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Pharmacological and toxicological study of a chemical-standardized ethanol extract of the branches and leaves from *Eysenhardtia polystachya* (Ortega) Sarg. (Fabaceae)

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ARTICLE INFO

Chemical compounds studied in this article:
Tramadol hydrochloride (PubChem CID:63013)
glibenclamide (PubChem CID:3488)
naproxen sodium (PubChem CID: 23681059)
naloxone (PubChem CID:5464092)
dimethyl sulfoxide (PubChem CID:679)
pentobarbital (PubChem CID: 4737)
loperamide hydrochloride (PubChem CID:71420)
clonazepam (PubChem CID:2802)
D-pinitol (PubChem CID:164619)
and ondansetron (PubChem CID:4595)

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ABSTRACT

Eysenhardtia polystachya is used for the empirical treatment of cancer, infections, diarrhea, inflammation, and pain. This study identified, using GC-MS, the main chemical components in an ethanol extract of *E. polystachya* branches and leaves (EPE) and tested its cytotoxic, antimicrobial, anti-diarrheal, anti-inflammatory, and antinociceptive effects. The *in vitro* and *in vivo* toxicity of EPE was evaluated using the comet assay in human peripheral blood mononuclear cells (PBMC) and the acute toxicity test in mice, respectively. The cytotoxic and the antimicrobial effects were performed using the MTT assay and the minimum inhibitory concentration (MIC) test, respectively. The levels of pro-inflammatory mediators in LPS-stimulated macrophages were measured to evaluate the *in vitro* anti-inflammatory effects of EPE. The antidiarrheal (castor oil test, small intestine transit, and castor oil-induced enteropooling), and anti-inflammatory activities (TPA and carrageenan) of EPE were also performed. The anti-nociceptive actions of EPE were carried out with the following tests: acetic acid, formalin, and hot plate. The hypnotic and locomotor effects were analyzed using pentobarbital and a rotarod system, respectively. The main component in EPE was D-pinitol (26.93%). The antidiarrheal and antinociceptive effects of D-pinitol were also evaluated. EPE showed low *in vitro* toxicity (DNA damage in PBMC at concentrations higher than 200 μg/ml), and low *in vivo* toxicity (LD_{50} > 2000 mg/kg i.p. and p.o.). Furthermore, EPE lacked cytotoxic activity (IC_{50} > 300 μg/ml) on human cancer cells, but showed good antimicrobial effects in *E. coli* (MIC = 1.56 μg/ml) and *S. aureus* (MIC = 0.78 μg/ml). In multi-drug resistant microorganisms, EPE showed MIC > 100 μg/ml. EPE exerted *in vitro* anti-inflammatory effects, mainly, by the decrease in the production of H_2O_2 (IC_{50} = 43.9 ± 3.8 μg/ml), and IL-6 (73.3 ± 6.9 μg/ml). EPE (ED_{50} = 7.5 ± 0.9 mg/kg) and D-pinitol (ED_{50} = 0.1 ± 0.03 mg/kg) showed anti-diarrheal activity, and antinociceptive effects in the acetic acid test with ED_{50} = 117 ± 14.5 mg/kg for EPE and 33 ± 3.2 mg/kg for D-pinitol. EPE showed also antinociceptive activity in the phase 2 of the formalin test (ED_{50} = 48.9 ± 3.9 mg/kg), without inducing hypnotic effects or altering the locomotor activity in mice. The results here presented corroborate the folk medicinal use of *Eysenhardtia polystachya* in the treatment of infections, diarrhea, inflammation, and pain. D-pinitol, the main metabolite of EPE, showed antinociceptive and antidiarrheal effects with similar potency compared to standard drugs.

Abbreviations: CNZ, clonazepam; EPE, ethanol extract of branches and leaves of *Eysenhardtia polystachya*; GC-MS, Gas chromatography mass spectroscopy; IND, indomethacin; NPX, naproxen; PBMC, Peripheral blood mononuclear cells;; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; TRD, tramadol

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1. Introduction

Eysenhardtia polystachya (Ortega) Sarg. (Fabaceae) is a small tree (2–8 m in high) native from Mexico, commonly known as "palo dulce" or "palo azul", "tlapalezpatli" in Nahuatl dialect, and "urza" in otomi dialect (Argueta et al., 1994). The infusion of bark, branches, and leaves produces a sweet flavor and shows a golden color. *E. polystachya* is used in the Mexican traditional medicine as diuretic, anti-inflammatory, spasmolytic, wound healing, and anticonceptive agent, it is also employed for the empirical treatment of genitourinary infections, cancer, arthritis, diarrhea, fever, cough, vomiting, bronchitis, and bladder disorders (Argueta et al., 1994; Alonso-Castro et al., 2011; García-Regalado, 2015; Perez-Gutierrez et al., 2016; personal communication). Traditionally, one or two small pieces of the bark, or approximately 10 g of branches and leaves of *E. polystachya* are prepared as infusion, decoction, or maceration (Pablo-Pérez et al., 2016, personal communication). In veterinary, *E. polystachya* is used as an antimicrobial agent for poultry (García-Regalado, 2015). The phytochemical investigation with *E. polystachya* resulted in the isolation of chalcones, flavonoids, and phytosterols (Beltrami et al., 1982; Burns et al., 1984; Álvarez et al., 1998; Álvarez and Delgado, 1999; Perez-Gutierrez et al., 2016).

