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The antinociceptive effects of a standardized ethanol extract of the *Bidens odorata* Cav (Asteraceae) leaves are mediated by ATP-sensitive K^+ channels

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ARTICLE INFO

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Tramadol hydrochloride (PubChem CID:63013)
glibenclamide (PubChem CID:3488)
naproxen sodium (PubChem CID: 23681059)
naloxone (PubChem CID:5464092)
ketorolac (PubChem CID:3826)

ABSTRACT

Ethnopharmacological relevance: Bidens odorata Cav (Asteraceae) is used for the empirical treatment of inflammation and pain.
Aim of the study: This work evaluated the *in vitro* and *in vivo* toxicity, antioxidant activity, as well as the anti-inflammatory and antinociceptive effects of an ethanol extract from *Bidens odorata* leaves (BOE).
Materials and methods: The *in vitro* toxicity of BOE (10–1000 μg/ml) was evaluated with the comet assay in PBMC. The *in vivo* acute toxicity of BOE (500–5000 mg/kg) and the effect of BOE (10–1000 μg/ml) on the level of ROS in PBMC were determined. The *in vivo* anti-inflammatory activity of BOE was assessed using the TPA-induced ear edema in mice. The antinociceptive activities of BOE (50–200 mg/kg p.o.) were assessed using the acetic acid and formalin tests. The antinociceptive mechanism of BOE was determined using naloxone and glibenclamide.
Results: BOE lacked DNA damage, and showed low *in vivo* toxicity (LD_{50} > 5000 mg/kg p.o.). BOE inhibited ROS production (IC_{50} = 252.13 ± 20.54 μg/ml), and decreased inflammation by 36.1 ± 3.66%. In both antinociceptive test, BOE (200 mg/kg) exerted activity with similar activity than the reference drugs.
Conclusion: B. odorata exerts low *in vitro* and *in vivo* toxicity, antioxidant effects, moderate *in vivo* anti-inflammatory activity, and antinociceptive effects mediated by ATP-sensitive K^+ channels.

1. Introduction

Bidens odorata Cav (Asteraceae), commonly known as "Mozoquelite" or "aceitilla", is a herb that is about 10–80 cm in height and grows in warm, semi-dry, and temperate climates. *B. odorata*, native to Mexico and Guatemala, is used in the traditional medicine of Mexico for the empirical treatment of diarrhea, bronchitis, cough, fever, stomachache, and pain, among other ailments (Bello-González et al., 2015). This study describes, for the first time, the chemical composition, the *in vitro* and *in vivo* toxicity, antioxidant activity, as well as the anti-inflammatory and antinociceptive effects of an ethanol extract from *B. odorata* leaves.

2. Materials and methods

2.1. Plant material and preparation of ethanol extract of Bidens odorata leaves (BOE)

Samples of *Bidens odorata* were collected at the Mercado de Sonora, Mexico City. A voucher specimen (FEZA-15784) was deposited at the herbarium of the Facultad de Estudios Superiores Zaragoza, Universidad Nacional Autonoma de México (FEZA). Powdered dried

Abbreviations: BOE, ethanol extract from Bidensodorata leaves; BSTFA, N, O-bis (trimethylsilyl) trifluoroacetamide; NPX, Naproxen; OTM, Olive tail moment; PBMC, Peripheral blood mononuclear cells; ROS, Reative oxygen species; TPA, 12-O-tetradecanoylphorbol-13-acetate; TRD, Tramadol

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leaves of *Bidens odorata* (35 g) were extracted with ethanol as previously described (Alonso-Castro et al., 2016).

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

Approximately 100 µl of BOE was concentrated to dryness and dissolved in 20 µl of pyridine, later 80 µl of N, O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) were added. At the end of reaction, 100 µl of isooctane was added and transferred to a glass tube. A GC System Agilent Technologies model 7890A coupled to an electron impact ionization mass spectrometer Agilent technologies model 5975 was used for the analysis. The chromatography phase was done in an Agilent J & W DB-1MSUI capillary column (60 m × 250 µm × 0.25 µm). The data obtained were examined with the software Mass Hunter Workstation version B.06.00 (Agilent Technologies, Inc.). Mass spectra library software and Database NIST MS Search version 2.0 (National Institute of Standards and Technology, 2008) was used for compounds identification.

2.3. Animals

Male Balb/c mice (25–30 g), obtained from the Universidad of Guanajuato animal facility. The experiments were carried out according to national and international standards (Zimmerman, 1983; NOM 062-ZOO-1999).

