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Antinociceptive effect and gastroprotective mechanisms of 3,5-diprenyl-4-hydroxyacetophenone from *Ageratina pichinchensis*

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ABSTRACT

The present study aimed to evaluate the antinociceptive activity (in inflammatory and neuropathic pain models) and gastroprotective effect of the 3,5-diprenyl-4-hydroxyacetophenone (HYDP), isolated from *Ageratina pichinchensis*. The gastroprotective activity of this plant was previously reported by our workgroup, finding encesanescin to be one active compound. The present results show that HYDP reduced nociception in a dose-dependent manner in carrageenan and L5/L6 spinal nerve ligation, with efficacies of 72.6 and 57.1%, respectively, at doses of 100 and 562 mg/kg. HYDP also showed gastroprotective activity in the model of ethanol-induced gastric lesion, with a 75.59% maximum inhibition of ulcers at a dose of 100 mg/kg. This gastroprotective effect was attenuated by N^G-nitro-L-arginine methyl ester, indomethacin and N-ethylmaleimide, indicating that NO, prostaglandins and sulfhydryl groups are involved in the mechanisms of action. This is the first evidence, to our knowledge, of the antinociceptive and gastroprotective activities of HYDP.

1. Introduction

Today few available drugs are effective in treating neuropathic pain [1], which is produced by damage or injury to the peripheral or central nervous system. Conventional analgesics have limited efficacy in the treatment of this condition, and the effectiveness of other drugs prescribed for treatment of neuropathic pain is variable for each patient. These other drugs include anticonvulsants, antidepressants and N-methyl-D-aspartate antagonists [2,3]. On the other hand, the NSAIDs are commonly used to treat inflammatory states and reduce the conditions of pain. However, their use is associated with an increased of the risk of ulcer development in the gastrointestinal tract and its complications (e.g. hemorrhages and perforations) [4].

These facts evidence the need for new research to discover substances that could be effective for managing pain and avoiding or reducing side effects. An attractive source of new biomolecules is represented by medicinal plants, especially considering that some have already been reported as antinociceptive and gastroprotective agents [5,6]. One such plant is *Ageratina pichinchensis*, previously denominated *Eupatorium aschembornianum* [7]. This plant, popularly known as axihuitl, is used to treat pain and gastric ulcers [8,9] in the Mexican state of Morelos, where it is an endemic herb that grows in the Tepozteco National Park.

Hence, after isolating 3,5-diprenyl-4-hydroxyacetophenone (HYDP) from *Ageratina pichinchensis*, we evaluated its antinociceptive effect in carrageenan-induced thermal hyperalgesia and L5/L6 spinal nerve ligation-induced tactile allodynia pain models. Since our research group previously

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reported the gastroprotective activity of this plant, finding encesanescin to be one compound participating in this effect [9], we herein assessed the gastroprotective effect of 3,5-diprenyl-4-hydroxyacetophenone (HYDP) in the ethanol-induced gastric ulcer model. Finally, we explored the participation of endogenous NO, prostaglandins and sulfhydryl groups in the gastroprotective mechanism of HYDP.

2. Experimental

2.1. General methods

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The ^{13}C and ^{1}H NMR spectra were recorded on a Varian VNMRS with tetramethylsilane (TMS) as internal standard.

2.2. Plant material

The leaves of *A. pichinchensis* were collected in the municipality of San Mateo de Tlanepantla, in the state of Morelos, during August of 2011. A voucher specimen can be found in the Hortorio Jorge Espinosa Herbarium at the Universidad Autónoma Chapingo, with the voucher number 1835.

2.3. Extraction and isolation

The leaves of *A. pichinchensis* were dried at room temperature (22 ± 2 °C) in the shade. After grinding, 2.0 kg of leaves were extracted by maceration with hexane during 3 successive days. Evaporation of the solvent in vacuum left 45 g of crude extract. A sample of hexane extract (35 g) was fractioned by open-column chromatography into silica gel (525 g) by using a step gradient of hexane and hexane/EtOAc mixtures (150 fractions of 50 mL). Fractions 45–60 (hexane) yielded a white solid (3.5 g, mp 90–92 °C), which was identified as 3,5-diprenyl-4-hydroxyacetophenone (Fig. 1A) by comparing its spectral data (^{1}H and ^{13}C NMR) with that of the literature [10]. From the fractions 115–130 (hexane/EtOAc 9:1), we obtained encecanescin (Fig. 1B), a compound with known gastroprotective activity [9].

