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Gastroprotective activity of ferruginol in mice and rats: effects on gastric secretion, endogenous prostaglandins and non-protein sulfhydryls

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Abstract

The gastroprotective mechanism of the natural diterpene ferruginol was assessed in mice and rats. The involvement of gastric prostaglandins (PGE_2), reduced glutathione, nitric oxide or capsaicin receptors was evaluated in mice either treated or untreated with indometacin, N-ethylmaleimide (NEM), N-nitro-L-arginine methyl ester (L-NAME) or ruthenium red, respectively, and then orally treated with ferruginol or vehicle. Gastric lesions were induced by oral administration of ethanol. The effects of ferruginol on the parameters of gastric secretion were assessed in pylorus-ligated rats. Gastric PGE_2 content was determined in rats treated with ferruginol and/or indometacin. The reduction of gastric glutathione (GSH) content was determined in rats treated with ethanol after oral administration of ferruginol, lansoprazole or vehicle. Finally, the acute oral toxicity was assessed in mice. Indometacin reversed the gastroprotective effect of ferruginol (25 mg kg^{-1}) but not NEM, ruthenium red or L-NAME. The diterpene (25 mg kg^{-1}) increased the gastric juice volume and its pH value, and reduced the titrable acidity but was devoid of effect on the gastric mucus content. Ferruginol (25, 50 mg kg^{-1}) increased gastric PGE_2 content in a dose-dependent manner and prevented the reduction in GSH observed due to ethanol-induced gastric lesions in rats. Single oral doses up to 3 g kg^{-1} ferruginol did not elicit mortality or acute toxic effects in mice. Our results showed that ferruginol acted as a gastroprotective agent stimulating the gastric PGE_2 synthesis, reducing the gastric acid output and improving the antioxidant capacity of the gastric mucosa by maintaining the GSH levels.

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Introduction

In recent years different diterpenes isolated from plants have been shown to possess gastroprotective activity against gastric lesions induced by different agents in experimental animals (Lewis & Hanson 1991; Souza-Brito et al 1998; Rodríguez et al 2001; Matsuda et al 2002; Melo et al 2003). The study of the mechanism underlying this protective effect has pointed out that many of these compounds act by reinforcing the defensive factors in the gastric mucosa such as stimulation of prostaglandin synthesis, increase of mucus production or improvement of the mucosal antioxidant capacity (Onoda et al 1990; Wada et al 1997; Ichikawa et al 2000; Rodríguez et al 2005). On the other hand, some of these gastroprotective diterpenes seem to reduce the activity of the aggressive factors in the stomach such as pepsin or acid secretion (Pearson & Roberts 2001; Hiruma-Lima et al 2002).

Ferruginol is an abietane diterpene occurring in plants belonging to the families Podocarpaceae (Cambie et al 1984), Cupressaceae (Sharp et al 2001), Lamiaceae (Ulubelen & Topcu 1992) and Verbenaceae (Ono et al 1999) among others. Recently, it was reported that ferruginol displayed gastroprotective activity against gastric damage induced

by HCl/ethanol in mice (Rodríguez et al 2006). The mechanism involved in this gastroprotective effect was partially assessed using some in-vitro models. Those studies suggested that ferruginol did not have free radical scavenging activity, did not protect against cellular damage induced by sodium taurocholate nor modified the reduced glutathione content in the gastric mucosa; but may have protected the epithelial cells against lipid peroxidation and stimulated an increase of the prostaglandin content (Rodríguez et al 2006).

The use of in-vitro tests to assess the bioactivity of potential new drugs may give an incomplete picture of their pharmacological effects because in-vitro systems do not reproduce properly the conditions found in the whole organism. Different factors occurring in the animals such as absorption, distribution, transport, metabolism and xenobiotic clearance capacity, among others, may modify the biological response observed during in-vitro testing (Houghton et al 2007). To determine the pharmacological effect of ferruginol in-vivo, we have assessed different mechanisms that could be involved in the gastroprotective activity of this diterpene in rats and mice.

