humoral factors, and capsaicin-sensitive sensory neurons (CPSN) as neural elements [3, 4].

Even though many pills have been efficaciously employed within the remedy of gastro-duodenal ulcer and peptic diseases, all of those compounds have proven predominant shortcomings, which include the healing screw ups observed in certain instances, or the damaging results and high value [2]. In the look for new healing alternatives, conventional medicinal flowers are a supply of natural merchandise, which includes triterpenes, diterpenes, and flavonoids, among others with gastroprotective activity [5]. Hyperin, a flavonoid is an energetic phytochemical constituent present in numerous flowers such as *Hypericum perforatum*, *Drosera rotundifolia*, *Stachys byzantina*, *Prunella vulgaris, Rumex acetosella, Abelmoschus manihot,* and *Rhododendron arboreum*. Hyperin can have a protecting impact on cultured PC12 cells in opposition to cytotoxicity caused using hydrogen peroxide and tert-butyl hydroperoxide [6].

Hyperin, isoquercitrin, and quercetin isolated from ethyl acetate fraction of the basis *of A. chiisanensis* confirmed lipopolysaccharide-prompted nitrite manufacturing in rat peritoneal macrophages [7]. As a vital bioactive compound, hyperoside (hyperin) has been documented to possess antiviral activity [8, 9], antinociceptive [10, 11, 12], anti-inflammatory [13], cardio-protective [14, 15, 16], hepatoprotective [17, 18, 19] and gastric mucosal protective effect [20, 21]. Flowers of *Rhododendron arboreum* showed anti-nociceptive and anti-inflammatory activity [22]. Aqueous methanolic extract of *Rhododendron arboreum* showed *in-vitro α*-glucosidase inhibitory and antidiabetic activity [23].

MATERIAL AND METHODS:

Isolated Compound: The method of isolation of hyperin can be found from our earlier study [24]. Briefly, the air-dried powdered flowers were extracted with ethanol (50%) using a Soxhlet apparatus, which was then evaporated under vacuum and afford the ethanolic extract of flowers. A voucher specimen (NBRI/CIF/83/2009) was deposited in the institute for future reference. The extract thus obtained was partitioned with organic solvents afford the *n*-hexane, chloroform, ethyl acetate, and *n*-butanol fractions. The ethyl acetate fraction was applied to a Sephadex-LH 20 column (300 g) eluted with a gradient of ethyl acetate/methanol (70:30) to give four fractions. Fraction 2 was submitted to HPLC separation (RP-18; MeOH / H_2O 72:28; flow 9 mL/min; UV detection at 280 nm) gave compound hyperin.

Drugs and Chemicals: Omeprazole, ranitidine, and diclofenac sodium were sampled from Cipla Laboratories, Unique Chemicals and Pharmaceuticals Ltd., and Themis Pharmaceuticals, India respectively. Thiobarbituric acid (TBA), trichloroacetic acid (TCA), reduced glutathione, oxidized glutathione, and nicotinamide adenine dinucleotide-phosphate (NADPH) were obtained from Hi-media Laboratories, Mumbai, India. Epinephrine, histamine and 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Sigma Chemical Co., St Louis, MO, USA. All other chemicals were obtained from local sources and were of analytical grade.

Experimental Animals: Wistar rats (150-200 g) of both sexes were kept inside the departmental animal house of National Botanical Research Institute (NBRI), Lucknow at 25 ° ± 3 °C and relative humidity 50 ± 5%, 12 h light, and dark cycles respectively for one week before and for the duration of the experiments. They have been allowed free access to standard rat feed and water *ad libitum*. All of the research has been done by the guidelines for the care and use of laboratory animals, as followed and promulgated by the Institutional Animal Care Committee, (Reg. no. 222/2000/CPCSEA), India.

Preparation of Test and Reference Drug Solutions: Reference drugs *viz*., diclofenac sodium, omeprazole, and ranitidine were prepared as a suspension in 1% (w/v) aqueous carboxymethyl cellulose solution and used immediately. Toxicant drugs *viz*., aspirin and histamine were also prepared

as suspensions in 1% (w/v) aqueous carboxymethyl cellulose solution and administered immediately.