2.4. Animals

All the experiments were performed with male Wistar rats, weighing 180–220 g, obtained from the animal house of the Universidad Autónoma Metropolitana, Xochimilco campus. The animals were placed in single cages with wire-net floors and deprived of food 24 h before experimentation, but allowed free access to tap water throughout. Procedures involving animals and their care were conducted in accordance with the Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999) and the guidelines on Ethical Standards for investigation of Experimental Pain in Animals, and were approved by our local Ethics Committee.

2.5. Drugs

Ketorolac, pregabalin and carbenoxolone, used as reference drugs (purchased from Sigma Chemical Co. USA), were dissolved in water. HYDP was suspended in 0.5% Tween 80 and administered by the intragastric route. Control rats received the vehicle (water or 0.5% Tween 80) in the same volume (0.5 mL/100 g) and by the same route. Carrageenans, NG-nitro-L-arginine methyl ester (L-NAME), and N-ethylmaleimide (NEM) (purchased from Sigma Chemical Co., USA) were dissolved in saline solution and indomethacin was dissolved in $NaHCO_3$ (5 mM). All drugs were prepared freshly for each use. The scheduling of dosages was based on pilot studies performed with animal models in our lab.

2.6. Carrageenan-induced thermal hyperalgesia

To assess thermal hyperalgesia, a previously described paw thermal stimulator [11] was used. The device consists of a glass surface upon which the rats are placed individually in plexiglas cubicles. The glass surface temperature was maintained at 30 ± 0.1 °C. The thermal nociceptive stimulus, originating from a focused projection bulb, was manually manipulated to allow for separate stimulation of each hind paw. This stimulus was positioned under each footpad before and after a 1% carrageenan injection (50 μL). A timer was automatically activated with the light source, and the response latency was defined as the time required for the paw to be an abruptly withdrawn. In all cases, there was a cut-off at 20.48 s to avoid tissue injury.

Rats were acclimated to the test chamber for 20–30 min prior to testing, which was carried out immediately before the injection and every 30 min thereafter, for up to 6 h. Different groups of rats received the vehicle, HYDP at doses of 10, 32, 56 or 100 mg/kg (p.o.), or ketorolac (positive control; 10 mg/kg, p.o.). At the end of the experiments the rats were sacrificed in a CO_2 chamber. Data are presented as the percentage of the maximum possible effect (% MPE) obtained from the area under the curve,

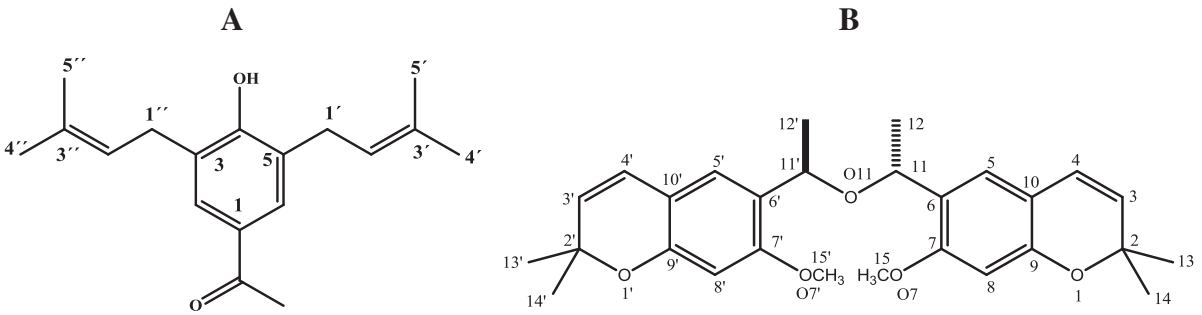

Fig. 1. Chemical structure of 3,5-diprenyl-4-hydroxyacetophenone (A) and encecanescin (B).