Gastroprotective Effect and Cytotoxicity of Semisynthetic Jatropholone Derivatives

Abstract

The gastroprotective effect of the diterpenes jatropholone A, jatropholone B and 16 semisynthetic derivatives was assessed in the HCl/ethanol-induced gastric lesion model in mice and the cytotoxicity was determined towards fibroblasts and AGS cells. In a dose-response study, jatropholone B reduced gastric lesions by 65% at 6 mg/kg and jatropholone A by 54% at 100 mg/kg. The jatropholone B derivatives 9–14 and the compounds 15–18 were compared at a single oral dose of 25 mg/kg while the jatropholone A derivatives 2–7 were assessed at 100 mg/kg. A decrease in gastroprotective activity was observed for the ether as well as for the ester derivatives of jatropholone B. The methyl and propyl ethers of jatropholone A were more gastroprotective than the natural product. The placement of an additional methyl group at C-2 in the jatropholone B derivatives led to a loss of selectivity, the methyl and propyl ethers lack a gastroprotective effect. Jatropholone B was not toxic towards AGS cells and fibroblasts. Jatropholone A was active only against AGS cells. The gastroprotective effect of the epimeric jatropholones was selective showing a higher effect for jatropholone B. These results further support that the stereochemistry of the methyl group at C-2 in the jatropholones plays a relevant role in preventing the gastric lesions in mice. The compounds 3, 5–7, 10 and 12–18 are described for the first time.

Key words
Jatropha isabelli · Euphorbiaceae · jatropholone · semisynthetic derivatives · gastroprotective activity · cytotoxicity

Abbreviations
AGS cells: human gastric adenocarcinoma cells
DMF: dimethylformamide
DMSO: dimethyl sulfoxide
FBS: fetal bovine serum
MEM: minimum essential Eagle's medium
MRC-5 cells: human lung fibroblasts
NRU: neutral red uptake

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

Introduction

Plants provide a rich source of bioactive molecules. A number of natural products or their derivatives displaying gastroprotective effect have been reported. Among these compounds we found different flavonoids [1], diterpenes [2], triterpenes [3], sesquiterpenes [4], tannins [5], coumarins [6], saponins [7], alkaloids [8] and xanthones [9].
Some diterpenes isolated from plants traditionally used to treat gastric lesions have been reported as powerful gastroprotective agents. Of particular interest are the clerodanes from the Brazilian *Croton cajinacra* (Euphorbiaceae) including trans-dehydrocrotonin (DHC) [2], [10], [11] trans-crotonin [12], crotonin [13] and DHC derivatives [14]. In addition, the gastroprotective activity of the diterpenes solidagone from *Solidago chliensis* (Asteraceae), ferrarigul from *Prumnopitys andina* (Podocarpaceae) and diterpenes from the *Araucaria araucana* (Araucariaceae) resin has been reported [15], [16], [17], [18], [19], [20]. Many of these compounds seem to stimulate the defensive factors of the gastric mucosa such as prostaglandin content, antioxidant capacity and epithelial restitution [10], [15], [16], [17].

The naturally occurring diterpenes jatrophone and jatropholones A and B isolated from the rhizomes of *Jatropha isabielli* (Euphorbiaceae) were shown to be powerful gastroprotective compounds, reducing gastric lesions as much as the reference drug lansoprazole at the same dose [21]. However, neither information on the gastroprotective effect of jatrophone derivatives nor the influence of some structural features on the gastroprotective activity is known.

Following our studies on the antiulcer activity of natural terpenes and their derivatives now we report the gastroprotective and cytotoxic effect of 16 semisynthetic jatrophone derivatives.

### Materials and Methods

#### Plant material

The jatrophoneolines A (1) and B (8) used for the preparation of semisynthetic derivatives were isolated from the rhizomes of *Jatropha isabielli* as described previously [21]. A voucher specimen has been deposited at the Herbarium of the Universidad de Talca, Chile (Schmeda N° 1594). The percentual w/w yields of the compounds from the combined petroleum ether and ethyl acetate extracts of the rhizomes were 0.02 and 0.02%, respectively.