Materials and Methods

Animals

Male Wistar rats (200–250 g; Central Animal House of the Universidade Estadual de Campinas (CEMIB/UNICAMP)) and male Swiss albino mice (26–34 g; CEMIB/UNICAMP or Instituto de Salud Pública de Chile) were used. The animals were fed on a standard certified diet with free access to water under standard conditions of 12-h dark–light period, 50% relative humidity and 23 ± 2°C room temperature. The experimental protocols were approved by the Institutional Committee for Ethics in Animal Experimentation (CEEA/UNICAMP) and the Universidad de Talca Institutional Animal Care and Use Committee that follows the recommendations of the Canadian Council on Animal Care (Olfert et al 1993).

Drugs

Ethanol, formalin and NaOH were purchased from Merck (Germany). Tween 80, indometacin, N-ethylmaleimide (NEM), ruhenium red, N-nitro-L-arginine methyl ester (L-NAME), lansoprazole, cimetidine, carbenoxolone, Alcian blue, sucrose, EDTA, trichloroacetic acid, tris, 5,5′-dithiobis-(2-nitrobenzoic acid) and salts were obtained from Sigma Chemical Co. (USA).

Acute oral toxicity

Acute oral toxicity of ferruginol was assessed on 12-h fasted male Swiss albino mice as described by Souza-Brito et al (1998). Increasing doses of ferruginol (500, 1000, 2000 and 3000 mg kg^{-1}, p.o.) were administered to groups of 10 animals for each dose level. Animals receiving the vehicle (12% Tween 80, 10 mL kg^{-1}) served as the control. The groups were observed at 0, 30, 60, 120, 180 and 240 min after ferruginol administration and then twice a day for the next 14 days. At the end of this period the number of survivors was recorded and the acute toxicological effect was inferred on the basis of mortality, expressed as LD50. The LD50 value was obtained using software based on the method of Litchfield & Wilcoxon (1949).

Ethanol-induced gastric lesions in indometacin-, NEM-, ruthenium red- and L-NAME-pretreated mice

The experiment was performed with mice according to Matsuda et al (2002). After 24-h fasting, groups of mice were treated with indometacin (30 mg kg^{-1}, s.c.), an inhibitor of prostaglandin synthesis, or saline; NEM (10 mg kg^{-1}, s.c.), an SH blocker (sulfhydryl compounds), or saline; N-nitro-L-arginine methyl ester (L-NAME) (70 mg kg^{-1}, i.p.), an inhibitor of NO synthase, or saline; and ruthenium red (3.5 mg kg^{-1}, s.c.), a vanilloid receptor antagonist, or saline. Thirty minutes later, the different groups received an oral dose of the vehicle (12% Tween 80, 10 mL kg^{-1}) or ferruginol (25 mg kg^{-1}). Animals treated with saline and Tween were used as the control group. After 60 min, all groups were orally treated with 1 mL absolute ethanol for gastric lesion induction. Animals were killed 1 h after administration of ethanol by cervical dislocation and the stomachs were fixed in 5% formalin for 30 min and opened along the greater curvature. Gastric damage visible to the naked eye was observed in the gastric mucosa as elongated black-red lines, parallel to the long axis of the stomach. The length (mm) of each lesion was measured, and the lesion index was expressed as the sum of the length of all lesions.

Ethanol-induced ulcer

The experiment was performed according to the method of Morimoto et al (1991). After 24-h fasting, rats received an oral administration of ferruginol (25, 50 mg kg^{-1}), lansoprazole (30 mg kg^{-1}) or 12% Tween 80 (10 mL kg^{-1}). One hour after treatment, all rats received 1 mL 99.5% ethanol to induce gastric ulcer. The animals were killed 1 h after treatment and the stomachs removed, inflated with 1 mL saline, fixed in 5% formalin for 30 min and opened along the greater curvature to determine the lesion index (mm^2).

