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Epiisopiloturine hydrochloride, an imidazole alkaloid isolated from *Pilocarpus microphyllus* leaves, protects against naproxen-induced gastrointestinal damage in rats

Lucas A.D. Nicolau[a], Nathalia S. Carvalho[b], Dvison M. Pacífico[c], Larisse T. Lucetti[a], Karoline S. Aragão[a], Leiz M.C. Véras[d], Marcellus H.L.P. Souza[a], José R.S.A. Leite[d], Jand Venes R. Medeiros[b,d,*]

[a] *Department of Physiology and Pharmacology, Federal University of Ceará, Fortaleza, CE, Brazil*
[b] *Post Graduation Program in Pharmacology, Federal University of Piauí, Teresina, PI, Brazil*
[c] *Post Graduation Program in Morphofunctional Sciences, Department of Morphology, Faculty of Medicine, Federal University Ceará, Fortaleza, CE, Brazil*
[d] *Post Graduation Program in Biotechnology, Federal University of Piauí, Parnaíba, Brazil*

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ABSTRACT

Objective: This study aimed to investigate the protective effect of epiisopiloturine hydrochloride (EPI), an imidazole alkaloid, on NAP-induced gastrointestinal damage in rats.
Methods: Initially, rats were pretreated with 0.5% carboxymethylcellulose (vehicle) or EPI (3, 10 and 30 mg/kg, *p.o.* or *i.p.*, groups 3–5, respectively) twice daily, for 2 days. After 1 h, NAP (80 mg/kg, *p.o.*) was given. The control group received only vehicle (group 1) or vehicle + naproxen (group 2). Rats were euthanized on 2nd day, 4 h after NAP treatment. Stomachs lesions were measured. Samples were collected for histological evaluation and glutathione (GSH), malonyldialdehyde (MDA), myeloperoxidase (MPO), and cytokines levels. Moreover, gastric mucosal blood flow (GMBF) was evaluated.
Results: EPI pretreatment prevented NAP-induced macro and microscopic gastric damage with a maximal effect at 10 mg/kg. Histological analysis revealed that EPI decreased scores of damage caused by NAP. EPI reduced MPO (3.4 ± 0.3 U/mg of gastric tissue) and inhibited changes in MDA (70.4 ± 8.3 mg/g of gastric tissue) and GSH (246.2 ± 26.4 mg/g of gastric tissue). NAP increased TNF-α levels, and this effect was reduced by EPI pretreatment. Furthermore, EPI increased GMBF by 15% compared with the control group.
Conclusion: Our data show that EPI protects against NAP-induced gastric and intestinal damage by reducing pro-inflammatory cytokines, reducing oxidative stress, and increasing GMBF.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are often used clinically because of their anti-pyretic, anti-inflammatory, and analgesic properties. However, the chronic administration of these drugs is restricted as a consequence of their capacity to cause damage to the gastrointestinal tract, such as ulceration, erosion, perforation, and hemorrhage [1]. Naproxen, a non-selective NSAID, is widely prescribed for disorders such as arthritis, and is also one of the NSAIDs most likely to induce gastrointestinal injury [2,3]. The pathophysiology of naproxen-induced gastric antral ulceration is characterized by the production of oxygen free radicals, lipid peroxidation, and increased neutrophil adherence and activation [4–6].

The plants of the *Pilocarpus* genus, commonly known as "jaborandi," contain 14 known alkaloids [7], including pilocarpine, which is a particularly important phytotherapeutic compound that is used in human and veterinary medicine, and produces therapeutic effects such as decreased intraocular pressure, reduced dry mouth, and stimulation of smooth muscle for the treatment of gastrointestinal complications [8]. Other alkaloids such as isopilosine, epiisopilosine, and epiisopiloturine (Fig. 1) have been isolated from jaborandi [*Pilocarpus microphyllus* Stapf (Rutaceae)] leaves and are structurally similar to pilocarpine [9]. Epiisopiloturine is the second-most concentrated alkaloid, and has

* Corresponding author at: LAFFEX/BIOTEC/UFPI, Av. São Sebastião, n° 2819, CEP 64202-020, Parnaíba, PI, Brazil.
E-mail address: jandvenes@ufpi.edu.br (J.V.R. Medeiros).

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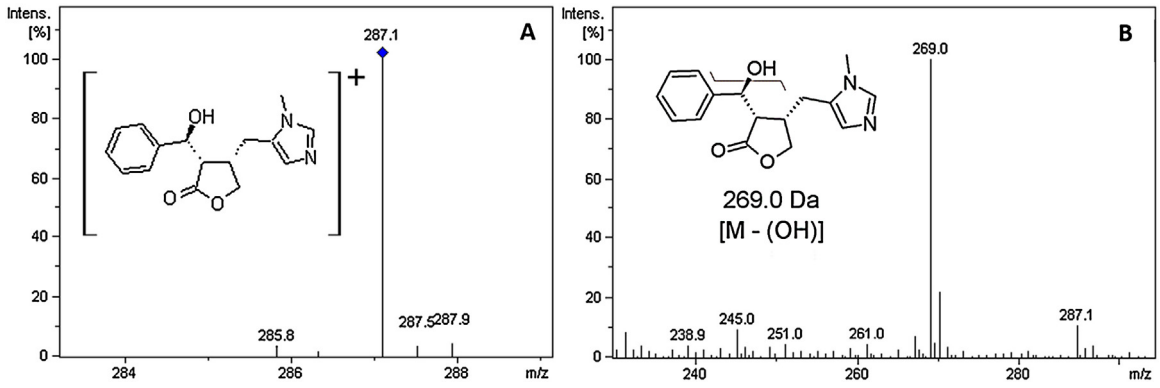

