Antispasmodic Activity of Fractions and Cynaropicrin from Cynara scolymus on Guinea-Pig Ileum

Fernanda EMENDÓRFER,a Fabiane EMENDÓRFER,a Fernanda BELLATO,a Vânia Floriani NOLDIN,a Valdir Cechinel-FILHO,*b Rosendo Augusto YUNES,b Franco Delle MONACHE,c and Alcibia Maia CARDozoa

a Programa de Mestrado em Ciências Farmacêuticas e Núcleo de Investigações Químico-Farmacêuticas (NIFQAR), University of Vale do Itajaí (UNIVALI); Itajaí, 88302–202, Santa Catarina, Brazil; b Curso de Pós-Graduação em Química, Federal University of Santa Catarina (UFSC); Florianópolis-SC, Brazil; c Centro Chimica Recettori, Istituto de Chimica, Universita Cattolica del Sacro Cuore; Rome, Italy; and d Department of Pathology, Federal University of Santa Catarina; Florianópolis, Santa Catarina, Brazil. Received September 27, 2004; accepted February 5, 2005

This study describes the antispasmodic activity of some fractions and cynaropicrin, a sesquiterpene lactone from Cynara scolymus, cultivated in Brazil, against guinea-pig ileum contracted by acetylcholine. The dichloromethane fraction showed the most promising biological effects, with an IC_{50} of 0.93 (0.49—1.77) mg/ml. Its main active component, the sesquiterpene lactone cynaropicrin, exhibited potent activity, with IC_{50} of 0.065 (0.049—0.086) mg/ml, being about 14-fold more active than dichloromethane fraction and having similar potency to that of papaverine, a well-known antispasmodic agent. The results confirm the popular use of artichoke for the treatment of gastrointestinal disturbances, and encourage new studies on this compound, in order to obtain new antispasmodic agents.

Key words Cynara scolymus; medicinal plant; folk medicine; antispasmodic activity; cynaropicrin

Cynara scolymus (Compositae), popularly known as “alca-chofra” or “artichoke”, is widely cultivated in Mediterranean and American countries, and its sprout is commonly eaten as a vegetable. Its leaves are frequently used in folk medicine in many countries, to treat several ailments, including hepatitis, hyperlipidemia, and obesity, dyspeptic disorder, among others. Clinical and pre-clinical trials have confirmed the therapeutic potential of this plant, particularly in the treatment of hyperlipidemia, and obesity, dyspeptic disorder, among others.

In previous studies conducted in our laboratories, we have described the chemical and biological activities (cytotoxic and diuretic) of artichoke cultivated in Brazil. Our results showed that the main constituents are glycosyl flavonoids (cyanaroside and scolymoside), along with cynaropicrin, which are indicated as the active principles of this plant.

In order to elucidate the antispasmodic component(s) of this plant, we carried out a bio-guided assay against acetylcholine in guinea pig-ileum.

MATERIAL AND METHODS

Plant Material, Preparation of Fractions and Isolation of Cynaropicrin Leaves of Cynara scolymus were picked, in February 2000, from the “Central de Plantas” at Curitiba, in the State of Parana, southern Brazil. Air-dried leaves (1900 g) were macerated with methanol at room temperature for approximately 7 d, followed by the removal of solvent under reduced pressure to obtain the crude methanolic extract (CME). Afterwards, the CME was suspended in water, 48 h prior to the experiments and their food was restrained 24 h before the experiments.

Animals Guinea-pigs (300—400 g) of both sexes were kept in automatically controlled temperature conditions (23±2 °C), in 12 h light–dark cycles and with food and water “ad libitum”. The animals were kept in the laboratory for 48 h prior to the experiments and their food was restrained 24 h before the experiments.

Evaluation of Pharmacological Activity. Guinea-Pig Ileum Guinea-pigs of both sexes were sacrificed and the ileum was removed. The terminal portions, about 10 to 20 mm in length, were taken after discarding the 15 cm portion nearest to the ileum-caecal junction. The intestinal content was eliminated by washing with Tyrode solution, and the mesenteric residues were removed. Preparations were set up for recording the isometric contractions in 5 ml jacketed organ baths containing Tyrode solution at 37 °C, continuously bubbled with air under 1 g load by means of a light lever (six fold amplification) recorded in kymograph. The Tyrode solution had the following composition (m M): NaCl 136.8; KCl 2.7; CaCl 2 1.3; NaHCO 3 12.0; MgCl 2 0.5; NaH 2 PO 4 0.14 and glucose 5.5. After an initial balance period of about 30—45 min, cumulative concentration–effect curves were obtained for acetylcholine (1 pM to 100 μM), in the absence or presence of the four different fractions...
(0.10—2.0 mg/ml), and for cynaropicrin (0.01—0.2 mg/ml), after incubation for 15 min. Six cumulative concentration–effect curves were obtained for each preparation, with a period of at least 20 min between each. The maximum response obtained from the first cumulative concentration–effect curve was taken as the 100% response value. In order to correct for spontaneous and/or vehicle-induced desensitization, control experiments were performed for acetylcholine in separate sets of experiments, in the presence of corresponding concentrations of vehicle.