Determination of gastric secretion

Gastric secretion was assessed by the method of Shay et al (1945) with modifications. Rats were fasted for 24 h, with free access to water. After ligation of the pylorus, ferruginol (25 mg kg^{-1}), cimetidine (100 mg kg^{-1}) or 12% Tween 80 (10 mL kg^{-1}) was administered intraduodenally. Cimetidine, instead of the antisecretory drug lansoprazole, was used as reference compound because this substituted benzimidazole interacts directly on the gastric mucosa and the compounds were administered intraduodenally in this experiment. Rats were killed by cervical dislocation 4 h later, the abdomen was opened, and another ligature was placed around the oesophagus close to the diaphragm. The stomachs were removed and the volume of gastric juice (mL) and pH were determined. Distilled water (5 mL) was added and the solution was centrifuged at 1000 *g* for 10 min. The total acid in the

gastric secretion was determined in the supernatant volume by titration to pH 7.0 with 0.01 M NaOH.

Determination of mucus in gastric content

This assay was carried out as described by Corne et al (1974) with slight modifications. Rats were fasted for 24 h and, under anaesthesia, the abdomen was incised and the pylorus ligated. Carbenoxolone (200 mg kg^{-1}), 12% Tween 80 (10 mL kg^{-1}) and ferruginol (25 mg kg^{-1}) were administered intraduodenally after pylorus ligation. Animals were killed by cervical dislocation 4 h after ligation and the glandular segments of the stomachs were removed and weighed. Each glandular segment was immediately immersed in 10 mL of the 0.1% Alcian blue solution (0.16 M sucrose/0.05 M sodium acetate, pH 5.8). After immersion for 2 h, excess dye was removed by two successive rinses with 10 mL 0.25 M sucrose, first for 15 min and then for 45 min. The stomachs were transferred to 0.5 M magnesium chloride and shaken for 2 h. A 4-mL portion of the blue extract was then shaken vigorously with an equal volume of ether. The resulting emulsion was centrifuged at 1050 *g* and the absorbance of the aqueous layer was read at 580 nm. The amount of Alcian blue extracted per gram of net glandular tissue was calculated from a standard curve.

Determination of gastric prostaglandin E_2 (PGE_2) content

Gastric prostaglandin content was determined in rats according to Curtis et al (1995). Animals were killed by cervical dislocation 30 min after treatment with 12% Tween 80 (10 mL kg^{-1}, p.o.), or Tween 80 (10 mL kg^{-1}, p.o.) plus indometacin (30 mg kg^{-1}, s.c.) or ferruginol (25 and 50 mg kg^{-1}, p.o.), or ferruginol (25 and 50 mg kg^{-1}, p.o.) plus indometacin (30 mg kg^{-1}, s.c.). A non-treated group of animals (sham) was included. The abdomen of the animals was opened; samples of the corpus (full thickness) were excised, weighed and suspended in 1 mL 10 mM sodium phosphate buffer, pH 7.4. The tissue was minced finely with scissors and incubated at 37°C for 20 min. The prostaglandin content of the buffer was measured using an enzymatic immunoassay kit (RPN 222, Amersham).

Determination of gastric glutathione (GSH) content

Gastric reduced GSH was determined in rats as described by Rafatullah et al (1990) with slight modifications. Animals were fasted for 24 h and then treated orally with 12% Tween 80, ferruginol (25 and 50 mg kg^{-1}) or lansoprazole (30 mg kg^{-1}). One hour after the treatment, all rats received 1 mL 99.5% ethanol to induce gastric lesions. A non-treated and non-ethanol-induced gastric lesion group of animals (sham) was included. After 1 h, animals were killed and the glandular portions of the stomachs removed, minced finely and homogenized using a Polytron homogenizer in ice-cold 0.02 M EDTA. Samples (5.0 mL) of the homogenates were mixed in 15-mL test tubes with 4.0 mL distilled water and 1.0 mL 50% trichloroacetic acid. The tubes were shaken intermittently for l0–15 min and centrifuged at 3000 *g* at 4°C. Samples (2 mL) of supernatants were mixed with 4.0 mL 0.4 M Tris buffer (pH 8.9). 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB) 0.1 mL was added and the sample shaken. Optical density was determined at 412 nm within 5 min of adding the DTNB. The protein content of the gastric glandular tissue was determined according to the method of Bradford (1976). The reduced glutathione content was calculated from a standard curve and expressed as μg (mg protein)$^{-1}$.