Antiulcer Activity: The effects of hyperin were evaluated in ethanol, aspirin, and histamine-induced ulcer models in rats. Omeprazole was used as a standard drug for the ethanol and aspirin-induced ulcer models and ranitidine was used as a standard drug for the histamine-induced ulcer model for comparing the antiulcer potential of hyperin.

Ethanol-Induced Gastric Ulceration: [25, 26] Albino wistar rats weighing 150-200g after acclimatization (6-7 days) in the animal quarters were randomly divided into five groups of six animals each and treated in the following way:

Group 1: Normal control (untreated);

Group 2: Toxicant control and received absolute ethanol (1 mL/200 g, p.o.);

Group 3: Standard and received omeprazole (20 mg/kg, p.o.) 30 min before absolute ethanol treatment (1 mL/200 g, p.o.);

Group 4: Received hyperin (25 mg/kg, p.o.) 30 min before absolute ethanol treatment (1 mL/200 g, p.o.);

Group 5: Received hyperin (50 mg/kg, p.o.) 30 min before absolute ethanol treatment (1 mL/200 g, p.o.).

All rats were fasted for 24 h but allowed free access to water. The standard drug and the test drugs were given orally to the respective groups. Thirty minutes after their pre-treatment, all animals were gavaged with absolute ethanol. They were humanely sacrificed 1 h later by cervical dislocation; the stomachs were excised and opened along the greater curvature. Ulcers formed in the glandular portion of the stomach were observed under a magnifying glass for measuring the ulcer score and the ulcer index. The stomachs were weighed, chilled and washed with ice-cold saline after evaluation of the above parameters. A stomach homogenate (10% w/v) was prepared in 1.15% (w/v) KCl. An aliquot of the homogenate was used for the estimation of lipid peroxidation (LPO). The homogenates were centrifuged at 7000×g for 10 min at 4 ºC and the supernatants were used for the assays of reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidize (GPx) and glutathione reductase (GR).

Aspirin-induced Gastric Ulceration: [27] Albino wistar rats were randomly divided into five groups of 6 animals each and treated in the following way:

Group 1: Normal control (untreated);

Group 2: Toxicant control, which received aspirin (200 mg/kg, p.o);

Group 3: Standard and received omeprazole (20 mg/kg,p.o.) 1 h prior to aspirin administration (200 mg/kg, p.o);

Group 4: Received hyperin (25 mg/kg, p.o.) 1 h prior to aspirin administration (200 mg/kg, p.o);

Group 5: Received hyperin (50 mg/kg, p.o.) 1 h before aspirin administration (200 mg/kg, p.o).

All rats were fasted for 24 h but allowed free access to water. The standard drug and the test drugs were administered orally to the respective groups. One hour after their pre-treatment, all animals were gavaged with aspirin. After 4 h, they were humanely sacrificed by cervical dislocation. Ulcers formed in the glandular portion of the stomach were observed under a magnifying glass for measuring the ulcer score and the ulcer index. The stomach was further tested for LPO, GSH, SOD, CAT, GPx, and GR.

Histamine-Induced Gastric Ulceration: [28, 29] Albino wistar rats were randomly divided into five groups of 6 animals each and treated in the following way:

Group 1: Normal control (untreated);

Group 2: Toxicant control, which received histamine (300 mg/kg, i.p.);

Group 3: Standard and received ranitidine (50mg/kg,p.o.) 1 h prior to histamine injection (300mg/kg, i.p.);

Group 4: Received hyperin (25 mg/kg, p.o.) 1 h before histamine injection (300 mg/kg, i.p.);

Group 5: Received hyperin (50 mg/kg, p.o.) 1 h before histamine injection (300 mg/kg, i.p.).

