

Antihyperalgesia Induced by *Heliopsis longipes* Extract

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Abstract

Heliopsis longipes is an herbaceous plant found in Mexico. *Heliopsis longipes* is traditionally used for its analgesic and anesthetic properties. Plant extracts may represent a therapeutic advantage for the clinical treatment of pain. Therefore, the main objective of this study was to determine the possible antihyperalgesic effect produced by the *Heliopsis longipes* ethanolic extract (HLEE) in the Hargreaves model of thermal hyperalgesia in the mouse. HLEE was administered systemically to mice and the antihyperalgesic effect was evaluated using the thermal hyperalgesia test. Oral Administration of HLEE produced a dose-dependent antihyperalgesic effect. Previously, it was reported that *Heliopsis longipes* extract was able to release GABA in mice temporal cortex slices. Therefore, it is likely that the antihyperalgesic effect observed in our study could result from GABA liberation and its inhibition of excessive excitation of nociceptive circuits in the thalamus and cortex evoked by tissue injury. Our results suggest that HLEE may represent a therapeutic advantage for the clinical treatment of inflammatory pain.

Introduction

Heliopsis longipes is an herbaceous plant found in Mexico [1]. Analgesic and anti-inflammatory effects induced by *Heliopsis longipes* extracts have been demonstrated in dental and oral pathologies in humans [2,3]. Similarly, a *Heliopsis longipes* extract and a pure compound derived from *Heliopsis longipes*, showed an antinociceptive effect in the acetic acid-induced writhing test in mice [4]. More recently, it has been reported that a solution of dichloromethane extract from *Heliopsis longipes* showed analgesic activity determined by gamma-amino butyric acid (GABA) release in mice brain slices [5]. Hence, the purpose of the present study was to characterize the antihyperalgesic effect of the systemic administration of the *Heliopsis longipes* ethanolic extract (HLEE) in the Hargreaves model of thermal hyperalgesia.

Material and Methods

Animals. Balb/c male mice (weight range, 20-28 g) from our own breeding facilities had free access to water before experiments, while food was restricted 12 hr before. Efforts were made to minimize animal suffering and to reduce number of animals used. Mice were used once only. All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals [6]. In addition, the study was approved by the Institutional Animal Care and Use Committee (Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico City, Mexico).

Evaluation of Thermal Hyperalgesia. Anti-hyperalgesia was assessed by the Hargreaves model of thermal hyperalgesia [7]. A plantar test (Ugo Basile apparatus) was used to measure the withdrawal latencies of the hind paws from a radiant heat stimulus. Mice were manually restrained and no pre-experiment habituation to the test environment was carried out. The thermal nociceptive stimulus originated from a high intensity projector lamp bulb was manually manipulated and positioned under each footpad before and after the intraplantar injection of carrageenan (25 µl; 2%) into the right hind paw. A timer was automatically actuated with the light source, and the paw withdrawal latencies measured (PWLs) was defined as the time required for the paw to show an abrupt withdrawal. A cut-off time of 22 sec was used to prevent tissue damage. Measurements of PWLs were made immediately before and 1, 2, 3, 4, 5 and 6 hr after carrageenan injection.

Drugs and *Heliopsis longipes* Ethanolic Extract (HLEE).

Carrageenan (Type IV, Lambda) was purchased from Research Biochemical International (Natick, MA, USA). *Heliopsis longipes* (Gray) Blake (Asteraceae) specimens, as authenticated by José García Pérez from the Herbarium of the University San Luis Potosí (SLP), were collected in the mountain zone of Rio Verde in the state of SLP, Mexico at an altitude of 1795 meters above sea level. Voucher specimens (*H. Longipes* 41523) were deposited at the above mentioned institution. Dry roots were ground and extracted with absolute ethanol in a

continuous extraction system (Tecator, Soxtec System HT 1043 Extraction Unit) for 2 hr at 80°C. The extracts were filtered through Whatman paper No. 4 and the ethanolic extract was freed from solvent in a rotary evaporator (Büchi model R 3000) at 60°C under reduced pressure. HLEE and carrageenan were dissolved in 0.9% saline solution.

Study Design. In order to assess the antihyperalgesic effect, thirty min before the carrageenan injection, animals were pre-treated with oral (*p.o.*) administration of vehicle or increasing doses of HLEE (10-300 mg/kg). The injection volumes were 100 μ l. Mice in all groups were observed regarding behavioral or motor function changes induced by the treatments. This was assessed by testing the animals' ability to stand and walk in a normal posture. All observations were carried out by a blinded investigator.

Data analysis and statistics. All experimental results are given as the means \pm S.E.M. for 6-8 animals per group. Data are expressed as the area under the PWLs against time curve (AUC). Analysis of variance (ANOVA), followed by Tukey's test was used to compare differences between treatments. Differences were considered to reach statistical significance when $p < 0.05$.

