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Evaluation of antinociceptive effects of *Crassocephalum bauchiense* Hutch (Asteraceae) leaf extract in rodents

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ABSTRACT

Ethnopharmacological relevance: The leaves of *Crassocephalum bauchiense* have long been used in traditional Cameroonian medicine for the treatment of epilepsy, pain, inflammatory disorders, arthritis and intestinal pain.
Aim of the study: In this study, we attempted to identify the possible antinociceptive action of the aqueous extract and the alkaloid fraction prepared from the leaves of *Crassocephalum baucheiense*.
Materials and methods: Using acetic acid induced abdominal constrictions, formalin-, capsaisin- and glutamate-induced nociception, and hot plate assay procedures, the antinociceptive effects of the aqueous extract and the alkaloid fraction was assessed after oral administration in mice. Morphine sulfate was used as reference analgesic agent. Mice were submitted to the rota-rod task and open-field test in order to assess any non-specific muscle-relaxant or sedative effects of the extracts of *Crassocephalum bauchiense*. Male and female Swiss mice were used to assess acute toxicity of these extracts.
Results: The aqueous extract and the alkaloid fraction of *Crassocephalum bauchiense* produced a significant antinociceptive effects in the acetic acid, formalin, glutamate, capsaicin and hot plate tests. These antinociceptive effects of *Crassocephalum bauchiense* were significantly attenuated by pretreatment with naloxone. The extracts of *Crassocephalum bauchiense* did not alter the locomotion of animals in the open-field or rotarod tests, which suggest a lack of a central depressant effect. The animals did not exhibit any acute toxicity to the aqueous extract and the alkaloid fraction, so it was not possible to calculate the LD_{50}.
Conclusion: The results confirm the popular use of *Crassocephalum bauchiense* as an antinociceptive, and contribute to the pharmacological knowledge of this species because it was shown that the aqueous extract and the alkaloid fraction of *Crassocephalum bauchiense* produced dose related antinociception in models of chemical and thermal nociception through mechanisms that involve an interaction with opioidergic pathway.

1. Introduction

The genus *Crassocephalum* belongs to the very large and widely distributed Asteraceae family in the tribe Senicioneae. It has been reported that *Crassocephalum* genus consists of nearly 24 species (Wagner et al., 1999). Many of these species are used widely as food additives or in traditional medicine, prompting phytochemical investigations that have in turn uncovered a variety of alkaloids, diterpenes and coumarins (Asada et al., 1985; Kongsaeree et al., 2003; Mohamed-Elamir et al., 2008). Well

Abbreviations: AE, aqueous extract; AF, alkaloid fraction; Fr, fraction; ID_{50}, dose of extract necessary to reduce the response by 50% relative to the control value; LD_{50}, median lethal dose; NIH, National Institutes of Health; S.E.M., standard error of the means; v, volume; w, weight.

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accepted that many plant-derived compounds possess analgesic and anti-inflammatory properties. Recent studies have shown that the labdane diterpene of the air-dried parts of *Crassocephalum mannii* has anti-inflammatory activity through its cyclooxygenase inhibitory activity (Heras et al., 2007; Liua et al., 2006; Mohamed-Elamir et al., 2008). Although the potent activity of non-steroidal anti-inflammatory drugs is noteworthy, they have also many severe adverse effects. The aim is therefore to identify medicinal plant agents with very little side effects as substitute therapeutics.

Crassocephalum bauchiense Huch (Asteraceae) is commonly used in traditional medicine in the north of Cameroon. The leaf extract has been used to treat several diseases, including epilepsy, pain, arthritis, intestinal pain and colics (Arbonnier, 2000). A decoction of the leaves has been reported to be useful in relieving bronchitis and the attendant fever. According to Cameroonian traditional healers, the plant is also effective in cases of cerebral deficit, behavioral disturbances in mentally retarded children, inflammatory disorders and neuropathic pain. However, there is no detailed study on the alleged antinociceptives properties of this medicinal plant. To provide scientific evidence for its antinociceptive activities known in folk medicine, the main purpose of the present study was to evaluate the effects of the aqueous extract and the alkaloid fraction of *Crassocephalum bauchiense* leaves using different assays of the chemical nociception and the thermal model of nociception.

2. Materials and methods

2.1. Plant material

Fresh leaves of *Crassocephalum bauchiense* used in this study were harvested in the Mawi area of Ngaoundéré, Cameroon in July 2007. Botanical identification was performed at the National Herbarium, Yaoundé, Cameroun. Voucher specimen No. 7954/SRF/Cam was deposited at the Yaoundé herbarium.

2.2. Extraction and fractionation

For the preparation of the aqueous extract of *Crassocephalum bauchiense*, 100 g of dried and powdered leaves was soaked in 1000 ml of distilled water for 72 h and filtered. The filtrate was then dried in the oven (Gallenhamp®, England) at 60 °C to give an extract with a 7.5% yield (w/w).