All rats were fasted for 24 h but allowed free access to water. The standard drug and the test drugs were

administered orally to the respective groups. One hour after their pre-treatment, all animals were treated with histamine (300 mg/kg, i.p.). After 4 h, they were humanely sacrificed by cervical dislocation. Ulcers formed in the in the glandular portion of the stomach were observed under a magnifying glass for measuring the ulcer score and the ulcer index. The stomach was further tested for LPO, GSH, SOD, CAT, GPx, and GR.

Ulcer Assessment: [30] The stomachs were harvested, opened along the greater curvature and the mucosa was exposed to macroscopic evaluation. The ulcerated area was assessed using planimetry and the ulcer index (UI) was calculated for each treatment.

Mean Scoring: A score for the ulcer was made as follows:

- 0 : Normal colouration;
- 0.5 : Red colouration;
- 1 : Spot ulcers;
- 1.5 : Haemorrhagic streaks;
- 2 : Ulcers >3 mm but < 5
- 3 : Perforation.

Ulcer Index: Ulcer index was calculated as:

$$\text{Ulcer index} = 10/x$$

Where x = Total mucosal area/Total ulcerated area

Lipid Peroxidation: The quantitative estimation of LPO was performed by determining the concentration of thiobarbituric acid reactive substances (TBARS) in the gastric tissue using the method of Ohkawa *et al*. [31] The amount of malondialdehyde (MDA) formed was quantified by reaction with TBA and used as an index of lipid peroxidation. The results were expressed as nanomole of MDA/mg protein using molar extinction coefficient of the chromophore (1.56×10^{-5}/M/cm) and 1, 1, 3, 3-tetraethoxypropane as the standard **Fig. 1**.

Estimation of Antioxidants in Gastric Tissue: The GSH level in the gastric tissue was determined according to the method of Ellman [32]. Gastric SOD activity was estimated by the method of Sun and Zigman [33]. CAT activity was estimated by the Clairborne *et al*. method [34]. GPx estimation was carried out using the method of Rotruck *et al*. [35] GR activity was determined by using the method of Mohandas *et al*. [36]

Statistical Analysis: The results of antiulcer and antioxidant activities are expressed as mean ± SEM. Results were statistically analyzed using one-way ANOVA, followed by the Dunnett's post-test for individual comparisons. $P<0.05$ was considered to be significant.

RESULTS:

Ethanol-Induced Ulceration in Rats: Absolute ethanol administration caused marked damage to the mucosa as seen in the ethanol control group of rats. However, there was a marked reduction in damage in the hyperin and omeprazole treated groups when compared with the ethanol control group, as seen in the images below **Fig. 1a-e**.