#### General procedure

Melting points were determined on a Koffler hot stage apparatus (Electrothermal 9100) and were uncorrected. Optical rotations were obtained for solutions in CHCl₃ (concentrations expressed in g/100 mL) on a Jasco DIP 370 polarimeter. IR spectra were recorded on a Nicolet Nexus FT-IR instrument. ¹H-NMR spectra were recorded at 400 MHz and ¹³C-NMR data were obtained at 100 MHz on a Bruker spectrometer (δ scale). Mass spectra are presented as m/z (rel. int. %). Si gel 60 (Merck, 63 - 200 μm particle size; Santiago, Chile) was used for column chromatography, precoated silica gel plates (Merck, Kieselgel 60 F₂₅₄, 0.25 mm) were used for TLC analysis. TLC spots were visualized by spraying the chromatograms with p-anisaldehyde-ethanol-acetic acid-H₂SO₄ (2:170:20:10 v/v) and heating at 110 °C for 3 min. All reactions were carried out under an inert dry nitrogen atmosphere.

#### Preparation of derivatives

The methyl and propyl ethers of jatropholones A and B were prepared by treating a mixture of jatropholones A and B in DMF with a stoichiometric amount of NaH and then adding methyl iodide to form the methyl ethers 2 and 9 or propyl iodide to obtain the propyl ethers of jatropholone A (3) and jatropholone B (10). The resolution of the mixtures was achieved by column chromatography followed by preparative TLC.

A mixture of the propyl ethers 3 and 10 in DMF was treated with NaH in excess and then methyl iodide was added, yielding the compound 16 (67% w/w yield). To a mixture of 2 and 9 in DMF, NaH in excess and then propyl iodide were added to afford compound 17 (53% w/w yield). Compounds 15 and 18 were prepared by treating the mixture of jatropholones A and B in DMF with NaH in excess and either methyl iodide to form compound 15 (61%) or propyl iodide to generate compound 18 (56%). Acetylation of 1 and 8 yielded compounds 4 (82%) and 11 (96%), respectively. Reaction of the mixture of jatropholones A and B with 3-chloropropionyl chloride, 4-nitrobenzoyl chloride and 4-chlorobenzoyl chloride in DMF afforded the jatropholone A derivatives 5, 6 and 7 and the jatropholone B derivatives 12, 13 and 14. The resolution of the mixtures was achieved by column chromatography followed by preparative TLC.

#### Animals

Animals were purchased from the Instituto de Salud Pública de Chile (Santiago, Chile). Male Swiss albino mice weighing 30 ± 3 g were fed on certified Champion diet (Champion S.A.; Santiago, Chile) with free access to water under standard conditions of a 12 h dark-light period, 50% relative humidity and 22 °C room temperature.

#### HCl/ethanol-induced gastric lesions

Mice were fasted for 24 h prior to the experiment. Compounds were suspended in a 12% solution of the non-ionic detergent Tween 80. Jatrophone B was administered orally at doses of 25, 50 and 100 mg/kg, while jatrophone B was given orally at: 6, 12, 25, 50 and 100 mg/kg. These dose-response experiments led to the selection of a single dose, namely 100 mg/kg for the jatrophone B derivatives (compounds 2–7) and 25 mg/kg for the jatrophone B derivatives (compounds 9–14). The products 15–18 were tested at 25 mg/kg. Fifty min after administration of the compounds, the antisecretory drug lansoprazole [2-[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyril][methyl][sulfanyl] benzimidazol; Genexpress; Santiago, Chile] (20 mg/kg) or the vehicle 12% Tween 80 (10 mL/kg) for all animals was given orally. Animals were sacrificed 1 h after the administration of HCl/ethanol. The ulcerated stomachs were excided, inflated by injection of saline (1 mL) 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 lesion index was expressed as the sum of the length of all lesions [18]. The protocols were approved by the Universidad de Talca Institutional Animal Care and Use Committee that follows the recommendations of the Canadian Council on Animal Care [22].

