Mary Ann Foglio[1]
Patrícia Corrêa Dias[1,2]
Marcia Aparecida Antônio[1,2]
Ana Possenti[1]
Rodney Alexandre Ferreira Rodrigues[1]
Érica Ferreira da Silva[1]
Vera Lúcia Garcia Rehder[1]
João Ernesto de Carvalho[1,2]

Antiulcerogenic Activity of Some Sesquiterpene Lactones Isolated from *Artemisia annua*

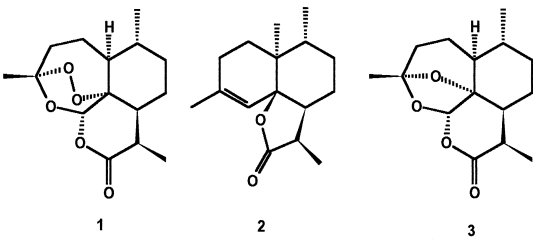
Abstract

Artemisinin **1**, dihydro-epideoxyarteannuin B **2** and deoxyartemisinin **3** were isolated from the sequiterpene lactone-enriched fraction obtained from the crude ethanolic extract of *Artemisia annua* L. These compounds were tested on ethanol and indomethacin-induced ulcer models. Compound **1** did not afford cytoprotection under the experimental models tested. Only compounds **2** and **3** decreased the ulcerative lesion index produced by ethanol and indomethacin in rats. These compounds did not demonstrate antiulcerogenic activity when tested on the ethanol-induced ulcer model, with previous administration of indomethacin, suggesting that the antiulcerogenic activity is a consequence of prostaglandin synthesis increase.

Key words
Artemisia annua · Asteraceae · antiulcer activity · sesquiterpenes

Introduction

Artemisia annua L. (Asteraceae) has received much attention due to the antimalarial activity of artemisinin **1** (Fig. **1**), a sesquiterpene lactone with an endoperoxide group [1]. Sesquiterpene lactones are common in most tribes of Asteraceae, with more than 4000 known structures. A diversity of bioactivities has been reported for this class of compounds, such as anti-inflammatory, antitumor, antiulcerogenic, cytotoxic, diuretic and cardiotonic, among others [2]. The genus *Artemisia* is widely known for its sesquiterpene lactone contents which have been mentioned as cytoprotective agents against the development of peptic ulcers [3]. The antiulcerogenic activity of many sesquiterpene lactones has been attributed to their α-methylene-γ-lactone moiety (3). The guaianolide dehydroleucodine isolated from *Artemisia douglasiana* Besser (Asteraceae) demonstrated cytoprotective activity by two different pathways, involvement of SH-compounds as well as the mucus synthesis [4].



1 2 3

Fig. **1** Artemisinin **1**, dihydro-epideoxyarteannuin B **2** and deoxyartemisinin **3**.

The present study was undertaken to evaluate the antiulcerogenic activity and to determine the probable pharmacological mechanism of three sesquiterpene lactones isolated from *Artemisia annua* L.

Affiliation
[1] Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas da Universidade Estadual de Campinas (CPQBA/UNICAMP), Campinas, SP, Brazil
[2] Departamento de Clínica Médica da Faculdade de Ciências Médicas da Universidade Estadual de Campinas (FCM/UNICAMP), Campinas, SP, Brazil

Correspondence
Dr. Mary Ann Foglio · Caixa Postal 6171 · CEP 13081 970 Campinas · São Paulo · Brazil ·
Phone: +(005519) 3884-7500 · Fax: +(005519) 3884-7811 · E-Mail: foglioma@cpqba.unicamp.br

Received August 24, 2001 · **Accepted** March 23, 2002

Bibliography
Planta Med 2002; 68: 515–518 · © Georg Thieme Verlag Stuttgart · New York · ISSN 0032-0943

Materials and Methods

Plant material and fractionation

Artemisia annua L. leaves (hybrid CPQBA 2/39 x PL5) were collected from the experimental field of CPQBA/UNICAMP. Voucher specimen is deposited at CPQBA/UNICAMP under registration number 229. This material was allowed to dry under air circulation (40 °C) and ground for use. The resulting powder (500 g) was submitted to dynamic maceration with ethanol during 4 hours (this procedure was repeated three times). Concentration of the extracts under reduced pressure gave 108 g of crude ethanolic extract (22 % yield).

A 10 % lead acetate solution (1 L) was added to the crude ethanolic extract (108 g) dissolved in ethanol (100 mL). This mixture was allowed to stand at room temperature for 30 minutes and filtered. The filtrate was extracted with chloroform (3 × 350 mL), dried over $MgSO_4$, filtered and dried under vacuum affording the sesquiterpene lactone enriched fraction (10 g – 9.3 % yield) [5].