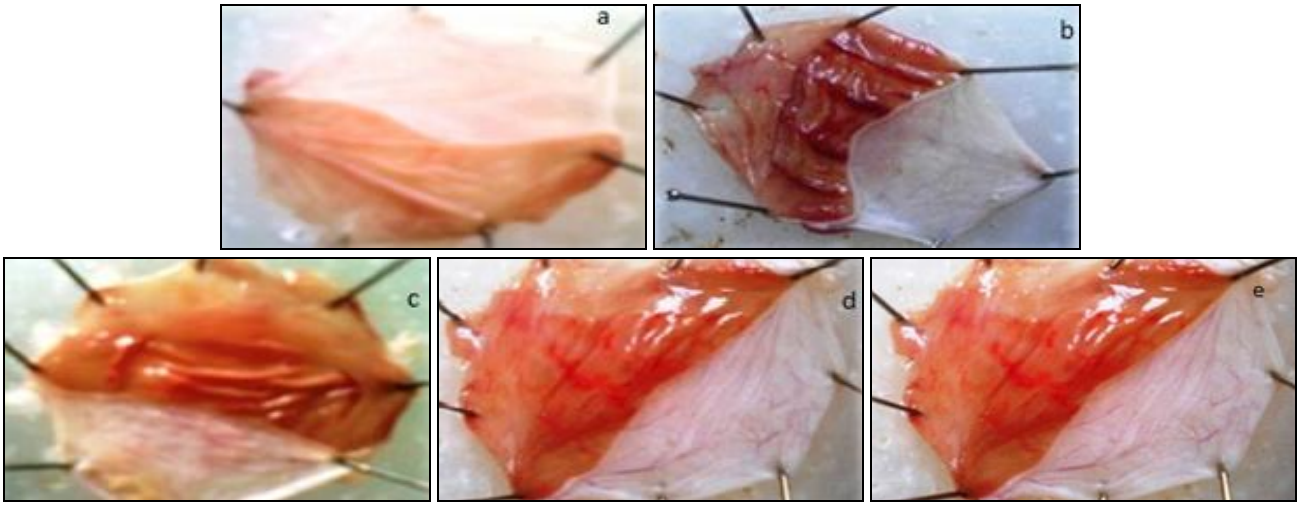

FIG. 1: OBSERVATIONS OF ULCER IN STOMACHS OF ETHANOL AND DRUG TREATMENT IN RATS. a: normal (untreated) group; b: absolute ethanol (1 mL/200 g) treated group; c: omeprazole (20 mg/kg) treated group; d: Hyperin (25 mg/kg) treated group; e: Hyperin (50 mg/kg) treated group.

The image of the stomach of ethanol-treated rat showed prominent ulcers, while the treatment groups, like omeprazole and hyperin 50 mg/kg showed only redness and no ulcers. The hyperin treatment showed a small amount of ulceration. The effect of hyperin 25 and 50 mg/kg treatments on mean ulcer score, ulcer index and percent inhibition are summarized in **Table 1**.

TABLE 1: EFFECT OF HYPERIN ON ULCER PARAMETERS IN ETHANOL-INDUCED ULCERATION IN RATS.

S. no.	Treatment group	Mean Ulcer Score	Ulcer Index	% Inhibition
1	Toxicant (absolute ethanol 1 mL/200 g)	2.9170 ± 0.0833	1.0180 ± 0.04528	–
2	Normal (untreated)	0.0833 ± 0.0833**	0.0000 ± 0.0000**	100
3	Standard (omeprazole 20 mg/kg)	0.9167 ± 0.2007**	0.2523 ± 0.0200**	75.22
4	Hyperin (25 mg/kg)	1.6670 ± 0.5110*	0.5012 ± 0.0873**	50.77
5	Hyperin (50 mg/kg)	1.1670 ± 0.2472**	0.2845 ± 0.0056**	71.05

Values are mean ± SEM; n = 6 in each group; one-way ANOVA followed by Dunnett's test is applied for statistical analysis.*P < 0.05. **P < 0.01 when the normal group and drug-treated groups were compared with the toxicant group.

TABLE 2: EFFECT OF HYPERIN ON STOMACH GSH, TBARS, SOD, CAT, GPx AND GR IN ABSOLUTE ETHANOL-INDUCED ULCERATION IN RATS

S. no.	Biochemical parameters	Normal control (untreated)	Toxicant control absolute ethanol (1 mL/kg)	Hyperin (25 mg/kg)	Hyperin (50 mg/kg)	Standard omeprazole (20 mg/kg)
1	GSH (mol/mg protein)	1.37 ± 0.03	0.82 ± 0.03a	1.05 ± 0.08z	1.34 ± 0.05x	1.35 ± 0.02x
2	TBARS (nmol MDA/mg protein)	0.55 ± 0.01	1.12 ± 0.06a	0.98 ± 0.04	0.75 ± 0.03x	0.69 ± 0.06x
3	SOD (U/mg protein)	48.12 ± 0.88	29.53 ± 1.91a	40.68 ± 2.21y	45.63 ± 1.95x	47.28 ± 1.33x
4	CAT (U/mg protein)	8.15 ± 0.07	4.00 ± 0.26a	5.01 ± 0.30x	7.09 ± 0.24x	7.57 ± 0.17x
5	GPx (U/mg protein)	8.96 ± 0.02	4.59 ± 0.44a	6.06 ± 0.38z	6.40 ± 0.42y	8.11 ± 0.22x
6	GR (U/mg protein)	54.16 ± 1.90	35.96 ± 2.24a	43.58 ± 2.18	50.38 ± 1.09x	50.52 ± 1.85x

Values are mean ± SEM; n = 6 in each group; one-way ANOVA followed by Tukey–Kramer post-test is applied for statistical analysis.1 unit of CAT = µmol H_2O_2 consumed/min/mg protein.1 unit of GPx = µg GSH utilized/min/mg protein.1 unit of GR = nmol NADPH oxidized/min/mg protein. aP<0.001 when toxicant control group compared with the normal control group. xP<0.001.yP<0.01.z P<0.05 when drug-treated groups compared with the toxicant control group.