E. polystachya has shown diuretic and antilithiatic effects (Pérez et al., 1998; Pablo-Pérez et al., 2016), moderate antibacterial effects against *Streptococcus mutans* (Rosas-Piñón et al., 2012), anti-diabetic and antioxidant activity (Gutierrez and Baez, 2014), and antifungal activity against two phytopathogens (Bernabé-Antonio et al., 2017). In this work, we evaluated some traditional uses (cytotoxic on cancer cells, analgesic, anti-inflammatory, antimicrobial, and anti-diarrheal) of *E. polystachya* using *in vitro* and *in vivo* pharmacological models. The *in vitro* and *in vivo* toxicity of *E. polystachya* was also evaluated. The antinociceptive and antidiarrheal effects of D-pinitol (Fig. 1), the main component of EPE were assessed.

2. Materials and methods

2.1. Reagents

Indomethacin (IND), clonazepam (CNZ), 3-(4,5-dimethylthiazol-2-yl)− 2,5-diphenyl tetrazolium bromide (MTT), tramadol (TRD), naproxen sodium (NPX), Lipopolysaccharides (LPS) from *Escherichia coli* 0111:B4, trypan-blue dye, sodium nitrite, Griess reagent, phenol red, λ-carrageenan, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), and D-pinitol (95% of purity according to the manufacturer) were acquired from Sigma Aldrich (St Louis, MO, USA). RPMI and fetal bovine serum (FBS) were from GIBCO BRL (Grand Island, NY). Cisplatin (CDDP) was from Accord Farma (Mexico City, México). ELISA assay kits for measuring mouse IL-1β, TNF-α, and IL-6 were obtained from Peprotech (London, UK).

2.2. Plant material

Samples of *Eysenhardtia polystachya* were collected in Ciudad Valles, State of San Luis Potosi, Mexico. Jose Garcia Perez (UASLP) identified the plant material. Voucher samples (SLPM-4588) were preserved for further reference in the herbarium Isidro Palacios of the Instituto de Investigación de Zonas Desérticas, Universidad Autónoma de San Luis Potosí (SLPM).

2.3. Preparation of the ethanol extract of E. polystachya branches and leaves (EPE)

Branches and leaves of *E. polystachya* were dried and ground. The powder (100 g) was extracted with ethanol (1 l) for 10 days. The extract was filtered, and the solvent was evaporated.

2.4. Gas chromatography mass spectrometry (GC-MS) analysis

The characterization of the main components in EPE was carried out following the method described by Alonso-Castro et al. (2016), using a gas chromatograph 6890 (Agilent Technology, Santa Clara, CA, USA) and a selective mass detector 5973. DB-5HT column (15 m × 0.25 mm ID, 0.10 μm film thickness), with some modifications. Briefly, 10 mg of EPE was relocated to glass tube and dissolved in 2 ml isooctane. For silylation, 100 μl BSTFA was added to the samples and incubated at 100 °C for 10 min in a CEM Discover microwave equipment at 150 W, 290 psi. The operating conditions of the column were as follows: oven temperature programmed from 100 °C (3 min) to 320 °C at 15 °C/min, and 2 min hold. The injector temperature was kept at 320 °C and the volume of injected sample was 1 μl. The MS ran in electron impact at 71 eV and mass spectral data were collected in the scan mode in the *m/z* range 33–800. The identity of compounds present in EPE was analyzed with the NIST 14 software (National Institute of Standards and Technology, USA).

2.5. In vitro assays

2.5.1. Cell lines and culture conditions

The human cancer cell lines of cervix (SiHa), breast (MDA-MB231), prostate (PC3), and glioblastoma (U87) were used in this study. The human keratinocytes HaCaT were used as non-tumorigenic cells. All cell cultures, obtained from ATCC (Manassas, VA, USA), were grown at 37 °C and 5% CO_2 in RPMI with 7% fetal bovine serum and antibiotics (100 U/ml penicillin and 100 pg/ml streptomycin).

2.5.2. Bacteria

The Gram-positive *Staphylococcus aureus* (ATCC 25923), and the Gram-negative *Escherichia coli* (ATCC 25922), as well as some clinical strains of microorganisms (*E. coli*, *Candida albicans*, and *Proteus vulgaris*) with multi-drug resistance, were used in this study. Microorganisms were grown in brain heart infusion broth, and the inoculum was adjusted to a 0.5 McFarland standard turbidity.

2.5.3. MTT assay

Cell lines were seeded at 5000 cells/well in 96-well microplates. After 24 h of incubation, cells were incubated with EPE at concentrations of 1–300 μg/ml, or the positive control cisplatin at 0.1–100 μg/ml. In HaCaT cells, EPE was tested at 1–1000 μg/ml. After 48 h of treatment, the MTT assay was performed following the protocol described by Jacobo-Salcedo et al. (2011). Optical density (O.D.) was recorded at 590 nm. IC_{50} values were calculated by lineal regression, and indicate the concentration that inhibit 50% of viability on cell line.

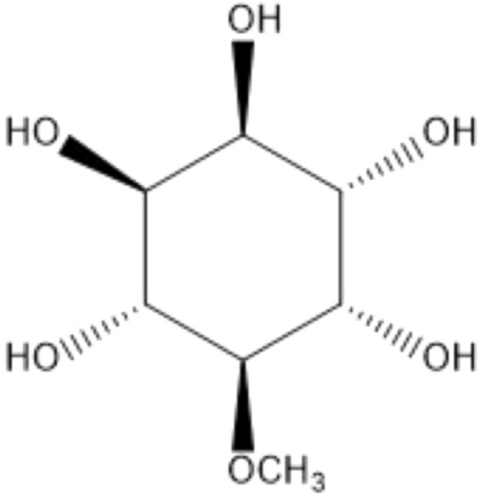

Fig. 1. Chemical structure of D-pinitol, the main component in EPE.