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Gastric anti-ulcer activity of silymarin, a lipoxygenase inhibitor, in rats

C. ALARCON DE LA LASTRA, M. J. MARTIN, E. MARHUENDA, *Departamento de Farmacia y Tecnología Farmacéutica, Laboratorio de Farmacodinamia, Facultad de Farmacia, 41012 Sevilla, Spain*

Abstract—Oral treatment with silymarin was found to be effective in the prevention of gastric ulceration induced by cold-restraint stress, in rats. Statistically significant ulcer index values with respect to the control group, were observed. In 6 h pyloric-ligated animals silymarin showed a significant reduction in the number and severity of the ulcers; however, it did not alter the gastric secretion volume or acidity although histamine concentration was significantly decreased. In absolute ethanol-induced ulcers, treatment with silymarin 1 or 2 h before the anti-ulcerogenic agent, did not prevent the formation of gastric lesions. Furthermore, the hexosamine content was decreased significantly, but the total protein output was enhanced, showing similar values to those with the standard drug, carbenoxolone. These results suggest that the anti-ulcerogenic effect of silymarin could be related to its inhibitory mechanism of enzymatic peroxidation by the lipoxygenase pathway, avoiding leukotriene synthesis.

Several flavonoids exert significant scavenging properties on oxygen radicals in-vivo and in-vitro affecting various steps in the arachidonate cascade via the cyclo-oxygenase or lipoxygenase pathway (Baumann et al 1980; Lonchampt et al 1989; Chen 1990). These radicals in particular seem to play an important role in ulcerative and erosive lesions of the gastrointestinal tract (Ogle & Cho 1989).

Silymarin, 5,7,4′-trihydroxy-3′-methoxy-flavanona-3-ol, is the major active principle of *Silybum marianum*, and is currently used therapeutically in hepatopathias caused by the action of a harmful agent. The effects of silymarin are partly due to inhibition of enzymatic peroxidation on biological membranes, via 5-lipoxygenase (Bindoli et al 1977a, b) and inhibition of biosynthesis of the leukotrienes. In contrast to prostaglandins the actions of leukotrienes may be deleterious to the integrity of gastric mucosa and therefore these eicosanoids are implicated as possible mediators of gastric damage and ulceration (Boughton-Smith 1989). Thus, it was of interest to establish the anti-ulcer activity of silymarin in various models of experimental gastric ulceration.

Materials and methods

Animals. Male Wistar rats, 180–200 g, were placed in single cages with wire-net floors in a controlled room (temperature 22–24°C, humidity 70–75%) and were fed a normal laboratory diet. The animals were deprived of food for 48 h before experimentation but allowed free access to tap water throughout.

Drug preparation and treatment. Silymarin (Madaus Cerafarm, SA, Spain) (25, 50 and 100 mg kg^{-1}), ranitidine (Alter, SA, Spain) (50 mg kg^{-1}) and carbenoxolone (Leo, SA, Spain) (80 mg kg^{-1}) were dissolved in saline (0·9% NaCl), prepared freshly each time and administered intragastrically. The control rats received saline in a comparable volume (1 mL/100 g) by the same route.

Cold-restraint-induced ulcers. Ulceration was induced by immobilizing the rat on a cylindrical cage enclosed at its ends and maintained at 3–5°C for 3 h (Senay & Levine 1967). Different groups of rats were administered the drugs 1 h before they were restrained. At the end of the experimental period, animals were killed by a sharp blow on the head. Their stomachs were removed and the number of ulcers was noted. The severity of mucosal ulceration was determined according to the scoring system of Cioli et al (1967). Mean ulcer scores for each animal were calculated and expressed as the ulcer index (UI).

Acute gastric ulcer induced by ethanol. Ulceration was induced according to the method described by Soldato et al (1984), by intragastric instillation of 1 mL of absolute ethanol. The drugs were administered to different groups, 1 and 2 h before ethanol administration. At the end of 1 h of intragastric administration of absolute ethanol, the animals were killed by a sharp blow on the head and their stomachs removed. The number of erosions per stomach was assessed for severity according to the scoring system of Adami et al (1964). Mean scores for each animal were calculated and expressed as the UI.

Measurement of gastric mucus output. The gastric mucus was obtained by scraping the mucosa with a glass slide and was immediately homogenized in 4 mL distilled water. Colorimetry was used to determine total proteins (Lowry et al 1951) and hexosamines (Boas 1953). In order to avoid variations in either the criteria of gastric mucosal lesion or scraping technique, they were performed by the same person.