Determination of gastric malondialdehyde (MDA) content

The level of MDA in the gastric mucosa as an index of membrane lipid oxidative damage was determined according to Ohkawa et al (1979) with slight modifications. Animals were fasted for 24 h and then treated orally with 12% Tween 80, ferruginol (25 or 50 mg kg^{-1}). One hour after the treatment, all rats received 1 mL 99.5% ethanol. A non-ethanol-treated group of animals (sham) was included. One hour after ethanol administration the mucosa was scraped using two glass slides kept cold on ice, weighed, and homogenized in 10 mL KCl (10%). The samples were supplemented with 20% acetic acid, 8.1% sodium lauryl sulfate and 0.8% thiobarbituric acid (TBA), and boiled for 1 h. After cooling, the samples were supplemented with 2.5 mL n-butanol, shaken vigorously during 1 min, and centrifuged for 10 min at 2600 *g*. Absorbance was measured at 532 nm, and the results were expressed as nmol MDA (g gastric tissue)$^{-1}$.

Statistical analysis

The results were expressed as the mean ± s.d. After the Bartlett's test for homogeneity of variance, statistical differences between the treated and control group were determined by one-way analysis of variance followed by Dunnett's test with the level of significance set at $P < 0.05$. All statistical analyses were performed using the software Statistica 5.1 (StatSoft, Inc.).

Results

Acute oral toxicity

At doses up to 3 g kg^{-1}, oral administration of ferruginol did not show any observable symptom of toxicity or mortality in mice. Therefore, the oral LD50 for this diterpene in mice was higher than 3 g kg^{-1} and was not determined.

Ethanol-induced gastric lesions in indometacin-, NEM-, ruthenium red- and L-NAME-pretreated mice

To assess the effect of ferruginol on the gastric lesions induced by oral administration of ethanol in mice pretreated with indometacin, NEM, ruthenium red or L-NAME, a single oral dose of the diterpene at 25 mg kg^{-1} was used. This dose was selected because in a previous report ferruginol was active as a gastroprotective agent at the same dose (Rodríguez et al 2006). In all cases, ferruginol showed a strong gastroprotective activity, reducing the formation of

gastric lesions by 75–79%. This effect was reversed significantly when the animals were pretreated with indometacin but not by NEM, ruthenium red or L-NAME (Table 1). Neither L-NAME nor indometacin increased the lesion index of the controls. These results fully agree with the findings reported by Matsuda et al (2002).

Effect of ferruginol on gastric secretion

To determine the dose to be used in the next rat experiment, we assessed the gastroprotective activity of ferruginol in the model of gastric lesions induced by ethanol. At 25 and 50 mg kg^{-1} the compound significantly reduced the lesions by 49 and 60%, respectively (Figure 1).

At the dose of 25 mg kg^{-1} ferruginol significantly increased the pH and reduced the titrable acidity of the gastric juice similar to cimetidine at 100 mg kg^{-1} (Table 2). In the same experiment, an increase of the gastric secretion volume induced by ferruginol was also observed.

Effect of ferruginol on gastric mucus, PGE_2, GSH and MDA content

While carbenoxolone (200 mg kg^{-1}) increased significantly the gastric mucus content, ferruginol 25 mg kg^{-1} was devoid of activity (data not shown).

At 25 and 50 mg kg^{-1}, ferruginol provoked a significant increase of the gastric PGE_2 content in rats. This effect was partially reversed by pretreatment with indometacin (Table 3).

Oral administration of ethanol in non-pretreated rats (control) elicited a reduction of gastric GSH content compared with sham animals. Pretreatment with ferruginol at 25 or 50 mg kg^{-1} before ethanol administration led to a significant recovery of gastric GSH levels (Figure 2). In the same model, ethanol increased the gastric MDA levels and this effect was significantly inhibited by ferruginol (25 and 50 mg kg^{-1}) (Figure 3).