Fig. 1. Mass chromatogram for epiisopiloturine hydrochloride (EPI). Mass spectrum obtained from ESI+/ion trap. (A) Dissociated EPI with a pseudo-molecular ion *m*/*z* of 287.1 Da $[M+H]^+$, and (B) MS^2 with characteristic fragment at *m*/*z* of 269.0 Da $[M–H_2O]^+$ with proposed chemical structure.

demonstrated anti-parasitical, anti-inflammatory, and antinociceptive effects [10,11]. Natural products represent an excellent source for drug discovery; these products can be used directly to treat human diseases or can serve as a valuable starting point for drug discovery programs [12]. Although careful studies of the biological activities of *Pilocarpus* alkaloids and their underlying molecular mechanisms have led to the identification of novel modes of action and targets of relevance to the treatment of human diseases, no studies have examined the potential of epiisopiloturine to protect against ulcerogenic side effects associated with the use of NSAIDs. In this context, some imidazole derivatives were found to be versatile scaffolds with which to design anti-inflammatory compounds, because the imidazole rings are known to possess gastric protective and ameliorative effects [13]. Furthermore, a range of alkaloids have been shown prominent place in research as scope of drugs able to improve peptic ulcers [14]. Thus, the aim of this study was to investigate the ameliorative effect of epiisopiloturine hydrochloride, a derivative imidazole alkaloid, on naproxen-induced gastrointestinal damage in rats.

2. Materials and methods

2.1. Plant material

A specimen of *Pilocarpus microphyllus* was collected in October 2008 near Matias Olímpio City (Piauí, Brazil) and was identified by Dr. Ivanilza Moreira de Andrade of the Department of Biology, Federal University of Piauí. A voucher specimen (TEPB 27.152) was deposited at the Graziella Barroso Herbarium (Teresina, Piauí, Brazil).

2.2. Characterization

Epiisopiloturine (EPI), shown in Fig. 1, was obtained from waste produced by pilocarpine extraction from *P. microphyllus* leaves according to a previous study [10]. For being an alkaloid, the natural form of EPI is presented as basis without charge. In reaction with an acid, formed a soluble salt that was obtained by slow evaporation of the solution and exhibited MS/MS data consistent with values in the literature, as determined with an AmaZon SL mass spectrometer (Bruker Daltonics, Bremen, Germany) [15].

2.3. Drugs and reagents

Naproxen, carboxymethylcellulose, omeprazole, histamine, and ranitidine were purchased from Sigma-Aldrich Chemical (Saint Louis, MO, USA). Naproxen was dissolved in 0.5% carboxymethylcellulose (w/v). EPI was dissolved in 0.9% NaCl.

2.4. Animals

Male Wistar rats, weighing 120–150 g, were housed in temperature-controlled rooms and received water and food ad libitum. The animals were deprived of food for 18–24 h before the experimentation, but had free access to water. All surgical procedures and animal treatments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD) and were approved by the local ethics committee (Protocol number 008/2012).

2.5. Effect of EPI on naproxen-induced gastrointestinal damage

Rats were pretreated with 0.5% carboxymethylcellulose (vehicle), epiisopiloturine EPI (3, 10, and 30 mg/kg, *p.o.*; 3, 10, and 30 mg/kg, *i.p.*, groups 3–8 respectively) or omeprazole (10 mg/kg, *p.o.*, group 9) twice per day (at 09:00 and 21:00) for 2 days. One hour after EPI administration, naproxen (80 mg/kg, *p.o.*) was administered (at 10:00 and 22:00) for 2 days as described by Kim et al. [3] with modifications. The control group received only vehicle (group 1) or vehicle + naproxen (group 2). The rats were killed on the second day, 4 h after the naproxen treatment. The stomachs were promptly excised, opened along the greater curvature. The gastric damage was measured using digital calipers (Mitutoyo®, IL, USA). To study intestinal damage the abdomens were opened, and after identification of the intestine, a 5-cm portion of the medial intestine was removed for the evaluation of macroscopic scores by the criteria described by Martin and Wallace [16] with some modifications. All scoring of damage was performed in a randomized manner by an observer who was unaware of the treatments that the rats had received. Samples of the stomachs and small intestine were used for posterior analysis.

2.6. Glutathione (GSH) level

A segment from stomach and small intestine was homogenized in 5 mL of cold 0.02 M EDTA solution (1 mL per 100 mg/tissue). Aliquots (400 mL) of the tissue homogenate were mixed with 320 mL of distilled water and 80 mL of 50% (w/v) trichloroacetic acid in glass tubes and centrifuged at 3000 rpm for 15 min (Eppendorf® centrifuge 5810r, Germany). Next, 400 mL of each supernatant was mixed with 800 μL of Tris buffer (0.4 M, pH 8.9) and 20 mL of 0.01 M 5,5′-dithiobis(2-nitrobenzoic acid). The