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Original article

Moringa oleifera, a species with potential analgesic and anti-inflammatory activities☆

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ABSTRACT

Moringa oleifera has long been used in large demand in folk medicine to treat pain. The present study was undertaken to examine the antinociceptive and anti-inflammatory spectrum of *M. oleifera* leaf extracts discriminating the constituents' nature by using different kind of experimental models in rats. Pharmacological evaluation of a non-polar and/or polar extracts at several doses (30–300 mg/kg, p.o.) was explored through experimental nociception using formalin test, carrageenan-induced paw edema and arthritis with subcutaneous injection of collagen in rats. Basic morphology characterization was done by scanning electronic microscopy and laser scanning confocal microscopy. Not only polar (from 30 or 100 mg/kg, p.o.) but also non-polar extract produced significant inhibition of the nociceptive behavior with major efficacy in the inflammatory response in different assessed experimental models. This antinociceptive activity involved constituents of different nature and depended on the intensity of the induced painful stimulus. Phytochemical analysis showed the presence of kaempferol-3-glucoside in the polar extract and fatty acids like chlorogenic acid, among others, in the non-polar extract. Data obtained with *M. oleifera* leaf extracts give evidence of its potential for pain treatment.

1. Introduction

Pain and inflammatory responses in the peripheral and central nervous systems play key roles in the development and persistence of many pathological pain states [1]. A variety of natural compounds are able to alleviate pain targeting inflammation by reducing the synthesis of inflammatory mediators, or modulating inflammatory and nociceptive pathways [2]. Undoubtedly nature has always been a meaningful origin of therapeutic remedies for centuries in different cultures all around the world. Current drugs are derived from ancestral knowledge and nowadays plants compounds are a very valuable source of active pharmaceuticals [3]; but most of all to isolate the specific compounds with a pharmacological potential use, it is essential to identify and characterize properties of herbal remedies [4].

Moringa oleifera Lamarck (synonymy *Moringa pterygosperma* Gaertn.) is a species belonging to the Moringaceae family (Papaverales Order), which possesses additional 13 species of trees and shrubs spread in several countries [5,6]. It is recognized for its nutritional properties [7,8], but also because of its fascinating medicinal uses [5,6,9,10,11]. *M. oleifera* leaves have

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been used in traditional medicine to alleviate pain mainly as an anti-inflammatory. In India, poultices of leafs have been used to treat glandular inflammation [9]. In Ayurveda, *M. oleifera* is known as Shigru, it is used during and after the body cleansing process called Panchakarma with the specific indication of antibacterial, antifungal, antibiotic, hepatoprotective, anticancer and anti-inflammatory [12]; decoction of leaves is used for Udar shool which means gastritis [13]. Based on human, animal, and in vitro studies, and the extrapolation of results from animal studies to humans, various preparations of *M. oleifera* leaves including aqueous extracts appear to be exceedingly safe at the doses and in the amounts commonly utilized [8]. All these antecedents recommend this species for the cure of pain in several diseases.

The nature of the bioactive constituents of *M. oleifera* leaves is scarcely known; it has been described that this species is rich in compounds containing the simple sugar, rhamnose, and a fairly unique group of compounds called glucosinolates and isothiocyanates [14]. Its leaves act as a good source of natural antioxidant due to the presence of compounds such as ascorbic acid, flavonoids, phenolics and carotenoids [15,16]. It is a rich source of minerals, high concentrations in iron (Fe), calcium (Ca), phosphorus (P), copper (Cu), vitamins A and B, α-tocopherol, riboflavin, nicotinic acid, folic acid, pyridoxine, β-carotene, protein, and in particular essential amino acids such as methionine, cystine, tryptophan and lysine present in leaves and pods make it a virtually ideal dietary supplement [15]. They can also be part of the medicinal properties on pain therapy [17]. Currently, analgesic and anti-inflammatory effects of *M. oleifera* have already been described for flowers, roots and seeds extracts [18,19], although, little is reported about these activities in the case of leaves. In this study, we examine a pharmacological spectrum of antinociceptive activity after enteral administration by comparing non-polar and polar extracts assessed in experimental pain models.