**Drugs** The drugs used were: acetylcholine iodide (from Sigma Chemical Company, St. Louis, U.S.A.), stored as 0.1 M stock solution for up to one week at −4 °C and diluted to the desired concentrations in saline 0.9%, just before use. Glucose, NaCl, KCl, CaCl$_2$·2H$_2$O, MgCl$_2$·6H$_2$O, NaHCO$_3$ and NaH$_2$PO$_4$ were acquired from Merck KGA, Darmstadt, Germany. The vegetal extracts were dissolved in 10% ethanol. Ethanol, at the final concentration in the bath, had no effect per se on the tonus of the preparations or on agonist-induced contractions.

**Statistical Analysis** Data are shown as media±S.E.M., except for the IC$_{50}$ (concentration of drugs causing half maximal responses) which are presented as geometric means, accompanied by their respective 95% confidence intervals. The IC$_{50}$ values were determined for individual experiments, at a minimum of four different concentrations, using a computer program for regression analysis. Statistical analyses were performed by means of the unpaired Student “t” test or by analysis of variance followed by the Tuckey test, when appropriate, and $p<0.05$ was considered significant.

**RESULTS AND DISCUSSION**

In order to locate the active principles of *C. scolymus*, we tested the four fractions (hexane, dichloromethane, ethyl acetate and butanol) obtained from the methanolic extract. Since dichloromethane fraction showed the best pharmacological action, the main component of this fraction, the sesquiterpene lactone cynaropicrin, was also analysed as an antispasmodic agent.

The results obtained with increasing the concentrations of hexane and butanolic fractions from *C. scolymus* (0.1—2.0 mg/ml) after every 15 min do not inhibit the contractile response elicited by acetylcholine in isolated guinea pig ileum (results not shown).

On the other hand, dichloromethane and ethyl acetate fractions (results not shown), in the same concentrations, caused a significant inhibitory effect for the contractile response elicited by acetylcholine on guinea-pig ileum, which was characterized by a clear displacement to the right of the concentration–response curves with inhibition higher than 50%. The calculated IC$_{50}$ values (with 95% confidence limits, mg/ml) of 0.065 (0.049—0.086) (Fig. 2). This fraction was about 14-fold more active than the dichloromethane fraction. The results were characterized by a clear displacement to the right of the concentration–response curves with inhibition higher than 50%. Cynaropicrin did not interfere with the basal tension of the preparations, indicating that no agonistic effect was present, with restoration of the contractile response to agonist after successive washings.

The results of cynaropicrin were comparable to that of papaverine, a potent antispasmodic compound isolated from *Papaver somniferum*, and used by the pharmaceutical industry in the manufacture of commercial drugs.14)

The inhibitory effect of both dichloromethane fraction and cynaropicrin is probably due to the decrease in calcium concentration available for contractile machinery in smooth muscle, which undergoes a biphasic mechanical response when exposed to acetylcholine. In fact, this effect could be caused by a reduction of calcium influx through the calcium channels, the tonic response, which consists of a slower, more sustained increase in tension that is usually of a lesser magnitude; and/or through the inhibition of calcium release from intracellular stores, the phasic response, which consists of a
rapid increase in tension reaching a sharp peak, followed by a rapid reduction in tension. However, other experimental studies are necessary to confirm their exact mechanism of action.

Cynaropicrin, one of the main components of artichoke cultivated in Brazil, exhibits other interesting biological properties. Cheng and co-workers\(^\text{15}\) reported a possible central neurotoxic effect for this compound isolated from *Centaurea solstitialis*, while Hay and co-workers\(^\text{16}\) showed that cynaropicrin inhibited aortic smooth muscle contractility, also suggesting a toxic pharmacological profile. Cho and co-workers\(^\text{17}\) demonstrated that cynaropicrin exhibits inhibitory effects on the production of tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), a proinflammatory cytokine. Recently, its antihyperlipidemic,\(^\text{18}\) anticancer\(^\text{19}\) trypanocidal and antimicrobial\(^\text{20}\) activities were reported. Taking all these studies together, it seems that cynaropicrin may be a potential pharmacutic agent. Nevertheless, further studies regarding its toxicity are required.

In summary, the current study indicated that cynaropicrin is the main active compound responsible for the smooth muscle relaxant effect observed for extract and fractions of artichoke. Its potent antispasmodic activity prompts us to carry out new studies in order to confirm the effects described here using other *in vitro* experimental models, as well as to synthesize new derivatives by structural modifications, in order to obtain more potent antispasmodic agents. Finally, our results confirm the antispasmodic activity of *C. scolymus*, supporting and justifying its popular use as a remedy to treat gastrointestinal problems.

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**REFERENCES**