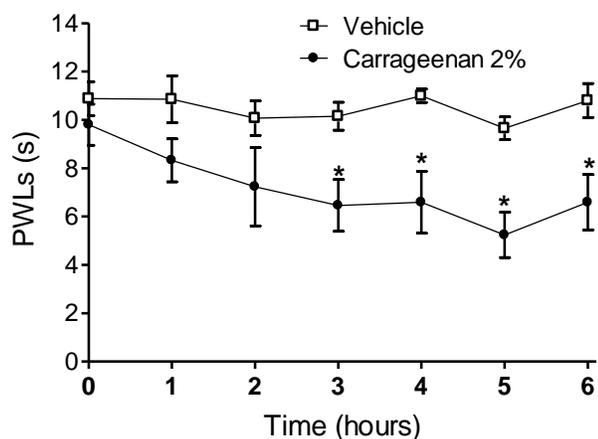


Figure 1. Time-course of carrageenan-induced hyperalgesia in the Hargreaves test. Mice were pretreated with vehicle or carrageenan (25 μ l; 2%) in the right hind paws. Curves were constructed plotting the PWLs as a function of time. * Significantly different from vehicle ($p < 0.05$), as determined by ANOVA followed by the Tukey's test.

Results and Discussion

During the last several years, traditional systems of medicine have become a topic of global importance. Current estimates suggest that in many developing countries herbal medicine is a major component in all

indigenous peoples' traditional medicine. Although some traditional Mexican medicines have been used for centuries, our understanding of the scientific principles of these herbal compounds is still far from satisfactory. The mechanism of action and interactions, and the chemicals herbal compounds contain remain undetermined in most Mexican traditional medicines. There is need to further elucidated these using modern biological tools to provide scientific findings to justify the medicinal use of several plants in inflammation and pain treatment. In the present study, intraplantar carrageenan (25 μ l, 2%), but not vehicle (saline), produced a time-dependent thermal hyperalgesia in the right hind paw (Fig. 1). Oral administration of the HLEE (10-300 mg/kg, *p.o.*), but not vehicle, produced a dose-dependent reduction in the hyperalgesic effect induced by carrageenan ($P < 0.05$, Fig. 2). No side effects were observed in either group, control or treated.

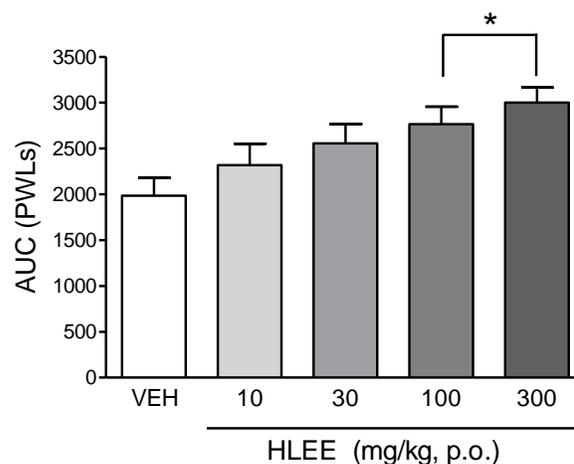


Figure 2. Antihyperalgesic effect of the HLEE (10-300 mg/kg, *p.o.*) in carrageenan-induced thermal hyperalgesia. Mice were pretreated with vehicle or HLEE 30 min before carrageenan injection. Data are expressed as the area under the PWLs against time curve (AUC). Bars are the mean \pm S.E.M. of 6-8 animals. * Significantly different from vehicle ($p < 0.05$) as determined by ANOVA followed by the Tukey's test.

Recently, Rios *et al.* [5] reported the release of GABA in temporal cortex slices in mice induced by an extract from *Heliopsis longipes* and the main active compound of *Heliopsis longipes* called Affinin, an alkylamide (N-isobutyl-2E-decenamide). Research suggested a feasible analgesic activity of *Heliopsis longipes* extract and affinin based on the neural mechanisms underlying the cortical modulation of pain by GABA [8]. Nevertheless, the *Heliopsis longipes* extract was not used *in vivo*. Consequently, we sought to demonstrate its possible analgesic action in a pain test different to the acetic

acid-induced writhing test [4]. In our study, systemic administration of HLEE was able to diminish the hyperalgesic effect induce by carrageenan in the mouse. Therefore, it is possible that the antihyperalgesic effect observed in our study could result from the release of GABA and its inhibition of extreme excitation of nociceptive circuits in the thalamus and cortex evoked by tissue injury. Our results suggest that HLEE may represent a therapeutic advantage for the clinical treatment of inflammatory pain.

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