#### MRC-5 cell culture

Human lung fibroblasts MRC-5 (ATCC CCL-171; ATCC, Manassas, VA, USA) were grown as monolayers in minimum essential Ea-
gle's medium (MEM; Bios Chile; Santiago, Chile), with Earle's salts, 2 mM L-glutamine and 2.2 g/L sodium bicarbonate, supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 IU/mL penicillin and 100 μg/mL streptomycin in a humidified incubator with 5% CO₂ in air at 37 °C. Cell passage was maintained between 10 and 16. Medium was changed every 2 days.

**AGS cell culture**

Human epithelial gastric cells AGS (ATCC CRL-1739) were grown as monolayers in Ham F-12 medium (Bios Chile) containing 1 mM L-glutamine and 1.5 g/L sodium bicarbonate, supplemented with 10% heat-inactivated FBS, 100 IU/mL penicillin and 100 μg/mL streptomycin in a humidified incubator with 5% CO₂ in air at 37 °C. Cell passage was maintained between 42 and 48. Medium was changed every 2 days.

**Cytotoxicity assay**

Confluent cultures of MRC-5 as well as AGS cells were treated with medium containing the diterpenes as well as the reference compound Lansoprazole at concentrations ranging from 0 up to 1000 μM. The products were first dissolved in DMSO and then in the corresponding culture medium supplemented with 2% FBS. The final content of DMSO in the test medium and controls was 1%. Cells were exposed for 24 h to the test medium with or without the compound (control). Each concentration was tested in quadruplicate together with the control and repeated three times in separate experiments. At the end of the incubation, the neutral red uptake (NUR) assay was carried out [23]. To calculate the IC₅₀ values (concentration that produces a 50% inhibitory effect on the evaluated parameter) the results were transformed to percentage of controls and the IC₅₀ values were graphically obtained from the dose-response curves.

**Statistical analysis**

Results were expressed as the mean ± S.D. In all experiments, statistical differences between several treatments and their respective control were determined by one-way analysis of variance (ANOVA) and when the F value was significant, post hoc differences were determined by the Dunnett's multiple comparison test. The level of significance was set at P < 0.05. All statistical analyses were performed using the software Statistica 5.1 (StatSoft, Inc.).

**Compounds**

*Jatrophone A acetate (4):* colorless crystals, m.p. 102–104 °C; [α]₀°C = +58.8 (c 0.17, CHCl₃); HR-MS: m/z = 338.1871 (calcld. for C₂₉H₂₉O₂: 338.1882).

*Jatrophone A 2-propenyl ester (5):* yellow oil; [α]₀°C = +36.4 (c 0.11, CHCl₃); IR (film): νmax = 2926, 1745, 1712, 1236, 888 cm⁻¹; HR-MS: m/z = 350.1893 (calcld. for C₂₉H₂₉O₃: 350.1882); MS: m/z (rel. int.%): 350 (100), 335 (15), 321 (14), 312 (9), 307 (30), 297 (13), 296 (37), 295 (31), 294 (30), 293 (17), 281 (29), 269 (10), 268 (25), 267 (22), 253 (31), 240 (27), 239 (23), 227 (16), 225 (14), 211 (15), 165 (15), 163 (9), 91 (9), 69 (50).

*Jatrophone A p-nitrophenyl ester (6):* yellow oil; [α]₀°C = +13.3 (c 0.15, CHCl₃); IR (film): νmax = 2926, 1742, 1712, 1530, 1255, 846 cm⁻¹; HR-MS: m/z = 445.1881 (calcld. for C₃₀H₂₉NO₃: 445.1889); MS: m/z (rel. int.%): 445 (71), 430 (23), 416 (11), 402 (39), 390 (30), 389 (39), 388 (14), 295 (20), 293 (8), 151 (8), 150 (100), 129 (29), 104 (24), 69 (4).

*Jatrophone A p-chlorophenyl ester (7):* yellow oil; [α]₀°C = +23.1 (c 0.13, CHCl₃); IR (film): νmax = 2925, 1740, 1712, 1254, 886, 754 cm⁻¹; HR-MS: m/z = 434.1658 (calcld. for C₃₀H₂₉ClO₃: 434.1649); MS: m/z (rel. int.%): 436 (13), 434 (32) 378 (11), 295 (6), 141 (28), 139 (100), 111 (16).