TABLE 3: EFFECT OF HYPERIN ON ULCER PARAMETERS IN ASPIRIN-INDUCED ULCERATION IN RATS

S. no.	Treatment group	Mean Ulcer Score	Ulcer Index	% Inhibition
1	Toxicant (absolute ethanol 1 mL/200g)	2.5830 ±0.2713	0.9257 ± 0.0338	-
2	Normal (untreated)	0.0833±0.0833**	0.0000 ± 0.0000**	100
3	Standard (omeprazole 20 mg/kg)	0.8333 ± 0.2108**	0.3083 ± 0.0303**	66.70
4	Hyperin (25 mg/kg)	1.5830 ± 0.3745*	0.5211 ± 0.1899*	43.71
5	Hyperin (50 mg/kg)	1.0000 ± 0.2582**	0.3240 ± 0.0140**	64.99

Values are mean ± SEM; n=6 in each group; one-way ANOVA followed by Dunnett's test is applied for statistical analysis.*P<0.05. **P<0.01 when the normal group and drug-treated groups were compared with the toxicant group.

Antioxidant Enzymes, GSH, and LPO: The effect of GIE on antioxidant enzymes (SOD, CAT, GPx, and GR), LPO and GSH in the gastric tissue homogenates is presented in **Table 2**. The free radical formation resulting in lipid peroxidation was measured in terms of MDA produced. There was a significant increase in MDA content in the stomach homogenates of the ethanol-treated group of rats when compared with the normal group. The levels of MDA elevated due to absolute ethanol were significantly attenuated by omeprazole and hyperin 50 mg/kg treatment. There was a significant decrease in GSH levels with absolute ethanol treatment. Pre-treatment with hyperin 50 mg/kg as well as omeprazole to alcohol administered rats prevented this decrease and restored the GSH levels to near normal. SOD activity in the ethanol-treated group of rats was examined to be strikingly lower than in the normal group. Both, hyperin (25 mg/kg and 50 mg/kg) and omeprazole treatments elevated the ethanol depleted SOD activities significantly.

CAT activity of the stomach homogenates of the ethanol-treated group of rats was measured to be significantly lower than that in the normal group. Treatment of hyperin (25 mg/kg and 50 mg/kg) and omeprazole to intoxicated ethanol rats restored significantly the ethanol-depleted CAT activities. Stomach GPx activity in the ethanol-treated group

of rats showed a significant decline when compared with the normal group of rats. Hyperin treatment elevated this GPx activity significantly. Both doses of hyperin induced a significant increase in GR activity depleted due to ethanol treatment to rats. The effect of hyperin 50 mg/kg was comparable to that of omeprazole in restoring the GR activity to near normal.