The sesquiterpene enriched fraction (10 g) was purified on successive column chromatography using silica gel (Merck 7734) (5 × 60 cm) with hexane/ethyl acetate (99 : 1), R_f **2**: 0.65 between 500 – 950 mL, $[\alpha_D^{20}]$: + 49.96° (*c* 0.025 g/mL, $CHCl_3$); hexane/ethyl acetate (99 : 2), R_f **3**: 0.61 between 1000 – 1700 mL, [hexane/ethyl acetate (99 : 3), $[\alpha_D^{20}]$: –149.8° *c* 0.045 g/mL, $CHCl_3$); R_f **1**: 0.57 between 1750 – 2550 mL, $[\alpha_D^{20}]$: + 80.93° (*c* 0.025 g/mL, $CHCl_3$).

Fractions were monitored by thin layer chromatography, eluent hexane/dicloromethane/methanol (20 : 79 : 1), detection anisaldehyde reagent. The physical and spectral data (mass, ^{1}H-NMR, ^{13}C-NMR) of compounds **1 – 3** were consistent with those of artemisinin [6], deoxyartemisinin [6] and dihydro-epideoxyarteannuin B [7].

Chromatographic analysis

The GC/MS analysis were carried out using a HP-5890/5970 system equipped with a J&W Scientific DB-5 fused capillary column (25 m × 0.2 mm × 0.33 m). Temperature program was 40 °C (5 °C/min) – 300 °C (10 min). Injector 250 °C, detector 300 °C. Helium was used as carrier gas (0.7 bar, 1 mL/min). The MS were taken at 70 eV. Scanning speed was 0.84 scans/s from 40 to 550. Sample volume was 1 μL. Split: 1 : 40.

Animals

Male Wistar rats (200 – 300 g), purchased from Animal Experimental Care Center of Campinas State University, were employed in all pharmacological experiments. These animals were maintained under standard conditions of light cycle (12 hours light, 12 dark) and temperature (23 ± 2 °C), for at least 7 days before the experiments. Animal's welfare guidelines were adopted during maintenance period and experimentation [8], [9].

Indomethacin-induced ulcer

Male Wistar rats (200 – 250 g), fasted for 24 hours, with free access to water, were divided in at least three groups, containing 4 – 7 animals, according to the respective treatment employed.

After 30 minutes of oral treatment, indomethacin (30 mg/kg) was administrated subcutaneously to all groups. After four hours, the animals were sacrificed, their stomachs were removed, and opened along the greater curvature [10]. The ulcerative lesion index of each animal was calculated by adding the following values [11]:

Loss of normal morphology	1 point
Discoloration of mucosa	1 point
Mucosal edema	1 point
Hemorrhages	1 point
Petechial points (until 9)	2 points
Petechial points(more than 10)	3 points
Ulcers up to 1 mm	number of ulcers × 2 points
Ulcers greater than 1 mm	number of ulcers × 3 points
Perforated ulcers	number of ulcers × 4 points

Ethanol-induced ulcer

Male Wistar rats (200 – 250 g), fasted for 24 hours, with free access to water, were divided in groups of 5 – 6 animals, according to the respective treatment employed.

After 30 minutes of oral treatment with saline, carbenoxolone, and compounds **1 – 3**, each animal received, 1 mL of absolute ethanol orally. After one hour, the animals were sacrificed and their stomachs were removed and opened along the greater curvature [12]. Ulcers were evaluated as above [11].

Determination of the role of prostaglandins in cytoprotection

The animals were divided in groups of 3 – 5 animals, according to the respective treatment. The control group received an intraperitoneal injection of saline solution, and the others an injection of indomethacin (5 mg/kg). After thirty minutes, all animal groups received the respective treatment orally. After one hour, the animals were orally administrated with 1 mL of absolute ethanol. These animals were sacrificed one hour later and their stomachs were removed, and opened along the greater curvature [13]. Ulcers were evaluated as above [11].

Statistical analysis

The results were expressed as mean ± SEM and the individual data were submitted to one way variance analysis with critical range at $p < 0.05$ and afterwards to Duncan"s test with the same critical range.

Results and Discussion

Previously we demonstrated that the resulting enriched sesquiterpene fraction from *Artemisia annua* L. crude ethanolic extract inhibited the ulcerative lesion index in all experimental models tested, in rats. The results mentioned therein suggested that the antiulcerogenic properties were afforded by cytoprotective mechanisms as result of active principles that increase the gastric mucosa prostaglandin level [14]. Prostaglandins released in the stomach protect the gastric mucosa by acid secretion decrease, mucosa blood flow, bicarbonate, and mucus production increase [15].