*Jatrophone B (8):* colorless crystals, m.p. 229–231 C; [α]₀°C = +76.2 (c 0.21, CHCl₃); HR-MS: m/z = 296.1727 (calcld. for C₂₀H₂₉O₂: 296.1776).

The spectroscopic data of compounds 1 and 8 are in agreement with literature values [24].

*Jatrophone B methyl ether (9):* colorless crystals, m.p. 93–95 C; [α]₀°C = +69.2 (c 0.13, CHCl₃); HR-MS: m/z = 310.1917 (calcld. for C₂₁H₃₀O₂: 310.1933).

*Jatrophone B propyl ether (10):* yellow oil; [α]₀°C = +35 (c 0.2, CHCl₃); IR (film): νmax = 2926, 1712, 1277, 1099, 888 cm⁻¹; HR-MS: m/z = 338.2249 (calcld. for C₂₂H₂₉O₃: 338.2246); MS: m/z (rel. int.%): 338 (100) 337 (7), 323 (18), 310 (9), 309 (12), 296 (13), 295 (48), 283 (7), 282 (31), 281 (28), 269 (8), 267 (7), 253 (14), 239 (11), 211 (7), 165 (7).

*Jatrophone B acetate (11):* colorless crystals, m.p. 131–133 C; [α]₀°C = +61.5 (c 0.13, CHCl₃); HR-MS: m/z = 338.1893 (100) (calcld. for C₂₂H₃₀O₃: 338.1882).

*Jatrophone B 2-propenyl ester (12):* yellow oil; [α]₀°C = +16.7 (c 0.12, CHCl₃); IR (film): νmax = 2925, 1744, 1712, 1236, 888 cm⁻¹; HR-MS: m/z = 350.1893 (calcld. for C₂₉H₂₉O₃: 350.1882); MS: m/z (rel. int.%): 350 (100) 335 (16), 321 (13), 312 (11), 307 (28), 297 (16), 296 (53), 295 (38), 294 (36), 293 (20), 281 (32), 269 (10), 268 (20), 267 (14), 253 (26), 240 (26), 239 (23), 227 (23), 225 (14), 211 (15), 165 (16), 163 (37), 91 (12), 69 (19).

*Jatrophone B p-nitrophenyl ester (13):* yellow oil; [α]₀°C = +20 (c 0.11, CHCl₃); IR (film): νmax = 2924, 1743, 1712, 1529, 1255, 846 cm⁻¹; HR-MS: m/z = 445.1894 (calcld. for C₃₀H₂₉NO₄: 445.1889); MS: m/z (rel. int.%): 445 (71) 430 (16), 416 (11), 402 (27), 390
Jatrophenolone B p-chlorophenyl ester (14): yellow oil; [α]_D^20 = +50 (c 0.1, CHCl₃); IR (film): ν_max = 2924, 1740, 1712, 1254, 886, 754 cm⁻¹; HR-MS: m/z = 434.1664 (calcd. for C_{22}H_{27}ClO₂; 434.1649); MS: m/z (rel. int.%) = 436 (9), 434 (24), 378 (10), 295 (5), 141 (31), 139 (100), 111 (12).

2-Methyljatrophenolone methyl ether (15): colorless crystals, m.p. 164–166°C; [α]_D^20 = +47.4 (c 0.19, CHCl₃); IR (KBr): ν_max = 2925, 1710, 1277, 1099, 886 cm⁻¹; HR-MS: m/z = 324.2086 (calcd. for C_{22}H_{28}O₂; 324.2089); MS: m/z (rel. int.%) = 324 (100), 309 (40), 296 (11), 282 (13), 281 (50), 269 (12), 268 (35), 267 (30), 255 (13), 253 (18), 225 (11), 165 (10), 155 (10), 69 (18).

2-Methyljatrophenolone propyl ether (16): yellow oil; [α]_D^20 = +28.6 (c 0.14, CHCl₃); IR (film): ν_max = 2926, 1711, 1278, 1105, 888 cm⁻¹; HR-MS: m/z = 352.2398 (calcd. for C_{23}H_{30}O₂; 352.2402); MS: m/z (rel. int.%) = 352 (100), 337 (23), 323 (12), 310 (30), 309 (47), 297 (10), 296 (29), 295 (28), 267 (16), 254 (11), 253 (20), 239 (13), 69 (14).