TABLE 4: EFFECT OF GIE ON STOMACH GSH, TBARS, SOD, CAT, GPx, AND GR IN ASPIRIN-INDUCED ULCERATION IN RATS

S. no.	Biochemical parameters	Normal control (untreated)	Toxicant control aspirin (200 mg/kg)	Hyperin (25 mg/kg)	Hyperin (50 mg/kg)	Standard omeprazole (20 mg/kg)
1	GSH (_mol/mg protein)	1.37 ± 0.03	0.89 ± 0.008a	0.99 ± 0.007y	1.30 ± 0.002x	1.31 ± 0.008x
2	TBARS (nmol MDA/mg protein)	0.55 ± 0.01	1.08 ± 0.08a	0.92 ± 0.03z	0.72 ± 0.11x	0.72 ± 0.009x
3	SOD (U/mg protein)	48.12 ± 0.88	30.28 ± 1.23a	35.87 ± 1.33y	44.98 ± 0.54x	46.29 ± 0.60x
4	CAT (U/mg protein)	8.15 ± 0.07	4.18 ± 0.08a	5.88 ± 0.81z	7.12 ± 0.04x	7.62 ± 0.15x
5	GPx (U/mg protein)	8.96 ± 0.02	4.83 ± 0.24a	6.28 ± 0.55y	8.107 ± 0.05x	8.59 ± 0.15x
6	GR (U/mg protein)	54.16 ± 1.90	31.59 ± 2.38a	48.85 ± 2.14x	51.22 ± 1.07x	52.09 ± 1.71x

Values are mean ± SEM; n=6 in each group, one-way ANOVA followed by Tukey–Kramer post-test is applied for statistical analysis. aP<0.001 when toxicant control group compared with the normal control group. xP<0.001. yP<0.01. zP<0.05 when drug-treated groups compared with the toxicant control group.

TABLE 5: EFFECT OF HYPERIN ON ULCER PARAMETERS IN HISTAMINE-INDUCED ULCERATION IN RATS

S. no.	Treatment group	Mean Ulcer Score	Ulcer Index	% Inhibition
1	Toxicant control (histamine 300 mg/kg)	2.8330 ± 0.1667	0.8916 ± 0.0027	-
2	Normal (untreated)	0.0833 ± 0.0833**	0.0000 ± 0.0000**	100
3	Standard (ranitidine 50 mg/kg)	0.9167 ± 0.0833**	0.2536 ± 0.0150**	71.56
4	Hyperin (25 mg/kg)	1.8330 ± 0.4595*	0.4716 ± 0.2085*	47.11
5	Hyperin (50 mg/kg)	1.0830 ± 0.1537**	0.3000 ± 0.0248**	66.35

Values are mean ± SEM; n=6 in each group; one-way ANOVA followed by Dunnett's test is applied for statistical analysis.*P<0.05. **P<0.01 when the normal group and drug-treated groups were compared with the toxicant group. Values are mean ± SEM; n=6 in each group, one-way ANOVA followed by Tukey–Kramer post-test is applied for statistical analysis. aP<0.001 when toxicant control group compared with the normal control group. xP<0.001.yP<0.01.zP<0.05 when experimental groups compared with the toxicant control group.

TABLE 6: EFFECT OF HYPERIN ON STOMACH GSH, TBARS, SOD, CAT, GPx AND GR IN HISTAMINE-INDUCED ULCERATION IN RATS

S. no.	Biochemical parameters	Normal control (untreated)	Toxicant control histamine (300 mg/kg)	Hyperin (25 mg/kg)	Hyperin (50 mg/kg)	Standard ranitidine (50 mg/kg)
1	GSH (_mol/mg protein)	1.37 ± 0.03	0.69 ± 0.02a	0.90 ± 0.06	1.38 ± 0.07x	1.30 ± 0.06x
2	TBARS (nmol MDA/mg protein)	0.55 ± 0.01	1.66 ± 0.02a	1.24 ± 0.10y	0.71 ± 0.07x	0.55 ± 0.04x
3	SOD (U/mg protein)	48.12 ± 0.88	33.10 ± 1.81a	39.85 ± 2.63	43.67 ± 1.66y	44.21 ± 2.40y
4	CAT (U/mg protein)	8.15 ± 0.07	3.97 ± 0.27a	6.01 ± 0.26x	8.13 ± 0.26x	8.23 ± 0.12x
5	GPx (U/mg protein)	8.96 ± 0.02	5.59 ± 0.16a	6.17 ± 0.15z	8.86 ± 0.07x	8.83 ± 0.08x
6	GR (U/mg protein)	54.16 ± 1.90	38.73 ± 2.02a	44.62 ± 3.01	53.41 ± 2.02y	52.09± 2.72y

Values are mean ± SEM; n=6 in each group, one-way ANOVA followed by Tukey–Kramer post-test is applied for statistical analysis. aP<0.001 when toxicant control group compared with the normal control group. xP<0.001.yP<0.01.zP<0.05 when experimental groups compared with the toxicant control group.

