Silymarin, an antioxidant bioflavonoid, inhibits experimentally-induced peptic ulcers in rats by dual mechanisms

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Abstract

Introduction: Antioxidants are reported to have antiulcer activity. We investigated silymarin, a bioflavonoid antioxidant, for antiulcer potential. **Materials and Methods**: Pylorus-ligated Shay rats (*n*=5) were used as the experimental gastric ulcer animal model. The rats, separated into three groups, were administrated silymarin (50 mg/kg), omeprazole (3.6 mg/kg), or saline (5 ml/kg) per orally daily for 5 days prior to ulcerogenic challenge. Nineteen hours after the challenge, the rats were sacrificed and their stomachs isolated. Formed gastric juice was collected for measurement of volume, titrimetric estimation of free and total acidity, and total acid output by the conventional methods. The ulcer index was calculated. Total acid output and free and combined acid quantities were calculated using the acidity value and the volume of formed gastric juice. **Results**: Silymarin exerted significant (*P*<.05) antiulcer activity (the ulcer index was reduced to 7.4 ± 1.0 from the control value of 19.8 ± 4.1). Silymarin also significantly reduced free and total acidity, gastric juice volume, total acid output, and combined acid content. The results were analyzed by ANOVA and Newman–Keuls multiple comparison test. **Conclusion**: This study demonstrates that silymarin has significant antiulcer activity. It perhaps acts by decreasing hydrochloric acid output and increasing buffering power (combined acid).

Key words: Antiulcer activity, omeprazole, Shay rat, silymarin

Introduction

Peptic ulcer disease (PUD) is a result of imbalance between the aggressive and defensive factors in stomach. Various pathophysiological mechanisms have been proposed for the development of ulcer disease. Recently, oxidative free radicals have been implicated as important factor in mediating PUD.[1] In the last few years, there have been experimental and clinical studies that have suggested that antioxidants such as a-tocopherol,[2] carotene,[3] and allopurinol[4] have protective effects in PUD.

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Silymarin, a bioflavonoid antioxidant, has been shown to have hepatoprotective activity in experimental and clinical studies.[5,6] A gastroprotective role of silymarin has not been established, although there is a single report in literature suggesting that silymarin may have gastroprotective action in PUD.[7] In view of this, the present study was undertaken to investigate the antiulcer potential of silymarin in experimentally-induced peptic ulcer in albino rats.

Materials and Methods

Fifteen albino rats of Wistar strain of either sex weighing 150–200 gm were randomly selected and divided into three groups of five animals each. The rats were maintained in separate cages under normal room temperature and a 12 hour:12 hour light:dark cycle. The animals were fed standard rat chow and provided water *ad lib*.

Animals of groups 1, 2, and 3 received, respectively, saline 5 ml/kg, omeprazole 3.6 mg/kg, and silymarin 50 mg/kg orally daily for 5 days. Omeprazole and silymarin were dissolved in 1 ml propylene glycol and 0.1% sodium bicarbonate,

respectively. Drug doses for rats were extrapolated from the clinical doses according to Paget and Barnes.[8]

On day 6, after overnight fasting, the animals were subjected to a pylorus ligation procedure as described by Shay *et al*.[9] Nineteen hours after ulcerogenic challenge, the animals were sacrificed and their stomachs were cut open along the lesser curvature and the gastric juice was collected. The wall of the emptied stomach was carefully examined with a lens for ulcers. The following parameters were recorded and calculated:
1. Gastric juice volume (GJV) in ml.
2. Free acidity (FA) at pH 3.8 and total acidity (TA) at pH 8.3 by titrating against 0.5N NaOH, with Toepfer's reagent and phenolphthalein, respectively, as indicators.[10]
3. Total acid output (TAO) in millimoles was calculated by the formula TAO = (X/5) × (V/2), where X = burette values of 0.5N NaOH required to reach pH 8.3, and V = volume of gastric juice in milliliters.
4. Ulcer index (UI) was calculated as a product of the ulcer numbers and ulcer severity score. The ulcer severity was scored by the method of Barret *et al*.[11]

The pH 8.3 was chosen as the end point for total acidity determination as it more accurately reflects H^+ secretion (acid output).[12] The quantity of free acid (QFA) in the gastric juice was calculated by the same formula as applied for TAO, except that the burette reading 'X' was taken at pH 8.3 as at this pH free hydrochloric acid (HCl) is totally neutralized.[13] The difference between TAO and QFA was taken as the quantity of combined acid (QCA), preferably labeled as 'buffer power,' which reflects the mucin content.[13]

The three calculated parameters TAO, QFA, and QCA help in monitoring the effects of the drugs on, respectively, HCl formed over a period of time, non-buffered HCl (QFA) in the juice, and combined acid (QCA), i.e., acid that has been mixed with mucus in the gastric juice.[14,15] The QCA reflects that part of the secreted HCl which has been complexed with protein buffers (like mucin) of the dissolved mucus in the gastric juice. The rolection has been well emphasized.[16]

Ethical clearance was obtained from the institutional ethical committee prior to the experiment. The chemical drug, i.e., silymarin and omeprazole were obtained *gratis* from Ranbaxy Co. Ltd.

Statistical analysis
Group means (±SE) were calculated for all parameters. These values were utilized to compare influence of pretreatment with saline , omeprazole , and silymarin (test drug). The results were analyzed by ANOVA, and the significance of differences between groups was calculated by post hoc multiple comparison test (Newman–Keuls method) as described by Portney and Watkins.[17]

RESULTS

There were striking differences between the means of the saline group and other two groups for all the parameters (P<.05). The mean values of the silymarin group were discernibly higher than those of the omeprazole group for almost all parameters except for free and total acidity [Table 1].

