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Pharmacological effects and toxicity of *Costus pulverulentus* C. Presl (Costaceae)

Angel Josabad Alonso-Castro [a,*], Juan Ramón Zapata-Morales [a], Marco Martin González-Chávez [b], Candy Carranza-Álvarez [c,**], Diego Manuel Hernández-Benavides [c,d], Alejandro Hernández-Morales [c]

[a] Departamento de Farmacia, División de Ciencias Naturales y Exactas, Universidad de Guanajuato, Guanajuato, México
[b] Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí, San Luis Potosí, México
[c] Unidad Académica Multidisciplinaria de la Zona Huasteca, Universidad Autónoma de San Luis Potosí, Ciudad Valles, San Luis Potosí, México
[d] El colegio de la Frontera Sur unidad San Cristóbal, San Cristóbal de las Casas, Chiapas, México

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Buprenorphine (PubChem CID: 644073)
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Naproxen sodium (PubChem CID: 23681059)
Ketamine (PubChem CID: 644025)
and cisplatin (PubChem CID: 441203)

ABSTRACT

Ethnopharmacological relevance: *Costus pulverulentus* C. Presl (Costaceae), a species endemic to Mexico, is used for the empirical treatment of cancer, pain, and inflammation.
Aim of the study: The objective of this study was to evaluate the toxicity, as well as the cytotoxic, anti-nociceptive, anti-inflammatory and sedative effects of an ethanol extract from *Costus pulverulentus* stem (CPE).
Materials and methods: The chemical characterization of CPE was performed by Gas chromatography–mass spectrometry (GC–MS). The toxicity of CPE was evaluated using the comet assay (10–1000 μg/ml during 5 h) and the acute toxicity test (500–5000 mg/kg p.o. and i.p. during 14 days). The cytotoxic effect of CPE (1–250 μg/ml) on human cancer cells was evaluated using the MTT assay. The antinociceptive effects of CPE (50–200 mg/kg p.o.) were evaluated using thermal-induced nociception tests (hot plate and tail flick) and the chemical-induced nociceptive tests (acetic acid and formalin). The sedative activity of CPE (50–200 mg/kg p.o.) was evaluated using the ketamine-induced sleeping time test.
Results: CPE showed the presence of compounds such as campesterol, stigmasterol β-sitosterol, vanillic acid, among others. In the comet assay, CPE at 200 μg/ml or higher concentrations induced DNA damage. In the acute toxicity test, the LD_{50} estimated for CPE was > 5000 mg/kg p.o. or i.p. CEP showed moderate cytotoxic effects on prostate carcinoma cells PC-3 cells ($IC_{50} = 179 \pm 23.2$ μg/ml). In the chemical-induced nociception models, CPE (100 and 200 mg/kg p.o.) showed antinociceptive effects with similar activity to 100 mg/kg naproxen. In the thermal-induced nociception tests, CPE tested at 200 mg/kg showed moderate antinociceptive effects by 28% (hot plate test) and by 25% (tail flick test). In the ketamine-induced sleeping time test, CPE showed no sedative effects.
Conclusions: *C. pulverulents* exerts moderate cytotoxic effects in human cancer cells, moderate anti-inflammatory and antinociceptive effects. *C. pulverulentus* induces antinociceptive effects without inducing sedation.

1. Introduction

Preclinical and clinical studies with medicinal plants have led to the development of phytomedicines that are now commercially available. Some examples include: *Galanthus spp./ Leucojum spp.* (Galanthamine), *Croton lechleri* Muell. Arg. (Crofelemer), *Euphorbia peplus* L. (Peplin), and *Cannabis sativa* L. (Sativex) (Heinrich, 2010). Therefore, the continuous searching of new phytomedicines is highly desirable.

In Mexican traditional medicine, many plants have been used for the empirical treatment of several diseases for centuries. Nevertheless, scientific studies that would validate their medicinal properties remain to be performed.

Abbreviations: (BNP), Buprenorphine; (CDDP), Cisplatin; (CNZ), Clonazepam; (CPE), Ethanol extract from *Costus pulverulentus* stem; (GC–MS), Gas chromatography–mass spectrometry; (IND), Indomethacin; (MTT), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; (NPX), Naproxen; (PBMC), Peripheral blood mononuclear cells; (TPA), 12-O-tetradecanoylphorbol-13-acetate

* Correspondence to: Cerro de la Venada S/N, Col. Pueblito de Rocha C.P. 36040, Guanajuato, Gto.
** Correspondence to: Romualdo del Campo 501, Fraccionamiento Rafael Curiel, Ciudad Valles, San Luís Potosí, 79060, México.
E-mail addresses: angeljosabad@ugto.mx (A.J. Alonso-Castro), candy.carranza@uaslp.mx (C. Carranza-Álvarez).