The dried and powdered leaves of *Crassocephalum bauchiense* (1000 g) were extracted with acetone/H_2O (7/3; 5 l) at room temperature. The combined extracts were evaporated in vacuo to afford a dark residue (652.45 g). The residue was suspended in warm water (1 l) and then extracted successively with ethyl acetate (0.5 l ×3) and n-butanol (0.5 l ×3), and concentrated to give residue A (207.13 g) and B (385.51 g), respectively. The latter was resolved in warm water (1 l), acidified with 1 mol/l HCl to pH between 4 and 5, and extracted with $CHCl_3$ (0.5 l ×3). The aqueous layer was neutralized with 1 mol/l NaOH to pH 9–10 and extracted with $CHCl_3$ (0.5 l ×3) once again and concentrated in vacuo to obtain the crude base (158.64 g; 41.15%). The crude base (158.64 g) was subjected to chromatography column on silica gel eluted using a gradient of $CHCl_3$:CH_3OH: 28% NH_4OH (from 50:1:0.1 to 3:1:0.1, v/v) to afford twelve fractions: Fr. I (16.54 g; 10.42%), Fr. II (16.34 g; 10.30%), Fr. III (12.68 g; 7.99%), Fr. IV (180.30 mg; 0.11%), Fr. V (2.44 g; 1.53%), Fr. VI (92.40 mg; 0.06%), Fr. VII (75.40 mg; 0.05%), Fr. VIII (41.27 mg; 0.03%), Fr. IX (39.20 mg; 0.02%), Fr. X (4.23 g; 2.66%), Fr. XI (135.20 mg; 0.08%) and Fr. XII (63.50 mg; 0.04%). The fraction Fr. II (16.30 g) was chromatographed on silica-gel chromatography column using a gradient of petroleum ether:Me_2CO:28%NH_4OH (from 30:1:0.2 to 2:1:0.1, v/v) to give Fr. XIII (47.14 mg; 0.28%), Fr. XIV (32.75 mg; 0.02%), Fr. XV (21.63 mg; 0.01%), Fr. XVI (18.71 mg; 0.01%), Fr. XVII (32.13 mg; 0.02%) and Fr. XVIII (29.34 mg; 0.18%). The aqueous extract and the alkaloid fraction of *Crassocephamum bauchiense* were dissolved in saline 0.90% containing dimethyl sulfoxide 2% (vehicle) and subjected to the following pharmacological studies.

2.3. Laboratory animals

Swiss albino mice (20–25 g) of either sex were used in this study. All animals were housed in a controlled environment, with free access to food and water and were maintained on a 12 h/12 h day/night cycle. Each animal was used only once. The investigation conforms to the Guide for the Care and Use of Laboratory Animal published by the US National Institutes of Health (NIH No. 85-23, revised 1996). In all the experimental studies each group consisted of six to eight animals and received approval of the local ethical committee for animal handling and experimental procedure.

2.4. Drugs and chemicals

Acetic acid, dipyrone, formalin, glutamate, morphine sulfate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Naloxone was obtained from Arkopharma (Carros, France). Dipyrone, glutamate and morphine were prepared in saline (0.90% NaCl) and contained 2% dimethyl sulfoxide. In all the pharmacological test naloxone was administered 15 min before the administration of the extracts of *Crassocephalum bauchiense*. Formalin stock solution was prepared in phosphate buffer solution (phosphate buffer solution concentration in mM: NaCl 137, KCl 2.70 and phosphate buffer 10). Acetic acid was prepared in saline (0.90% NaCl). Capsaicin stock solution (10^{-2} M) was prepared by successively dissolving capsaicin in 10% ethanol, 10% Tween 80 and 80% NaCl 0.90%. The stock solution was further diluted in saline upon administration to 80 mg/ml.

2.5. Pharmacological analysis

2.5.1. Abdominal constrictions induced by acetic acid

The aqueous extract of *Crassocephalum bauchiense* (20, 40, 80 and 160 mg/kg, p.o.), the alkaloids fractions from *Crassocephalum bauchiense* (Fr. I, Fr. II, Fr. III, Fr. IV, Fr. V, Fr. VI, Fr. VII, Fr. VIII, Fr. IX, Fr. X, Fr. XI, Fr. XII, Fr. XII, Fr. XIV, Fr. XV, Fr. XVI, Fr. XVII and Fr. XVIII; 40 mg/kg, p.o.), the aqueous extract + naloxone (160 mg/kg, p.o. + 1 mg/kg, i.p.), the alkaloid fraction + naloxone (Fr. XVI, 40 mg/kg, p.o. + 1 mg/kg, i.p.), morphine sulfate (positive control, 5 mg/kg, s.c.) or vehicle (10 ml/kg, p.o.) were administered 1 h prior to acetic acid treatment. Acetic acid (0.60%, 10 ml/kg) was injected i.p. and the number of abdominal constrictions (writhings) during the following 30 min period was observed (Taïwe et al., 2011). A significant reduction in the number of abdominal constrictions by any treatment compared with vehicle treated animals was considered as an antinociceptive response.

2.5.2. Formalin-induced nociception

The formalin test was carried out as described by Tjolsen et al. (1992). Mice were given the aqueous extract of *Crassocephalum bauchiense* (20, 40, 80 and 160 mg/kg, p.o.), the alkaloid fraction from *Crassocephalum bauchiense* (Fr. XVI; 5, 10, 20 and 40 mg/kg, p.o.), the aqueous extract + naloxone (160 mg/kg, p.o. + 1 mg/kg, i.p.), the alkaloid fraction + naloxone (Fr. XVI; 40 mg/kg, p.o. + 1 mg/kg, i.p.), morphine sulfate (positive control, 5 mg/kg, s.c.) or vehicle (10 ml/kg, p.o.) 1 h before injecting formalin. Pain was induced by injecting subcutaneously in the right hind paw 20 μl of 2.50% formalin (0.90% formaldehyde). The amount of time spent licking the injected paw was measured and considered as an indication of pain. The first phase of the nociceptive