In previous work we determined that the enriched sesquiterpene fraction had ulcer inhibition maximized at dose levels between 50 and 125 mg/kg [14]. Therefore, a dose of 100 mg/kg for compounds **1 – 3** would ensure an effect if the antiulcer activity re-

Table **1** Effect of oral administration of cimetidine, compound **1** and compound **2** on indomethacin induced ulcer model and effect of oral administration of carbenoxolone and compound **1**, on ethanol induced ulcer model

Treatment	*Dose (mg/kg)*	*n*	*Ulcer Model*	*ULI inhibition (%)*	*ULI (mean ± SEM) Negative Control – Test Group*
Cimetidine [A]	100	5	Indomethacin	92.7	52.4 ± 5.3 – 3.8 ± 0.5 [b]
Compound **1**[A]	100	7	Indomethacin	21.0	52.4 ± 5.3 – 41.4 ± 8.4
Carbenoxolone [B]	100	6	Ethanol	66.8	38.3 ± 6.2 – 12.7 ± 3.5 [a]
Compound **1**[B]	100	6	Ethanol	—	38.3 ± 6.2 – 50.2 ± 4.1
Cimetidine [C]	100	4	Indomethacin	92.2	38.3 ± 6.2 – 3.0 ± 0.6 [a]
Compound **2**[C]	200	6	Indomethacin	62.7	38.3 ± 6.2 – 14.3 ± 3.1 [a]

[A] ANOVA $F_{(2,16)}$ = 14.04, $p < 0.001$. Duncan's test: [b] $p < 0.001$.
[B] ANOVA $F_{(2,15)}$ = 16.51, $p < 0.001$. Duncan's test: [a] $p < 0.01$.
[C] ANOVA $F_{(2,13)}$ = 15.04, $p < 0.001$. Duncan's test: [a] $p < 0.01$.

Table **2** Effect of oral administration of carbenoxolone (100 mg/kg), compound **2** and compound **3**, in different doses, on ethanol induced ulcer model

Treatment	*Dose (mg/kg)*	*n*	*ULI inhibition (%)*	*ULI (mean ± SEM) Negative Control – Test Group*
Carbenoxolone [A]	100	5	76.,9	49.4 ± 4.9 – 11.4 ± 4.7 [c]
Compound **2**[A]	10	5	34.4	49.4 ± 4.9 – 32.4 ± 3.8 [a]
Compound **2**[A]	25	5	27.9	49.4 ± 4.9 – 35.6 ± 4.9 [a]
Compound **2**[A]	50	5	88.7	49.4 ± 4.9 – 5.6 ± 2.6 [c]
Compound **2**[A]	125	5	93.1	49.4 ± 4.9 – 3.4 ± 0.7 [c]
Compound **2**[A]	250	5	98.4	49.4 ± 4.9 – 0.8 ± 0.5 [c]
Carbenoxolone[B]	100	5	92.3	59.7 ± 7.7 – 4.6 ± 1.2 [c]
Compound **3**[B]	200	5	76.5	59.7 ± 7.7 – 14.0 ± 4.3 [c]
Compound **3**[B]	100	5	74.0	59.7 ± 7.7 – 15.5 ± 2.8 [c]
Compound **3**[B]	50	5	49.7	59.7 ± 7.7 – 30.0 ± 8.3 [b]

[A] ANOVA $F_{(6,33)}$ = 23.29 $p < 0.001$. Duncan's test: [a] $p < 0.05$; [c] $p < 0.001$.
[B] ANOVA $F_{(4,20)}$ = 14.11 $p < 0.001$. Duncan's test: [b] $p < 0.01$; [c] $p < 0.001$.

sults from one of the mentioned compounds, since they were obtained by chromatographic purification of the enriched sesquiterpene lactone fraction. Further bioassays were undertaken to evaluate the cytoprotective activity of these substances. On indomethacin and ethanol-induced ulcer model the, artemisinin **1** (100 mg/kg), did not inhibit the ulcerative lesion index, when administrated orally (Table **1**). When evaluated on the ethanol-induced model, artemisinin **1** (100 mg/kg) slightly increased the ulcerative lesion index. This can probably be explained due to the endoperoxide group present in the molecule which generates active oxygen species, one of the major causes for microcirculatory disturbance [16]. Otherwise compounds **2** and **3** inhibited the ulcerative lesion index on both experimental ulcer models. Dihydroepideoxyarteannuin B **2** administrated orally (100 mg/kg) on the indomethacin ulcer model, inhibited the ulcerative lesion index by 62.7 % (Table **1**). On the ethanol ulcer model, compound **2**, presented dose-dependent inhibition of the ulcerative lesion index, with an ED_{50} value of 55.6 mg/kg (Table **2**). Analogously, deoxyartemisinin **3** afforded an ED_{50} value of 87.5 mg/kg (Table **2**).

The dose-response evaluation on the ethanol-induced ulcer model demonstrated that compound **2** was more potent than compound **3**. This is the first mention of a pharmacological activity for dihydro-epideoxyarteannuin B **2** since it was first isolated and identified [7].