2-Propyljatrophenolone methyl ether (17): yellow oil; [α]_D^20 = +61.5 (c 0.13, CHCl₃); IR (film): ν_max = 2925, 1712, 1276, 1104, 887 cm⁻¹; HR-MS: m/z = 352.2399 (calcd. for C_{23}H_{30}O₂; 352.2402); MS: m/z (rel. int.%) = 352 (100), 337 (16), 323 (12), 310 (30), 309 (47), 297 (8), 296 (9), 295 (28), 267 (9).

2-Propyljatrophenolone propyl ether (18): yellow oil; [α]_D^20 = +66.7 (c 0.3, CHCl₃); IR (film): ν_max = 2926, 1712, 1277, 1105, 887 cm⁻¹; HR-MS: m/z = 380.2711 (calcd. for C_{25}H_{32}O₂; 380.2715); MS: m/z (rel. int.%) = 380 (100), 365 (15), 338 (25), 337 (49), 324 (28), 323 (20), 295 (10), 281 (13), 253 (8), 211 (8), 69 (13).

Supporting information

1H-NMR data and 13C-NMR data of compounds 1–18 are available as Supporting Information.

Results

The chemical structures of the compounds 1–18 are presented in Fig. 1. The 13C-NMR data of compounds 1–18 are summarized in Tables 15–35 (Supporting Information). The 13C-NMR data of compounds 1–18 are presented in Tables 45–65 (Supporting Information). All compounds prepared in this work exhibit spectroscopic data in agreement with the proposed structures. The compounds 3, 5–7, 10 and 12–18 were not found in the literature and are described for the first time.

As shown in the Table 1, compound 1 was less active than compound 8 in preventing the appearance of gastric ulcers. At the dose of 25 mg/kg compound 8 reduced the lesions by 83% while compound 1 inhibited them only by 36%. At 100 mg/kg all the derivatives of compound 1 were active. Compounds 4–6 showed a similar activity to that of their parent compound 1 while derivatives 2, 3 and 7 were the most active. Considering the derivatives of compound 8, at 25 mg/kg, compounds 10, 17 and 18 showed the best gastroprotective effect while compounds 14 and 15 were the less active and compound 16 was devoid of activity.

Regarding the cytotoxicity of the compounds, both jatropholones A (1) and B (8) showed IC_{50} values up to 1000 μM towards normal fibroblasts, however, a selective cytotoxic effect was observed for the jatropholone A against AGS cells (Table 1). Some derivatives of compounds 1 and 8 proved to be more cytotoxic than the parent compounds while other derivatives maintained their reduced cytotoxicity with IC_{50} values up to 1000 μM (Table 1).

![Fig. 1 Structures of jatropholone A (1), jatropholone B (8) and their semisynthetic derivatives.](image-url)
Table 1  Cytotoxicity and dose-response gastroprotective effect of jatropholone A and B (1 and 8) and gastroprotective activity of the derivatives 2 – 7 and 9 – 18 on the HCl/ethanol induced gastric lesions in mice

<table>
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<th>Compound (mg/kg)</th>
<th>Lesion index (mm)</th>
<th>% Reduction</th>
<th>Cytotoxicity (μM)</th>
<th>Compound (mg/kg)</th>
<th>Lesion index (mm)</th>
<th>% Reduction</th>
<th>Cytotoxicity (μM)</th>
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<tr>
<td>Control</td>
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<td>Lansoprazole</td>
<td>9.4 ± 1.2**</td>
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<td>306 / 162</td>
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<td>6</td>
<td>Compounds 8</td>
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<td>(100 mg/kg)</td>
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Results are expressed as means ± S.E.M. n = 8. Asterisks indicates significant difference from corresponding control (ANOVA followed by Dunnett’s test).

\*P < 0.05; \**P < 0.01 compared to the controls. Cytotoxicity data are presented as IC50 values.