2. Materials and methods

2.1. Animals

Male and female Wistar rats weighing 180–200 g were used in this study. Animals were housed in a temperature- and light-controlled room under a 12-h light/12-h dark cycle (lights on at 7:00 a.m.) with water and food *ad libitum*. Twelve hours before the experiments, food was suspended, though animals had free access to tap drinking water. All experimental procedures followed the Guidelines on Ethical Standards for Investigations of Experimental Pain in Animals [20], the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23; revised 1978), and the Norma Oficial Mexicana for the care and use of laboratory animals (NOM-062-ZOO-1999). In addition, the protocol was approved by the local Animal Ethics and Scientific Committee (Instituto Nacional de Psiquiatría "Ramon de la Fuente Muñiz", INP-NC2012). Efforts were made to minimize the number of animals used and their suffering. For each experimental procedure animal groups consisted of six rats.

2.2. Drugs

Dexamethasone was pharmaceutical grade purchased from Tecnofarma, S.A. de C.V. Crude extracts were diluted in vehicle (0.2% tween 80 in saline solution (0.9% NaCl, s.s.)). Naproxen, ketorolac and kaempferol-3-glucoside and all pure compounds were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Drugs were freshly prepared on the day of the experiments and administered by enteral (p.o.) or parenteral (i.p.) injection in a volume of 0.1 mL/100 g body weight. Control animals received the same volume of vehicle.

2.3. Vegetal material

M. oleifera Lam. leaves were bought from local cultivate in Rancho San Antonio, Culiacán Sinaloa, Mexico (minimum annual temperature mean is 10.5 °C, maximum temperature mean is 36 °C and mean annual rainfall is 790 mm). A voucher specimen (MEXUS-1420215) was previously identified by the plant biologist Dr. Mark Olson and deposited at the National Herbarium of the Biology Institute in the University of Mexico (UNAM).

2.4. Preparation of the extracts

Fresh leaves of *M. oleifera* (Fig. 1A) were naturally dehydrated and hand-milled. The dried mature leaves of the vegetal material (600 g) were extracted by successive maceration for 48 h with hexane (3 L × 3 times) at room temperature (22 ± 1 °C), followed by filtration. The residue was once again extracted but this time with absolute ethanol (3 L × 3 times), followed by filtration. The final residue was discarded. The filtrates were concentrated under vacuum to eliminate the solvent. As a result, two extracts were obtained: a dark green greasy hexane extract (24.30 g, 4.05%), and also a dark green semisolid ethanol extract (32.64 g, 5.44%). A conventional UHPLC analysis of the ethanol crude extract was included to identify a characteristic flavonoid (Kaempferol-3-glucoside) (Fig. 1A–B), whereas mass spectroscopy negative ion mode was applied to identify the presence of fatty acids in the non-polar extract (Fig. 1C).

2.5. Anatomical analysis

Fresh leaf samples were analyzed with a Quanta 3DFEG FEI scanning electronic microscope at low vacuum and electron energy used was 15 kV. A LSM 710 NLO Carl Zeiss laser scanning confocal microscope equipped with halogen and mercury vapor lamps in a wavelength emission between 422 and 724 nm was used to obtain images of the pollen fluorescence.

2.6. Pharmacological evaluation

2.6.1. Experimental design

Firstly, it was explored the antinociceptive and anti-inflammatory activities of *M. oleifera* leaves testing several doses of hexane and ethanol crude extracts (30, 100 and 300 mg/Kg, p.o.) by using the formalin test in rats. After these data, groups of at least six rats were administered with the most active crude extract and its effect was compared to that obtained with a reference drug (selected depending on the experimental test), such as: naproxen (10 mg/kg, p.o.), ketorolac (0.56 mg/kg, i.p.), or dexamethasone (0.1 mg/kg, i.p.), whose dosage were selected according to previous studies. All treatments were compared to rats receiving vehicle (saline solution or 0.2% tween 80) depending of the extract evaluated. Therefore, after a 30-min period from treatment administration, rats were tested in one of the following tests.

2.6.2. Antinociceptive activity

2.6.2.1. The formalin test. Male rats were placed in an open Plexiglass cylinder for 30 min to allow them to acclimate to their surroundings. A mirror was placed behind the cylinder to enable unhindered observation. The method used was similar to that described [21]. For this test, fifty microliters of diluted formalin at 1% was injected subcutaneously (s.c.) into the dorsal surface of the right hind paw with a 30-gauge needle. Nociception was observed immediately after formalin injection as a flinching behavior that happens in a biphasic manner. The initial acute phase (neurogenic, 0–10 min) is followed by a relatively short quiescent period, which