Table 1 Effect of ferruginol on the appearance of gastric lesions induced by oral administration of ethanol in indometacin-, ruthenium red-, L-NAME- and NEM-pretreated mice

Treatment	Dose (mg kg^{-1})	Lesion index (mm)
Control	–	39.1 ± 5.6
Indometacin	30	38.8 ± 5.6
Ferruginol	25	9.8 ± 4.2*
Indometacin + ferruginol	30 + 25	34.9 ± 7.3
Control	–	43.1 ± 6.4
Ruthenium red	3.5	38.9 ± 6.3
Ferruginol	25	10.2 ± 3.6*
Ruthenium red + ferruginol	3.5 + 25	10.9 ± 4.6*
Control	–	42.3 ± 5.1
L-NAME	70	39.8 ± 5.3
Ferruginol	25	9.5 ± 2.9*
L-NAME + ferruginol	70 + 25	10.9 ± 3.6*
Control	–	44.1 ± 6.5
NEM	10	39.5 ± 7.7
Ferruginol	25	9.4 ± 3.3*
NEM + ferruginol	10 + 25	16.1 ± 4.1*

Results are expressed as means ± s.d. n = 7–8. Analysis of variance followed by Dunnett's test. *P< 0.01 compared with respective control group.

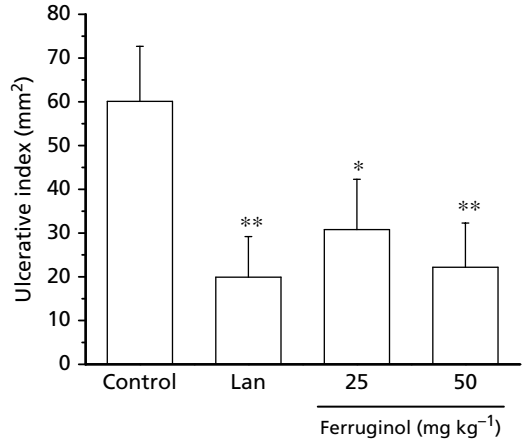

Figure 1 Effects of lansoprazole (Lan) (30 mg kg^{-1}) and ferruginol (25 and 50 mg kg^{-1}) on ethanol-induced gastric lesions in rats. The columns represent the mean ± s.d., n = 6. Analysis of variance followed by Dunnett's multiple comparison test. *P <0.05, **P <0. 01 compared with control group.

Discussion

Terpenes with gastroprotective properties act by several mechanisms, namely: mucus production, increase of the prostaglandin and NO levels, and improvement of the gastric antioxidant capacity i.e. GSH levels (Onoda et al 1990; Kinoshita et al 1995; Wada et al 1997; Ichikawa et al 2000; Rodríguez et al 2005). The triterpenic derivative carbenoxolone stimulates gastric mucus production (Lewis & Hanson 1991); the clerodane diterpene *trans*-dehydrocrotonin as well as the abietane diterpene derivative 12-sulfo-dehydroabietic acid disodium salt (ecabet) and the gastroprotective triterpene oleanolic acid proved to increase the gastric prostaglandin content (Kinoshita & Tamaki 1997; Hiruma-Lima et al 1999). Additionally, the inhibition of pepsin activity in the human gastric juice and an improvement on the eradication of *Helicobacter pylori* from the gastric mucosa, as well as enhancement of mucin metabolism, induced by ecabet has been reported (Ichikawa et al 2000; Adachi et al 2001; Pearson & Roberts 2001). The gastroprotective diterpenes cordatin, aparisthman and *trans*-crotonin decrease the gastric juice acidity (Hiruma-Lima et al 2000, 2001, 2002).

Ferruginol is a very active gastroprotective abietane diterpene occurring in plants. A preliminary assessment of the mechanism underlying the gastroprotective effect of ferruginol was reported by Rodríguez et al (2006). In that work, using only in-vitro testing, the authors concluded that ferruginol protected the gastric mucosa by increasing the prostaglandin content and preventing the lipoperoxidation of the mucosal cell membranes. In-vitro testing is a useful tool in

Table 2 Changes in gastric secretion parameters induced by treatment with ferruginol or cimetidine in pylorus-ligated (Shay model) rats

Treatment	Dose ($mg\ kg^{-1}$)	Gastric secretion ($mL\ (4\,h)^{-1}(100\,g)^{-1}$)	pH	Total titrable acidity ($\mu Eq\ (4\,h)^{-1}(100\,g)^{-1}$)
Control	–	0.8657 ± 0.19	2.068 ± 0.18	58.8 ± 11.2
Cimetidine	100	0.9234 ± 0.26	2.884 ± 0.23*	35.6 ± 8.4*
Ferruginol	25	1.3211 ± 0.17*	2.924 ± 0.19*	30.4 ± 7.9*

Results are expressed as means ± s.d. n = 6. Analysis of variance followed by Dunnett's test. *$P < 0.05$ compared with control group.