Pylorus-ligated gastric secretion and ulceration. The pylorus-ligated rat model first described by Shay et al (1954) was used. The drugs were administered orally 1 h before starting the experiments. The pylorus was tied under light ether anaesthesia. Care was taken not to damage the blood supply. The animals were allowed to rest for 6 h, then were killed using an overdose of anaesthetic. The cardia was ligated and the whole stomach was removed. The gastric content was collected and centrifuged to obtain the clear fluid. The total volume was measured. Samples (1 mL) were analysed for hydrogen ion concentration by potentiometric titration with 0·01 M NaOH. The stomach was cut open along the greater curvature and washed gently under running tap water. On examination, the ulcers produced were measured and expressed in terms of the UI (mm^2).

Biochemical assay of pepsin, histamine, sodium and potassium electrolytes. The colorimetric method of Lowry et al (1951) was used to determine the total protein in samples, expressed as mg pepsin (mL of gastric content)$^{-1}$.

Histamine output was measured by fluorimetry following the method of Lorenz (1972).

Gastric concentrations of sodium and potassium were determined using an Elvi 655 flame photometer.

Statistical analysis. Values are given as arithmetic means ± s.e.m. The statistical significance ($P < 0.05$) of observed differences between groups was evaluated by the Mann-Whitney U-test for non-parametric data.

Results

In cold-restraint ulcers, silymarin (100 mg kg^{-1}) was considerably more potent than ranitidine with a calculated UI value of

Correspondence: C. Alarcon de la Lastra, Departamento de Farmacia y Tecnologia Farmacéutica, Laboratorio de Farmacodinamia, Facultad de Farmacia, 41012 Sevilla, Spain.

Table 1. Effects of ranitidine and silymarin on cold-restraint-induced ulcers in rats.

Treatment (mg kg^{-1})	Ulcerated stomachs (%)	Haemorrhagic stomachs (%)	UI (mm^2) (mean ± s.e.)
Control	87·5	37·5	2·37 ± 0·65
Ranitidine			
50	50	0·0	0·66 ± 0·26**
Silymarin			
100	33·3	12·5	0·58 ± 0·34**
50	50	12·5	0·87 ± 0·11**
25	50	12·5	1·14 ± 0·51*

*$P < 0·05$, **$P < 0·01$ compared with vehicle-treated control. There were 8 animals in each group.

0·58 ± 0·34 (Table 1, $P < 0·01$). Pretreatment with silymarin 1 or 2 h before administration of absolute ethanol to rats did not prevent the formation of gastric lesions, however, a significant increase was observed in the mucus content. The highest increase was observed in rats receiving 25 mg kg^{-1} silymarin 2 h before the start of ethanol administration. In comparison, carbenoxolone (80 mg kg^{-1}) for a 1–2 h period almost completely prevented the formation of gastric lesions. The hexosamine content decreased significantly as compared with control, but the total protein output was enhanced (Table 2). In 6 h-pyloric ligated animals, silymarin showed a significant reduction in number and severity of ulcers, but no dose-dependency was observed and the results are lower than those obtained with ranitidine (Table 3). Under the same conditions, pepsin and

Table 2. Effects of carbenoxolone and silymarin on ethanol-induced gastric mucus secretion and ulceration in rats.

Treatment (mg kg^{-1})	UI (mm^2) (mean ± s.e.)	Mucus content (g)	Total proteins (mg mL^{-1})	Hexosamines (μg mL^{-1})
Control				
1 h	7·30 ± 0·33	0·345 ± 0·04	4·69 ± 0·37	83·32 ± 1·64
2 h	8·00 ± 0·0	0·444 ± 0·03	5·62 ± 0·36	114·62 ± 2·28
Carbenoxolone				
80, 1 h	2·50 ± 0·09**	0·791 ± 0·02**	10·44 ± 0·27**	103·22 ± 2·78*
80, 2 h	0·66 ± 0·33**	1·194 ± 0·07**	12·46 ± 0·02**	146·50 ± 7·06**
Silymarin				
25, 1 h	8·00 ± 0·0	0·544 ± 0·07	6·28 ± 0·35	53·62 ± 0·96
25, 2 h	8·00 ± 0·0	1·077 ± 0·06**	8·53 ± 0·33*	63·17 ± 3·9**
50, 1 h	7·57 ± 0·07	0·648 ± 0·02*	7·75 ± 0·34*	49·92 ± 3·13**
50, 2 h	7·16 ± 0·40	0·852 ± 0·11*	9·94 ± 0·26*	82·00 ± 1·65*

*$P < 0·05$, **$P < 0·01$ compared with vehicle-treated control. There were 6 animals in each group.

Table 3. Effects of ranitidine and silymarin on secretion and ulceration in pylorus-ligated rats.

