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Original Article

Neuropharmacological effects of the ethanolic extract of *Sida acuta*

Dora M. Benjumea[a,*], Isabel C. Gómez-Betancur[a], Julieta Vásquez[a], Fernando Alzate[b], Andrea García-Silva[c], José A. Fontenla[c]

[a] *Ophidism/Scorpionism Program, University Research Center, University of Antioquia, Medellin, Colombia*
[b] *Institute of Biology, Faculty of Natural Sciences, University of Antioquia, Medellin, Colombia*
[c] *Laboratory of Pharmacology of the Central Nervous System, Department of Pharmacology, Faculty of Pharmacy, University of Santiago de Compostela, Santiago de Compostela, Spain*

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ABSTRACT

Sida acuta Burm. f., Malvaceae, is regarded as astringent, tonic and useful in treating urinary diseases and blood disorders, bile, liver and as treatment for nervous diseases. Different methods were developed: sodium pentobarbital-induced sleeping time, anxiolytic activity, test for muscle-effects, pentylenetetrazole (PTZ)-induced seizures, effect on normal body temperature. All experiments were performed in an isolated room with 12/12 h light/dark cycles at 22 ± 1 °C. The effects described in this work for *Sida acuta* are according to what is known in traditional medicine, where is used as sedative agent. At the higher doses used in this work (500 and 1000 mg/kg), the *Sida acuta* extract reduced the latency time (T1) and increased the sleeping time (T2) induced by pentobarbital, indicating a sedative and hypnotic effect of the plant's extract. The extract of *Sida acuta* shows an increase in open arm exploration (anxiolytic activity). Results obtained in the rota-rod test showed that only the elevated dose (750 mg/kg) of *Sida acuta* extract, acutely administered, promotes significant changes, at 60 and 120 min post-administration, in the time of permanence in the rod. The ethanolic extract from the leaves and stems of *Sida acuta*, causes effects on the central nervous system in experimental animals.

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Introduction

Malvaceae is a family of flowering plants containing over 200 genera with close to 2300 species. The main economic use of Malvaceae plants is as a source of natural fibers, the family is also used for food, beverages, timber, in traditional medicine and in horticulture. The largest genera are *Hibiscus* (300 species), *Streculia* (250 species), *Dombeya* (225 species), *Pavonia* (200 species) and *Sida* (200 species) (Rizk and Soliman, 2014). *Sida acuta* Burm. f., locally known as "escobabosa" and "kuala" in Cuna, "samampiisa" in Burkina Faso (Nadembega et al., 2011), "arbre à balai" in French and "zon-raaga" in Mooré, is a perennial shrub widely distributed in the subtropical regions, found in bushes, in farms, around habitations. Grows abundantly on cultivated fields, waste areas, roadsides and highways, in damp or dry, between 0 and 1800 masl (Mejía et al., 1994; Karou et al., 2007).

In Colombia, the whole plant of *S. acuta* is widely used in traditional medicine of the Indigenous Tribes *Embera*, *Wounaan*, *Cunas* and *Katíos*, and in others regions of Antioquia, prepared as drinks, ointements and external baths against snakebite (Otero et al., 2000a,b,c; Vásquez et al., 2015). It is also used as stomachic, diaphoretic and antipyretic. It is regarded as astringent, tonic, useful in urinary diseases treatment (diuretic) and also blood disorders (stops bleeding), bile and liver and nervous diseases treatment (sedative) in Indian traditional medicine (Sreedevi et al., 2009; Govindarajan, 2010); in Mexico, smoked as marihuana substitute, and it is also used to treat asthma, renal inflammation, colds, gonorrhea, fever, bronchitis, malaria, diarrhea, headache, dysentery, abortion, breast cancer, skin diseases, hemorrhoids, insects' bites, erectile dysfunction, elephantiasis, rheumatism and ulcers (Napralert database, Bhardwaj et al., 2011; Kumar et al., 2012). It is claimed to have aphrodisiac properties (Govindarajan, 2010). The root's juice is applied to wounds and the barks are used for measles (Adetutu et al., 2011; Allabi et al., 2011). In Nigeria, *S. acuta* is one of the plants most commonly used for the treatment of hypertension, using its leaves, seeds and stems in different preparations (Gbolade, 2012).

The phytochemical screening of *S. acuta* species revealed the presence of alkaloids such as vasicine, ephedrine and cryptolepine (the main alkaloid in the plant) (Prakash et al., 1981; Karou et al., 2005), saponosides (unspecified type or hemolytic), coumarins,

* Corresponding author at: Departamento de Farmacia, Facultad de Química Farmacéutica, Universidad de Antioquia, Medellín, Colombia.
E-mail: dora.benjumea@udea.edu.co (D.M. Benjumea).

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steroids (ecdysterone, β-sistosterol, stigmaterol, ampesterol), tannins, phenolic compounds (evofolin-A, and B, scopoletin, loliolid and 4-ketopinoresinol, polyphenol, sesquiterpene and flavonoids (Konaté et al., 2010; Napralert database).

The tested pharmacological activities of the *S. acuta* involve stimulating smooth muscle, abortifacient, antiulcer, antiyeast, diuretic, antiplasmodial, antimicrobial, antiophidian, antioxidant, hepatoprotective, insecticidal, larvicidal-repellent and cytotoxic activities (Otero et al., 2000a; Karou et al., 2003; Banzouzi et al., 2004; Ekpo and Etim, 2009; Akilandeswari et al., 2010; Pieme et al., 2010; Upadhyay et al., 2010; Adeniyi et al., 2010; Ahmed et al., 2011; Koudouvo et al., 2011). Meanwhile, *S. acuta* extracts impact in the cardiovascular system function in zebrafish embryos (Kannan and Prakash, 2012). Additionally, it has been proved that the aqueous-acetone and ethanolic extracts of *S. acuta* leaves have analgesic activity and antidepressant-like properties tested in different animal models, proving that the plant contains psychoactive substances (Konaté et al., 2012; Ibironke et al., 2014).