Table 3 Changes on gastric PGE_2 content in rats treated orally with ferruginol and/or indometacin

Treatment	Dose ($mg\ kg^{-1}$)	PGE_2 ($\mu g\ (mg\ protein)^{-1}$)
Control	–	0.7872 ± 0.078
Indometacin	30	0.6953 ± 0.083
Ferruginol	25	1.4201 ± 0.211*
Ferruginol + indometacin	25 + 30	0.7208 ± 0.102†
Ferruginol	50	1.8120 ± 0.225*
Ferruginol + indometacin	50 + 30	0.9392 ± 0.174‡
Sham	–	0.7262 ± 0.041

Results are expressed as means ± s.d. n = 6–7. Analysis of variance followed by Dunnett's test. *$P < 0.05$ compared with control group. $^{\dagger}P < 0.05$ compared with ferruginol 25 $mg\ kg^{-1}$. $^{\ddagger}P < 0.01$ compared with ferruginol 50 $mg\ kg^{-1}$.

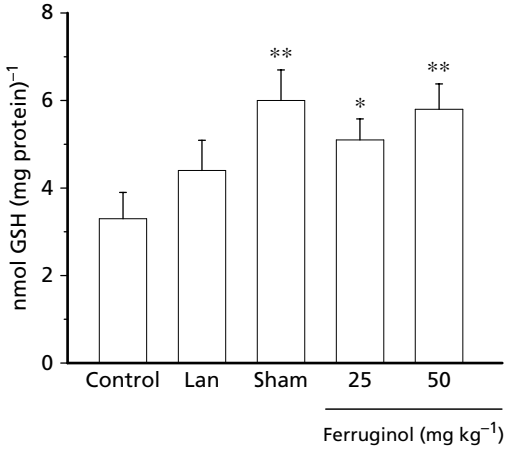

Figure 2 Effects of lansoprazole (Lan) (30 $mg\ kg^{-1}$) and ferruginol (25 and 50 $mg\ kg^{-1}$) on gastric GSH content in rats submitted to oral administration of ethanol. The columns represent the mean ± s.d., n = 6–7. Analysis of variance followed by Dunnett's multiple comparison test. *$P < 0.05$, **$P < 0.01$ compared with control group.

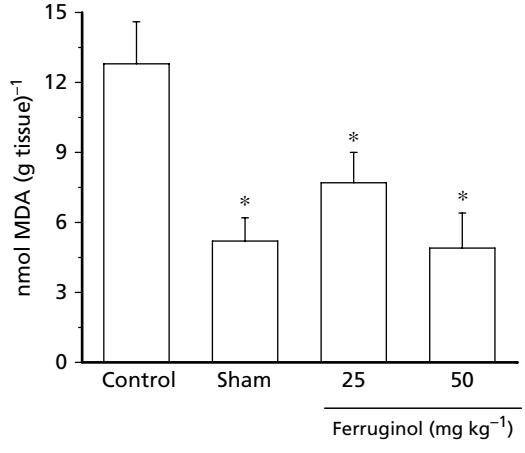

Figure 3 Effect of ferruginol on the malondialdehyde (MDA) gastric content in rats submitted to oral administration of ethanol. The columns represent the mean ± s.d., n = 7. Analysis of variance followed by Dunnett's multiple comparison test. *$P < 0.05$ compared with control group.

pharmacological research; however, such tests provide only a partial and incomplete picture of the complex interactions occurring in the organs and the whole organism (Houghton et al 2007). For this reason, we decided to assess the gastroprotective mechanism of ferruginol using different in-vivo assays.