Treatment (mg kg^{-1})	Ulcerated stomachs (%)	Haemorrhagic stomachs (%)	UI (mm^2) (mean ± s.e.)	Gastric content (mL) (mean ± s.e.)
Control	100	37·5	49·3 ± 15·9	10·0 ± 0·7
Ranitidine				
50	62·5	25·0	2·8 ± 1·8**	10·0 ± 0·4
Silymarin				
100	62·5	0·0	6·4 ± 2·8**	9·0 ± 1·1
50	62·5	0·0	6·3 ± 0·3**	10·1 ± 0·9
25	75	12·5	6·7 ± 1·8*	12·2 ± 0·7*

*$P < 0·05$, **$P < 0·01$ compared with vehicle-treated control. There were 8 animals in each group.

Table 4. Effects of ranitidine and silymarin on gastric acidity, pepsin, histamine, sodium and potassium output (mean ± s.e.)

Treatment (mg kg^{-1})	Acidity (mEq L^{-1})	Pepsin (mg L^{-1})	Histamine (μg L^{-1})	Na^+ (mEq L^{-1})	K^+ (mEq L^{-1})
Control	60·43 ± 6·80	18·66 ± 1·12	3·50 ± 0·34	56·42 ± 3·87	6·73 ± 0·77
Ranitidine					
50	16·23 ± 4·14**	18·66 ± 1·65	3·59 ± 0·33	84·98 ± 9·56*	11·03 ± 1·20*
Silymarin					
100	48·59 ± 17·08	16·78 ± 1·05	1·10 ± 0·14**	88·83 ± 7·24**	10·63 ± 1·97
50	51·50 ± 19·70	14·80 ± 1·11*	2·50 ± 0·28*	87·68 ± 6·80**	10·42 ± 2·02
25	65·49 ± 8·40	17·61 ± 1·40	3·37 ± 0·26	74·23 ± 3·73*	7·83 ± 0·45

*$P < 0·05$, **$P < 0·01$ compared with vehicle-treated control.

histamine concentration was significantly decreased with silymarin. Pretreatment with silymarin produced significant increases in Na^+ output. The maximal concentration obtained with the highest dose of silymarin ($88{\cdot}83 \pm 7{\cdot}2$ mEq L^{-1}) was comparable with that observed with ranitidine ($84{\cdot}98 \pm 9{\cdot}56$) (Table 4).

Discussion

Silymarin, a 5-lipoxygenase inhibitor, has been found effective in the various types of experimentally induced gastric ulcers, produced in the course of this study. Oral treatment with silymarin prevents, in a highly significant way, the ulceration induced by cold-restraint stress. It has been reported that lipoxygenase inhibitors are effective in the prevention of stress-induced ulceration (Ogle & Cho 1985). This observation suggests involvement of the leukotrienes in the aetiology of stress-induced ulcer formation (Ogle & Cho 1986, 1989). In stress, there appears to be an intense surge of vagal overactivity with rapid degranulation of the mast cell, resulting in a strong action by released histamine which adds to the other ulcerogenic effects of vagal stimulation (Brooks 1985). Silymarin has also been found to be effective in reducing the incidence of gastric mucosal damage in pylorus-ligated rats, with a statistically significant reduction in histamine output. It has been postulated that this autocoid might be involved in the formation of pylorus-ligated ulcers and play a mediating role in the gastric secretion stimulated by gastrin, vagal excitation and cholinergic agents (Parmar & Ghosh 1981).

It has been suggested (Goldyne 1984) that the ulcerogenic effects of lipoxygenase products could be indirectly through degranulation of the mucosal mast cells to release histamine, possibly through a direct histamine-like action. Findings using sulphasalazine, which blocks lipoxygenase activity, have also suggested that these eicosanoids may degranulate the gastric mast cells (Ogle & Cho 1985). The fact that silymarin does not reduce either acidity or pepsin secretion with the tested doses, suggests that leukotrienes may not play an important role in the control of gastric secretion. Thus, the anti-ulcerogenic effect of silymarin in both experimental models could be explained by means of its inhibitory mechanism of enzymatic peroxidation.

In absolute ethanol-induced ulcers, pretreatment with silymarin is ineffective; total mucus content is not significantly enhanced and it is not rich in hexosamines. Our results are in agreement with the study of Boughton-Smith & Whittle (1988), who observed that inhibitory compounds of the lipoxygenase pathway are not effective in reduction of ethanol-induced gastric mucosal damage. These findings, therefore, provide evidence against a role for leukotrienes as primary mediators in the pathogenesis of this class of ulcers.

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