Sida cordifolia (L.) extract has been reported to have central nervous system activity in experimental animals (Franco et al., 2005) and *Sida tiagii* Bhandari has been reported to have anxiolytic and anticonvulsant activity (Datusalia et al., 2008). In the present study, assesses the neuropharmacological properties of *S. acuta* leaves and stems, including sedative, anticonvulsant and anxiolytic activities.

Materials and methods

Plant material

Sida acuta Burm. f., Malvaceae, was collected from Medellín, in a University of Antioquia (Colombia), at 1525 m altitude above sea level, in February 2012. It was identified by Dr. Fernando Alzate Guarín, Institute of Biology, University of Antioquia (Medellín, Colombia), where voucher specimens have been deposited with the number 5111 in the collection.

Preparation of Sida acuta *extracts*

Leaves and stems (60:40) of *S. acuta* were air-dried in an oven at 40 °C for 4 days, then the dry plant was cut and ground to powder by mechanical milling. The dried powdered plant material was submitted to a continuous extraction in a Soxhlet extractor for 5 days using 100% ethanol as solvent. The solvent was then eliminated by vacuum distillation in a rotary vacuum evaporator (Büchi R – 124, Flawil, Switzerland), and it was lyophilized, representing an extraction yield of 3.1% of the dry material.

Animals

Male CD1 albino Swiss mice (weighing 22 ± 2 g) were obtained from the Central Animal House (University of Santiago de Compostela) and housed in groups of four, eight or twelve in standard Makrolon® cages (215 mm × 465 mm × 145 mm). The animals received standard laboratory chow (Scientific Animal Food and Engineering (SAFE), Augy, France) and tap water ad lib until the beginning of the experiments. Three days after laboratory arrival, both the housing and handling during experimentation were carried out according with the standards established in the directive 2010/63UE of the European Parliament and the Council and the Galician (Decreto 296/2008, DOGA number 11, January 16, 2009) and Spanish (Real Decreto 53/2013, BOE number 34, February 8, 2013) legislation on animal experimentation.

Drugs

All drugs were bought from Sigma–Aldrich (USA) or Merck (Germany). Drugs or the crude extract of *Sida acuta* were prepared immediately before use and orally administered (*per os*) in a total volume of 0.1 ml/10 g body weight (b.w.). Diazepam (DZP), sodium pentobarbital (PTB) and Pentylenetetrazole (PTZ) were dissolved in saline solution (NaCl 0.9%). The crude extract of *S. acuta* was suspended in a 1% (weight/volume) sodium carboxymethylcellulose (NaCMC) dispersion at doses of 50, 100, 300, 500 and 1000 mg/kg.

Pharmacological evaluation

All experiments were performed in an isolated room with 12/12 h light/dark cycles at 22 ± 1 °C, at the same time of day, in order to avoid potential variations caused by circadian rhythms.

The evaluation of time in open arms, number of entries, traveled distance, velocity, and another evaluated parameter in elevated plus maze (EPM) test and the open field test (OFT) were made with the video computerized animal observation system EthoVision V. 3.16 (Noldus Information Technology, Wageningen, The Netherlands).

All the procedures follow the guidelines of the research ethics committee at the University of Santiago de Compostela, according with the guidelines of the European Community Council Directive 86/609.

Sodium pentobarbital-induced sleeping time

The effect of *S. acuta* extract on pentobarbital sleeping time was performed in six groups of mice ($n = 4$). Four groups received graded doses of the extract (100, 300, 500 and 1000 mg/kg *p.o.*). One group received diazepam (5 mg/kg *i.p.*), while animals in the control group were administered NaCMC (0.1 ml/10 g *p.o.*). Thirty minutes post-treatment, sodium pentobarbital (40 mg/kg *i.p.*) was administered to each mouse. The elapsed time between the administration of pentobarbital until the loss of the righting reflex was recorded as the sleep latency (T1), and the time elapsed between the loss and recovery of the righting reflex (T2) was recorded as the sleep time (Carlini and Burgos, 1979; Ramírez et al., 1998; Wambebe, 1985).

Anxiolytic activity

Elevated plus maze (*EPM*): The EPM test was introduced in the research of new drugs with potential anxiolytic activity (Hanledy and Mithani, 1984). Experimental groups of four mice were treated with vehicle (NaCMC 1%, 0.1 ml/10 g *p.o.*), *Sida acuta* (50, 100, 300 and 500 mg/kg *p.o.*) or diazepam (1 mg/kg *i.p.*).

The elevated plus maze (EPM) is made of wood painted black. It has two arms 60 cm × 10 cm, arranged opposite to each other, and enclosed by walls 35 cm height. It also has two open arms of the same size and without flanges. The four arms are interconnected by a central square of 10 cm × 10 cm, forming a cross, and elevated to a distance of 75 cm from the ground. The device is illuminated by four independent tubes, arranged in a cross, located in the ceiling of the room, and the animal behavior was observed with a video camera disposed on the center.

Sixty minutes after the treatment, animals were placed individually at the center of the elevated plus maze with their nose facing the direction of one of the enclosed arms, and observed for 5 min (Pellow et al., 1985; Lister, 1987; Yemitan and Adeyemi, 2003). The maze platforms and walls were thoroughly cleaned with 70% ethanol between sessions and allowed to dry. Total residence time in open or closed arms, time ratio spent in open arms, and the number of entries (frequency) in the open or closed arms were recorded.

Open field test (*OFT*): This method is used to evaluate possible sedative or stimulating activities of animals (Carlini et al., 1986),

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