DISCUSSION: The hyperin, up to 2000 mg/kg when administered orally did not show any toxic or deleterious effects, indicating low toxicity at high doses. The LD_{50} value could not be determined as no mortality was observed at a dose of 2000 mg/kg. Hence, the hyperin was studied for its antiulcer activity using ethanol, aspirin and histamine-induced ulceration models in Wistar rats. Exposure of gastric mucosa to various ulcerogenic compounds, like absolute ethanol, aspirin, and histamine, has been shown to affect cellular integrity and such changes are associated with oxidative stress and mitochondrial damage [37]. The gastroprotective efficacy of the hyperin (25 mg/kg and 50 mg/kg) was evident from a significant reduction in the ulcer index obtained in hyperin

treatment groups. Flavonoids may prevent ulcer development due to their precipitating and vasoconstricting effects [38]. Their astringent action helps in precipitating microproteins on the ulcer site, thereby forming an impervious layer over the lining that hinders gut secretion and protects the underlying mucosa from toxins and other irritants.

Flavonoids are reported to protect the gastric mucosa by preventing the formation of lesions by various necrotic agents by scavenging ROS [39]. Flavonoid hyperin stabilize the ROS by reacting with them and getting oxidized in turn to more stable less reactive radicals. Presumably, the high reactivity of the OH group of flavonoids is responsible for this free radical scavenging activity. Ethanol-induced gastric ulcers have been widely used for the evaluation of the gastroprotective activity. Ethanol induces ulcers by reducing gastric mucosal blood flow and mucus production in the gastric lumen, by decreasing endogenous glutathione and prostaglandin levels. It also associated with increasing ischemia, gastric vascular permeability, acid "back diffusion," histamine release, efflux of sodium and potassium, an influx of calcium, generation of free radicals and production of leukotrienes [40]. It has been noticed that oxygen-derived free radicals are implicated in the mechanism of acute and chronic ulceration and scavenging these free radicals may help in healing these ulcers. Elevation in the levels of end products of lipid peroxidation, such as MDA was observed in the stomachs of ethanol treated rats. The increase in MDA levels in the stomach suggests enhanced peroxidation leading to tissue damage and failure of the antioxidant defense mechanisms to prevent the formation of excessive free radicals. Treatment with hyperin significantly reversed these changes.

Hence, it is likely that the mechanism of gastroprotection of hyperin is due to inhibition of LPO by its free radical scavenging activity. Reduced glutathione, one of the most abundant non-enzymatic biological antioxidants, is found in high concentrations in the gastric mucosa of rats and humans. Its functions include removal of ROS, such as H_2O_2, superoxide anions and alkoxy radicals, maintenance of membrane protein thiols and to act as a substrate for GPx and GR [41]. Glutathione is essential for the maintenance of mucosal integrity, and the depletion of glutathione from the gastric mucosa induces macroscopic mucosal ulceration [42]. Decreased GSH levels in ethanol administered rats may be due to its increased utilization for enhancing the activities of the GSH-related enzymes GPX and GR. The glutathione levels increased significantly with hyperin treatment. The effect of hyperin might be due to an initial reduction in stomach peroxidative activities followed by an inhibition of the activities of the GSH-related enzymes, thereby leading to restoration of GSH content flavonoid (OH) + R•→ Flavonoid (O•) + RH. It is known that SOD, CAT, and GPx constitute a mutually supportive team of antioxidant enzymes, which provides a defense system against ROS.