To ascertain whether or not the observed differences could have occurred by chance, the data was subjected to ANOVA [Table 2]. A multiple comparison followed [Table 3].

The observed differences between silymarin and omeprazole group means were significant (P<.05) for parameters like formed GJV, TAO, and QCA (buffer power), with the means of the silymarin group being higher in all cases. Further, calculation of 'buffer power' (the ratio of combined acid to the corresponding total acid) revealed that this ratio was highest for the silymarin group (74%), followed by that for the omeprazole (60%) and saline groups (50%). This suggests silymarin also promotes mucin synthesis in comparison to omeprazole.

DISCUSSION

The primary objective of this study was to ascertain the antiulcer potential of silymarin, an antioxidant bioflavonoid. The

Table 1: Results showing the various parameters in the treated groups

Parameters	Values (mean±SE) for different groups		
	Saline	**Silymarin**	**Omeprazole**
Ulcer index	19.8 ± 4.1	7.4 ± 1.0	2.2 ± 1.0
Gastric juice vol (ml)	19.8 ± 1.3	13.4 ± 1.2	9.6 ± 0.3
Total acidity (mEq/l)	28.8 ± 3.5	13.1 ± 1.6	11.8 ± 0.54
Free acidity (mEq/l)	7 ± 1.5	3.3 ± 0.3	2.6 ± 0.2
Total acid output (mmoles)*	23 ± 2.2	8.8 ± 0.43	4.68 ± 0.23
Total free acidity (mmoles)*	10.13 ± 2.1	2.29 ± 0.13	1.04 ± 0.07
Total combined acid (mmoles)Å	13.10 ± 1.5	6.6 ± 0.45	2.94 ± 0.19

*Over the 19-hours period of observation post pylorus ligation; Åin gastric juice collected over 19 hours.

Table 2: ANOVA results for the three treatment groups

Parameters	MSb	MSe	SSb	SSe	F#
Ulcer index	339	64	677	959	5.2
Gastric juice vol (ml)	168	2.5	337	31	6.7
Total acidity (mEq/L)	142	30	283	447	4.7
Free acidity (mEq/L)	38	4	76	64	9.5
Total acid output (mmoles)*	461.36	8.05	922.77	96.60	7.3
Total free Acid (mmoles)*	109.17	5.8	218.34	70.49	18.8
Total combined acid (mmoles)Å	137.15	4.08	274.31	48.17	34.28

MSb = SSb / dfb; MSe = SSe / dfe; F = MSb / MSe; N=15, K=3. #The calculated F ratio for each parameter is large than the table F ratio value (3.89) for the given dfb 2 and dfe 12 at P=.05.*Over the 19-hours period of observation post pylorus ligation; Åin gastric juice collected over 19 hours; MSb- mean square between treatment groups; MSe-mean square within group (error); SSb-sum of squares between treatment groups; SSe-sum of squares within treatment group(error); N-total no of animals (15); K-no of replicates; dfb-degree of freedom (number of treatments n-1); dfe-degree of freedom {error (N-1)-(n-1)}

Table 3: Results of multiple comparison test by Newman–Keuls method for determining minimum significant difference

Parameters	Saline silymarin	Saline omeprazole	Silymarin omeprazole
Ulcer index	12.4* (7.7)	17.6* (12)	5.2 (7.7)
Gastric juice vol (ml)	6.4* (2.17)	10.2* (2.6)	3.8* (2.0)
Total acidity (mEq/l)	16* (7.5)	17* (8.2)	1 (7.5)
Free acidity (mEq/l)	4* (2.7)	4.4* (2.9)	0.4 (2.7)
Total acid output (mmoles)#	14.2* (3.9)	18.32* (4.7)	4.20* (3.9)
Total free acid (mmoles)#	7.84* (5.8)	9.09* (4.06)	1.25 (5.8)
Total combined acid (mmoles)Å	6.5* (2.7)	10.16* (3.4)	3.66* (2.78)

*Actual differences between concerned means are significantly (P<.05) higher than corresponding MSD given in parenthesis alongside. #Over 19-hours period of observation post pylorus ligation; Åin gastric juice collected over 19 hours

secondary objective was to identify the probable mechanisms of action. The results confirm the antiulcer activity of silymarin and provide some understanding the possible mechanisms involved.

The cytoprotective action of silymarin could be by prevention of peroxidative processes. There are a few reports suggesting that silymarin, by increasing superoxide dismutase (SOD) and glutathione levels, increases the endogenous levels of antioxidants.[16] The other possible mechanism is that silymarin stimulates DNA-dependent RNA polymerase, leading to increased protein synthesis and thus promoting healing and reparative processes as explained by Alarcon de la lastra et al.[7]

Thus, this study shows that silymarin has significant antiulcer activity by dual mechanisms: an ability to decrease the HCl secreted by gastric glands in pylorus-ligated rats and by a cytoprotective potential. The results of present study are consistent with the findings of Alarcon de la lastra et al.[7] The above-suggested mechanism of antiulcer activity of silymarin is perhaps due to its antioxidant property of scavenging active oxidative radicals. The antiulcer action of silymarin is similar to that of rebamapide which is used in some Asian countries for treatment of peptic ulcer; the latter acts by cytoprotective effects as well as by scavenging oxidative radicals.[18] Silymarin seems to have an additional property of being able to decrease HCl secretion.

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