It is well known that prostaglandins, NO, capsaicin receptor stimulation and GSH protect the gastric mucosa submitted to an ulcerative challenge (Kinoshita et al 1995; Blandizzi et al 2005; Petersson et al 2007). The protective effect of ferruginol against gastric lesions induced by ethanol in mice was reversed when the animals were pretreated with indometacin

but not by L-NAME, ruthenium red or NEM. These results suggested the involvement of gastric prostaglandins, but not of NO, capsaicin receptor or GSH, in the gastroprotection displayed by ferruginol. These assumptions were supported by the direct determination of PGE_2 content in the gastric tissue. It has been reported that some terpenes, like carbenoxolone, enhance the gastric prostaglandin content due to their inhibitory action on the catabolic enzymes 15-hydroxy-PG-dehydrogenase and Δ^{13}-PG-reductase (Lewis & Hanson 1991). The fact that indometacin reversed the effect of ferruginol, as well as the increase on PGE_2 content induced by ferruginol, was correlated with the decrease in lesion index, giving further support to the opinion that the gastroprotective activity of the diterpene was mainly related to an increase in prostaglandin synthesis.

Some gastroprotective diterpenes act by increasing the gastric wall mucus content, while others may increase the gastric prostaglandins without changes on the gastric mucus (Hiruma-Lima et al 1999). We observed that ferruginol did not modify the mucus content. However, the intraduodenal administration of ferruginol had a significant effect in increasing the gastric juice volume, reducing the total acid output and raising the gastric juice pH value. Rodríguez et al (2006) reported that ferruginol did not seem to modify the gastric acid output. This discrepancy with our findings could be explained because in that study ferruginol was administered orally and the gastric secretion parameters were evaluated within the next 2 h. Other gastroprotective diterpenes have proved to modify the gastric juice characteristics when administered by the intraduodenal route (Hiruma-Lima et al 2000, 2001, 2002). It is well known that antisecretory drugs and prostaglandins markedly accelerate the gastric ulcer healing in rats (Konturek et al 2001; Ishihara & Ito 2002). The fact that ferruginol exhibited both antisecretory properties and a stimulating effect on the prostaglandin synthesis may explain the gastric ulcer healing activity of this diterpene (Rodríguez et al 2006).

It has been reported that oral administration of ethanol or HCl/ethanol to induce gastric ulcers in rats provokes an increase in thiobarbituric acid-reactive substances (TBARS), accompanied by a reduction in the gastric content of GSH (Arafa & Sayed-Ahmed 2003; Natale et al 2004). Rodríguez et al (2006) reported that ferruginol strongly inhibited the lipoperoxidation induced by *t*-butylhydroperoxide in human red blood cell membranes. Peroxidation of the lipids in the cell membranes may originate differently to TBARS, increasing the damage of the gastric mucosa. Our results showed that the gastroprotective effect of ferruginol in rats receiving ethanol was not reversed by pretreatment with NEM. On the other hand, the terpene prevented the reduction of the gastric GSH levels induced by ethanol in rats, and the MDA generated after oral ethanol administration was lowered by the compound. These findings suggested that in addition to the effect observed on gastric prostaglandins, the gastroprotective effect of ferruginol could be mediated by a direct inhibitory action on the membrane lipid peroxidation rather than changes on gastric GSH content.

Only a few reports on the oral toxicity of gastroprotective compounds in animals have been published. Probably the most studied of these compounds has been the natural diterpene *trans*-dehydrocrotonin. In a study of acute oral toxicity in mice this compound showed an LD50 value of 876 mg kg^{-1} (Souza-Brito et al 1998), while in a sub-chronic study hepatotoxic effects were reported (Rodríguez et al 2004). Ferruginol proved to be much more active as a gastroprotective agent and several times less toxic than *trans*-dehydrocrotonin.

Conclusions

The mechanism underlying the gastroprotective activity of ferruginol was assessed in mice and rats. We observed that the compound maintained the GSH levels and inhibited the MDA generated in the gastric mucosa challenged by ethanol, and modified the gastric juice by increasing its volume and reducing its total acid content. In addition, an increase in the gastric prostaglandin content was elicited by ferruginol, supporting previous findings using a cell culture model. The strong gastroprotective activity of ferruginol and its low toxicity should encourage the pharmacological study of this compound and its derivatives as potential new gastroprotective drugs.

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