SOD is an important defense enzyme that catalyzes the dismutation of superoxide anions [43]. CAT is a heme-protein that catalyzes the reduction of H_2O_2 and protects the tissue from highly reactive oxygen free radicals and hydroxyl radicals [44]. GPx, an enzyme with selenium, catalyzes the reduction of H_2O_2andhydroperoxides to non-toxic products [45]. In the present study, SOD activity decreased significantly in ethanol-treated animals. The activities of H_2O_2 scavenging enzymes CAT and GPx also decreased significantly after ethanol treatment. The decline in these enzyme activities can be explained by the fact that excessive superoxide anions may inactivate SOD, thus, resulting in inactivation of the H_2O_2 scavenging enzymes. Administration of hyperin effectively prevented the decrease in SOD, CAT and GPx activities, which may be attributed to the scavenging of radicals by hyperin, resulting in protection of these enzymes.GR is a cytosolic enzyme involved in the reduction of GSSG (an end product of GPx reaction) to GSH [46]. With ethanol treatment, there was a marked reduction in GPx activity, leading to reduced availability of substrate for GR, thereby decreasing the activity of GR. Pre-treatment of hyperin to ethanol administered rats restored the activity of GR, which accelerates the conversion of GSSG to GSH.

Thus, from the present investigation, it can be concluded that the hyperin afforded significant antiulcer activity in ethanol-induced gastric mucosal injury by enhancing the antioxidant potential of the gastric mucosa, thereby reducing mucosal damage.

NSAIDs (Non-steroidal anti-inflammatory drugs), such as aspirin are known to be potent prostaglandin (PG) biosynthesis inhibitors [47]. In the stomach, prostaglandins play a vital protective role in stimulating the secretion of bicarbonate and mucus, in maintaining mucosal blood flow, in regulating mucosal cell turnover and repair, and in decreasing the aggressive factors, such as acid and pepsin [48]. Thus, the suppression of prostaglandin synthesis by aspirin results in increased susceptibility to mucosal injury and gastroduodenal ulceration. Exposure of gastric mucosa to aspirin has been shown to affect cellular integrity, and such changes are correlated with oxidative stress and mitochondrial damage [49].

In this study, exposure of animals to aspirin caused severe mucosal lesions. However, a reversal in mucosal damage was observed by hyperin 25 mg/kg and hyperin 50 mg/kg treatment. The gastroprotective activity of hyperin seems to be related to a reduction in damage to the mucosa induced by free radicals and this activity may be due to its antioxidant action. These findings could be efficiently correlated with the images of the stomach sections of the aspirin as well as hyperin treated animals.

The aspirin-treated group of animals showed an increase in LPO (MDA levels) and a decrease in GSH levels and SOD, CAT, GPx and GR activities, which was reversed in hyperin 25 mg/kg and hyperin 50 mg/kg treatment groups. This was probably by a mechanism similar to that explained in the ethanol-induced ulceration model. Histamine is a powerful gastric secretagogue and evokes a copious secretion of acid from parietal cells by acting on the H_2 receptors [50].

Gastric acid is considered as an important factor in the development of acute and chronic gastric mucosal lesions. Levels of MDA in animals treated with histamine reflect the excessive formation of free radicals and greater formation of lipid peroxides, resulting in severe damage to the gastric tissue. The histamine-elevated MDA levels were significantly attenuated by the hyperin treatments, probably by preventing the formation of lipid peroxides. Decreased GSH levels in histamine treated rats may be due to its increased utilization to augment the activities of GPx.

The GSH levels depleted by histamine were significantly restored by hyperin oral administration. It may be that an increase in GSH levels is due to its enhanced synthesis or improved GR activity in the presence of hyperin. Histamine treatment to rats significantly decreased the activities of the antioxidant enzymes SOD, CAT, GPx and GR. Administration of hyperin to histamine treated rats effectively prevented this depletion, which may be due to the free radical scavenging activity of it. In conclusion, the two major mechanisms responsible for the gastroprotective activity of hyperin are scavenging of ROS and protection against GSH depletion. To summarize, the hyperin showed significant gastroprotective activity probably by an underlying antioxidant activity.

CONCLUSION: Based on the results, we confirmed the popularly known benefits of the hyperin for the treatment of gastric ulcers. These benefits involved the gastroprotective activity of the hyperin using experimental models of ulcer prevention and cure. This compound is dependent on the antioxidant potential for antiulcer activity.

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