2.4. Toxicity assays

2.4.1. Comet assay

PBMC, isolated and seeded as previously described (Alonso-Castro et al., 2016),were treated with DMSO 0.1% (as the vehicle group), BOE ranging in concentrations from 10 to 1000 µg/ml, or 70 µM H_2O_2 (as the positive control) for 5 h. The comet assay was used to evaluate the DNA damage in PBMC (Singh et al., 1988). The olive tail moment (OTM) was calculated as follows: OTM = [(tail mean−head mean) × tail % DNA/100]. The software Comet score version 1.5 (TriTek, Corp, Summerduck, VA) was used in the analysis of the comets.

2.4.2. Acute toxicity test

The acute toxicity of the BOE was evaluated following the procedure described previously (Lorke, 1983).

2.5. Intracellular reactive oxygen species (ROS) assay

The level of intracellular ROS was determined using the fluorescent probe H_2DCFDA. Approximately 5 × 10^5 PBMCs/well were exposed to BOE (10–1000 µg/ml) for 3 h. After incubation, cells were washed and then incubated with 70 µM H_2O_2 at 37 °C for 30 min. PBMC were incubated with 50 mM H_2DCFDA at 37 °C. The degree of fluorescence was analyzed using a cytometer.

2.6. Acute inflammation with 12-O-tetradecanoylphorbol-13-acetate (TPA)

The induction of ear edema by TPA application in mice was carried out according to the method described by De Young et al. (1989). The following treatments were topically administered 30 min prior to the application of (TPA): vehicle (ethanol), BOE at 2 mg/ear, and indomethacin (IND) at 2 mg/ear, as the positive control. After 6 h, the mice were sacrificed. Sections (6 mm in diameter) from the right (treated) and the left (non-treated) ears were weighed

2.7. Antinociceptive activity

2.7.1. Acetic acid-induced constrictions

The induction of constrictions by the administration of acetic acid (i.p.) was performed according to the method described by Koster et al. (1959). The following treatments were orally administered 60 min prior to the administration of acetic acid: vehicle (saline solution), BOE (50–200 mg/kg), and 100 mg/kg naproxen (NPX), as the positive control. Each group consisted of 8 animals. Mice were administered with 10 ml/kg (i.p.) of an acetic acid solution (1% v/v). Abdominal constrictions were counted for 30 min.

2.7.2. Formalin test and study of the mechanism of action

The formalin-induced paw edema in mice was performed according to the protocol described by Hunskaar and Hole (1987). The following treatments were administered 60 min prior to the administration of formalin: saline solution p.o. (the vehicle group), 30 mg/kg i.p. tramadol (TRD), 10 mg/kg ketorolac, and BOE (50–200 mg/kg p.o.). Each group consisted of 8 animals. Additional groups were pre-treated with naloxone (2 mg/kg i.p.), a non-selective opioid receptor antagonist, or glibenclamide (10 mg/kg i.p.), an ATP-sensitive K+ channel inhibitor, 15 min before administration of 200 mg/kg CPE, 30 mg/kg TRD, or 10 mg/kg ketorolac. Mice received 30 µl of 2.5% formalin solution into the subplantar space of the right hind paw. After formalin injection, the time of paw licking was counted at first phase (0–15 min) and second phase(15–45 min).

2.8. Statistical analysis

All experimental values are expressed as the mean ± the standard error media (S.E.M) of two independent experiments. Statistically significant differences were identified by ANOVA test with post hoc Tukey test for paired data. The level of $p \leq 0.05$ was used to determine statistical significance. All calculations were performed using the Graph Pad Prism V.3 software system (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Chemical composition of BOE

The ratio of the herbal substance to the native herbal drug preparation (DER native) was 24:1. The identification of compounds found in BOE and retention times are shown in Table 1.

3.2. Genotoxicity

The positive control 70 µM H_2O_2 induced DNA damage in PBMC, whereas BOE (10–1000 µg/ml) lacked genotoxicity in PBMC.

3.3. Acute toxicity

The LD_{50} values of BOE in mice were higher than 5000 mg/kg i.p. and higher than 5000 mg/kg p.o.

3.4. Inhibition of ROS production

BOE inhibited ROS production in PBMC with IC_{50} = 252.13 ± 20.54.

3.5. BOE exerts moderate anti-inflammatory effects in acute TPA-induced ear edema

IND (2 mg/ear) and BOE (2 mg/ear) significantly ($p \leq 0.05$) decreased the inflammation in mice ears by 63.8 ± 2.47%, and 36.1 ± 3.66%., respectively, in comparison to the vehicle group.

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