The previous treatment with indomethacin, a cyclooxygenase inhibitor, blocked the antiulcerogenic activity of compounds **2**

Table **3** Effect of oral administration of compound **2** and compound **3**, on ethanol induced ulcer model, with previous administration of indomethacin

Treatment	*Dose (mg/kg)*	*n*	*ULI (mean ± SEM)*	*ULI Inhibition (%)*
Saline		5	43.6 ± 5.6	
Carbenoxolone	100	3	7.0 ± 3.1 [b]	83.9
Saline + indomethacin		5	69.6 ± 3.0 [a]	–
Compound **2** + indomethacin	50	5	52.2 ± 5.8	–
Compound **3** + indomethacin	100	5	49.8 ± 6.0	–

ANOVA $F_{(4,18)}$ = 15.04 $p < 0.01$. Duncan's test: [a] $p < 0.01$; [b] $p < 0.001$.

and **3** on ethanol ulcer model. (Table **3**). These results suggest that the antiulcerogenic activity of compounds **2** and **3** have a straight relationship with an increase of prostaglandin synthesis [14].

References

[1] Acton N, Klayman BL. Artemisinin, a new sesquiterpene lactone endoperoxide from *Artemisia annua*. Planta Medica 1985; 47: 442 – 5

[2] Marles RJ, Pazos-Sanou L, Compadre CM, Pezzuto JM, Bloszyk E, Arnason JT. Sesquiterpene lactones revisited, 13. Phytochemistry of Medicinal Plants. In: Arnason JT, Mota R, Romero JT, editors. New York: Plenum Press, 1995: 333 – 56

[3] Giordano OS, Guerreiro E, Pestchanker MJ, Guzman J, Pastor D, Guardia T. The gastric cytoprotective effect of several sesquiterpene lactones. Journal of Natural Products 1990; 53: 803 – 9

[4] Guardia T, Guzman JA, Pestchanker MJ, Guerreiro E, Giordano OS. Mucus synthesis and sulfhydryl groups in cytoprotection mediated by dehydroleucodine, a sesquiterpene lactone. Journal of Natural Products 1994; 57: 507 – 9

[5] Lonergan G, Routsi E, Georgiadif T, Agelis G, Hondrelis J, Matsoukas J, Larsen LK, Caplan FR. Isolation and NMR studies in biological activities of onopordopicrine from *Centaurea sonchifolia*. Journal of Natural Products 1992; 55: 225 – 8

[6] Roth RJ, Acton N. The isolation of sesquiterpenes from *Artemisia annua*. Journal of Chemical Education 1989; 66: 349 – 50

[7] Brown GD. Two new compounds from *Artemisia annua*. Journal of Natural Products 1992; 55: 156 – 76

[8] Guide for the Care and Use of Laboratory Animals 1996. NIH Publication n° 82 – 23 revised

[9] International Guiding Principles for Biomedical Research Involving Animals (CIOMS). Genebra: 1985

[10] Morimoto Y, Shimohara K, Oshima S, Takayuki S. Effects of the new anti-ulcer agent KB-5492 on experimental gastric mucosal lesions and gastric mucosal defensive factors, as compared to those of teprenone and cimetidine. Japanese Journal of Pharmacology 1991; 57: 495 – 505

[11] Gamberini MT, Skorupa LA, Souccar C, Lapa AJ. Inhibition of gastric secretion by a water extract from *Baccharis triptera* Mart. Memórias do Intituto Oswaldo Cruz 1991 Supplement II; 86: 137 – 9

[12] Robert A. Cytoprotection by prostaglandins. Gastroenterology 1979; 77: 761 – 7

[13] Konturek SJ, Brozozowsky T, Pytko-Polonczyk J, Drozdowicz D. Comparison of cholecystokinin, pentagastrin and duodenal oleate in gastroprotection in rats. Scandinavian Journal of Gastroenterology 1995; 30: 620 – 30

[14] Dias PC, Foglio MA, Possenti A, Nogueira DCF, Carvalho J.E. Antiulcerogenic activity of crude ethanolic extract and some fractions obtained from aerial parts of *Artemisia annua* L. Phytotherapy Research 2001; 15: 670 – 5

[15] Felix WL, Miller JC, Guth PH. Dissociated effects of misoprostol on gastric acid secretion and mucosal blood flow. Digestive Diseases and Sciences 1986 (Supplement Feb); 31 (2): 86S – 90S

[16] Yoshida M, Kitahora T, Wakabayashi G, Hirokazu T, Ono H, Otani Y, Shimazu M, Kubota T, Kumai K, Kitajima M. Active oxygen species in formation of acute gastric mucosal lesions induced by thermal injury in rats. Digestive Diseases and Sciences 1995; 40: 1306 – 10