Discussion

The Euphorbiaceae family has been shown to be a rich source of gastroprotective diterpenes. Most of the active compounds identified from these plants were isolated from crude drugs traditionally used to treat gastric lesions. Extensive studies on the gastroprotective activity of the clerodanes from Croton cajucara have been carried out by Brazilian research groups who reported the effects of trans-dehydrocrotinin (DHC) [2], [10], [11] trans-crotinin [12], crotinin [13] and DHC derivatives [14].

Some 16 jatropholone derivatives were prepared starting from jatropholone A and jatropholone B. The effect of an ether function at C-14 was investigated comparing the compound 1 (jatropholone A) with the methyl and propyl ethers 2 and 3 as well with the compound 8 (jatropholone B) and the methyl and propyl ethers 9 and 10, respectively. Regarding the jatropholone A derivatives, a strong increase in the toxicity towards fibroblasts was observed for the derivatives 2 and 3, while the effect on AGS cells was less remarkable and increased with a longer ether chain. In the jatropholone B derivatives 9 and 10, a strong increase in cytotoxicity was observed against both cell lines (56.6 and 48.3 μM for fibroblasts and 63.8 and 88.3 μM for AGS cells, respectively). In contrast, the parent compound presented a low toxicity with an IC50 value >1000 μM. Acetylation of the hydroxy function at C-14 also had effects on cytotoxicity. This effect on AGS cells was clear for jatropholone B (IC50 > 1000 μM) compared with the acetate 11 (148 μM).

Three esters were prepared from jatropholones A and B, including a vinyl and two p-substituted aromatic derivatives. While the compounds 5 and 12 presented marked cytotoxicity against both AGS cells and fibroblasts, the aromatic esters of jatropholones A and B, 6, 7 and 13, 14, respectively, were non-toxic to both cell lines with IC50 values >1000 μM.

Since there was a clear difference in cytotoxicity of jatropholones A and B, towards AGS cells, differing only in the stereochemistry of the C-16 methyl group, the effect of an additional alkyl group at C-2 was investigated. The cytotoxicity of C-2 methyl and propyl derivatives with either a methyl or propyl ether at C-14 were compared. The compounds 15 and 16, with a methyl group at C-2 differ in the ether chain length at the 14-OH function. While the derivative 15 was devoid of toxicity on both cell lines, the propyl ether proved to be cytotoxic with IC50 values of 55.1 and 74.8 μM on fibroblasts and AGS cells, respectively. A longer alkyl chain at C-2 (propyl) increases cytotoxicity on fibroblasts as can be seen comparing the IC50 values of the products 17 and 18 with the parent compounds 1 and 8.

Regarding the jatropholone B derivatives at 25 mg/kg, a decrease in gastroprotective activity was observed both for the ether as well as for the ester derivatives 9, 10 and 11 – 14, respectively, when compared with the parent compound presenting a free OH group at C-14. For the jatropholone A derivatives at 100 mg/kg, the methyl and propyl ethers presented better gastroprotective effects than the natural product (81 and 75% reduction of lesions compared with 54% of jatropholone A). Esterification of the phenolic OH reduced the gastroprotective effect except for the derivative 7.
The placement of an additional methyl group at C-2 led to a loss of stereoselectivity at this place in the molecule, and the methyl and propyl ether derivatives lack gastroprotective effects at 25 mg/kg. In the presence of an additional C3 alkyl chain at C-2 with either a methoxy or propoxy function at C-14 the activities did not differ from each other (see compounds 17 and 18, Table 1).

The diterpene jatrophonol B, proved to be more active as a gastroprotective agent than the Croton diterpenes, reducing gastric lesions by 65% at the lowest assayed dose (6 mg/kg) and with a better effect than Lansoprazole at 20 mg/kg. Other diterpenes from the Euphorbiaceae with effects on gastric lesions included aparisthman and cordinatin from Aparisthmium cordatum [25, 26] and jatrophone and derivatives from Jatropha isabellii [21].

The present study shows the selective gastroprotective effect of the epimeric jatrophonolines, with a much higher effect for jatrophonol B. The stereochemistry of the methyl group at C-2 plays a relevant role in preventing the gastric lesions in mice. This fact suggests the participation of a stereospecific receptor mechanism in the gastroprotective effect of these compounds. Further studies should be undertaken to disclose the action mechanism of natural and semisynthetic jatrophonol derivatives.

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