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The Costus genus encompasses approximately 70 species. The American species of Costus genus are most abundant in regions of heavy rainfall, high humidity, and temperature. Among these species, *Costus pulverulentus* C. Presl (Costaceae), (synonyms: *Costus ruber* C. Wright ex Griseb., and *Costus formosus* C.V. Morton), commonly known as "caña de jabalí" (wild boar cane), "caña agría" (sour cane) or "spiral ginger", is a plant distributed from Mexico, Central America, Caribbean, and along the west coast from South America. This plant is found from sea level to 540 m elevation. In traditional medicine, *C. pulverulentus* is used for the empirical treatment of inflammation, pain, fever, stomach ache, gastric ulcers, kidney problems, cancer, diabetes, and gonorrhea (Lentz et al., 1998; Leonti et al., 2001; Avelino-Flores, 2005; Andrade-Cetto, 2009; Zavala-Ocampo et al., 2013). For the empirical treatment of cancer, inflammation and pain, the stem and/or aerial parts of *Costus pulverulentus* is prepared by maceration of approximately 30 g of raw material and 500 ml of ethanol during at least 4 days. The maceration is rubbed onto the body. An alternative way of preparation is by the infusion of approximately 30 g of raw material in 500 ml of water. The infusion is taken three times per day (Avelino-Flores, 2005; Zavala-Ocampo et al., 2013; Granda-Calle, 2015; personal communication).

As part of our continuous investigation regarding the validation of pharmacological effects in Mexican medicinal plants, this study describes, for the first time, the chemical composition, as well as the cytotoxic, anti-inflammatory, antinociceptive, and sedative activities of an ethanol extract from *C. pulverulentus* stem.

2. Materials and methods

2.1. Reagents

Naproxen sodium (NPX) was obtained from Tripharma (Distrito Federal, Mexico), whereas clonazepam (CNZ) was from Tecnofarma (Mexico City, Mexico). Indomethacin (IND), hydrogen peroxide, N, O-bis (trimethylsilyl) trifluoroacetamide (BSTFA), MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, and 12-O-tetradecanoylphorbol-13-acetate (TPA) were acquired from Sigma Aldrich (St. Louis, MO, USA). Buprenorphine (BNP) was from Schering Plough Mexico (Distrito Federal, Mexico). DMEM and fetal bovine serum (FBS) were from GIBCO BRL (Grand Island, NY). Cisplatin (CDDP) was from Accord Farma (Distrito Federal, México).

2.2. Plant material

Samples of *C. pulverulentus* were collected, on June 23, 2015, in the municipality of Aquismón, San Luis Potosi State, Mexico (21° 58′52.7″ north latitude and 98° 58′38.3″ west longitude). A voucher specimen (SLPM-033070) was deposited at the herbarium Isidro Palacios of the Instituto de Investigación de Zonas Desérticas, Universidad Autónoma de San Luis Potosí (SLPM). Jose Garcia Perez (UASLP) identified the plant material.

2.3. Preparation of ethanol extract from Costus pulverulentus stem (CPE)

Powdered dried stem of *C. pulverulentus* (35 g) were extracted with ethanol (315 ml) using a closed system of microwave assisted extraction (Multiwave 3000 Solv, Anton Paar, Graz, Austria). The extraction was performed at 100 °C and 90 bars during 17 min. The extract was filtered and concentrated under reduced pressure to dryness and the residue was protected from light.

2.4. Sample preparation and gas chromatography–mass spectrometry (GC–MS) analysis

Approximately 10 mg of CPE was transferred 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.

Analysis of the CPE was performed on 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) was used for the analysis. 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 maintained 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 acquired in the scan mode in the *m*/*z* range 33–800. The identification of compounds was performed by comparing their mass spectra with data from NIST 11 (National Institute of Standards and Technology, USA), WILEY 09.

2.5. Cell lines and culture conditions

Cell lines of colorectal adenocarcinoma (SW-620), breast carcinoma (MDA-MB231), lung adenocarcinoma (SKLU1), and prostate carcinoma (PC-3) were maintained in DMEM supplemented by 7% fetal bovine serum and antibiotics (100 U/ml penicillin and 100 pg/ml streptomycin). All cell lines were obtained from ATCC (Manassas, VA, USA). All cell cultures were grown at 37 °C, in a humidified atmosphere of 5% CO_2.

2.6. Isolation of peripheral blood mononuclear cells (PBMC)

(PBMC) were obtained from healthy volunteers as described previously (Yañez et al., 2004). PBMC were seeded in RPMI medium supplemented with 10% fetal bovine serum and antibiotics (100 units/ml penicillin and 100 pg/ml streptomycin). Cell cultures were grown at 37 °C and 5% CO_2. All the procedures carried out in this study were approved by the Research Ethic Committee of Dixpertia (CEID-001B-2016), which is authorized and registered by the Mexican legislation (Official Mexican Standard NOM-012-SSA3-2012).

2.7. Animals

Male Balb/c mice weighing 25–30 g, from the Universidad of Guanajuato animal facility, were housed in isolated cages at 24 °C under a light-dark cycle of 12:12. The animals were supplied with food and water *ad libitum*. The experiments were carried out according to Official Mexican Norm NOM 062-ZOO-1999 (Technical specifications for the production, care, and use of laboratory animals). The research also followed the Guidelines on Ethical Standards for Investigations of Experimental Pain in Animals (Zimmerman, 1983).

2.8. Toxicity assays

2.8.1. Comet assay

PBMC were treated with the vehicle (DMSO 0.1%), with different concentrations of CPE (10, 50, 200, 500, and 1000 μg/ml) or 70 μM H_2O_2 (positive control) during 5 h. DNA damage in PBMC was assessed by comet assay (Singh et al., 1988). The olive tail moment [(tail mean − head mean) × tail %DNA/100] was determined using the software Comet score version 1.5 (TriTek, Corp, Summerduck, VA). The comet image magnification was 40 ×.