Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Ethnopharmacology 105 (2006) 187–195
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Toxicological evaluation of an ethanolic extract from *Chiococca alba* roots

Valeria E. Gazda [a,b], Maria Regina Gomes-Carneiro [a], Nancy S. Barbi [b], Francisco J.R. Paumgartten [a,*]

[a] *Laboratory of Environmental Toxicology, Department of Biological Sciences, National School of Public Health, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil*
[b] *Department of Clinical and Toxicological Analysis, School of Pharmacy, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*

Received 11 June 2005; received in revised form 28 September 2005; accepted 15 October 2005
Available online 17 November 2005

Abstract

The roots of *Chiococca alba* have been employed to treat rheumatic disorders and for other therapeutic purposes in Brazil and elsewhere. This study was undertaken to evaluate the toxicological properties of an ethanolic extract from *Chiococca alba* roots (EE), including mutagenicity in the *Salmonella* assay and acute and subacute toxicity to mice. Single oral doses of EE caused hypoactivity, but no deaths were noted up to the highest dose tested (2000 mg/kg). EE (500 mg/kg p.o.) reduced mouse locomotion in the open field test. EE was markedly more toxic when given by intraperitoneal (i.p.) and subcutaneous (s.c.) routes. Acute approximate lethal doses (ALD) were 125 mg/kg (males) and 250 mg/kg (females) and 250 mg/kg (both sexes) by i.p. and s.c. routes, respectively. Deaths after single doses were preceded by hypoactivity, ataxia and lethargy. Repeated administration of EE by gavage for 14 days caused no deaths. Activity of liver monooxygenases (pentoxy- and ethoxyresorufin-*O*-dealkylases) was not altered by repeated treatment with EE (2000 mg/kg/day p.o.). Administration of EE by the i.p. route for 14 days decreased weight gain and caused anemia, neutrophilia and deaths. The no-observed-adverse-effect level (NOAEL) for subacute treatment by the i.p. route was as low as 15.6 mg of EE/kg body weight (wt)/day. EE was not mutagenic in the *Salmonella*/microsome assay with TA100, TA98, TA97a and TA1535 strains. In summary, EE was not mutagenic and presented a low acute and subacute toxicity by the oral route. Toxicities by parenteral routes, however, were more pronounced.

Keywords: Rubiaceae; *Chiococca alba*; Mutagenicity; Central nervous system depression; Acute toxicity; Repeated-dose toxicity

1. Introduction

Chiococca alba (L.) Hitchc (Rubiaceae) is a tropical plant that has arching branches, dark green foliage, cream-colored blooms and snow-white berries and grows either as a scrambling shrub or as a woody vine that often climbs taller vegetation (Pio-Corrêa, 1931; Cruz, 1932). It is native to the New World, where it is found in South Florida (USA), the Bahamas, Mexico and through Central and South America up to Paraguay and Southeastern Brazil. *Chiococca alba* is also known by a variety of common names, such as West Indian milkberry, snow-berry, David's root, '*bejuco de berac*', '*buenda*', '*liane des sorciers*' and in Brazil, as '*cainca*', '*cipó-cruz*' '*raiz-de-frade*' and '*cruzeirinha*' (Pio-Corrêa, 1931; Cruz, 1932).

The decoction and infusions of roots and other parts of *Chiococca alba*, alone or mixed with other plant species, have long been used in folk medicine as emetic, purgative, diuretic, antidiarrheic, antipyretic, tonic, aphrodisiac, as well as for a variety of other purposes, such as to treat rheumatism, snakebites, flatulence, delayed menstruation, dementia, alcoholism, nervousness and kidney troubles (Costa, 1932). A decoction of the whole-plant was also reported to be effective as laxative and as a remedy for gonorrhea, skin infections and rheumatism (Cruz, 1932). It is of note that at least until the first half of the 20th century, *Chiococca alba* was listed in the Brazilian pharmacopeia as well as in those of several European countries (Schapoval et al., 1983). In Brazil, a medicine for rheumatic

Abbreviations: ALD, approximate lethal dose; CNS, central nervous system; EE, ethanolic extract; EROD, ethoxyresorufin-*O*-deethylase; HCT, hematocrit; HGB, hemoglobin; LD_{50}, lethal dose 50%; NOAEL, no-observed-adverse-effect level; PROD, pentoxyresorufin-*O*-depenthylase; RBC, red blood cells; WBC, white blood cells; wt, weight

* Corresponding author. Tel.: +55 21 38829044.
E-mail address: paum@ensp.fiocruz.br (F.J.R. Paumgartten).

0378-8741/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2005.10.017

disorders (Rheumoflora®) that contains an alcoholic extract of *Chiococca alba* roots, combined with a whole-plant extract of a fern (*Polypodium lepidopteris*), has been in the phytopharmaceutical market since 1924.

Literature on the chemical composition of *Chiococca alba* is relatively scarce. Two biologically active quinoline alkaloids (El-Abadi et al., 1989), an oleanane-type triterpene (Bhattacharyya and Cunha, 1992), an *ent*-kaurane (Borges-Argáez et al., 1997), an iridoid and a *seco*-iridoid (Carbonezi et al., 1999) and a *nor-seco*-pimarane known as merilactone (Borges-Argáez et al., 2001) have been isolated from *Chiococca alba* roots, while lignans, coumarins and two new keto alcohols (El-Hafiz et al., 1991) have been found in the leaves.

Studies on the pharmacological properties of *Chiococca alba* and its constituents have revealed anti-inflammatory effects (Schapoval et al., 1983), antimicrobial action against *Staphylococcus aureus* (Borges-Argáez et al., 1997) as well as anticancer activity (Carbonezi et al., 1997). Notwithstanding the widespread medicinal use of *Chiococca alba* root extracts in Brazil and elsewhere, as far as the authors are aware, there is no published study on their toxicological properties. It should be borne in mind that tradition in use, by no means, warrants that a medicinal plant is safe, particularly with regard to mutagenicity and carcinogenicity because cause–effect relationships in these areas are rather complex and not easily recognized by the population.

The present study was undertaken to provide data on the safety of an alcoholic extract of *Chiococca alba* roots, including its acute and subacute toxicities by different routes of administration as well as an evaluation of its mutagenicity using the *Salmonella*/microsome assay.

2. Materials and methods

2.1. Plant material

Chiococca alba (synonyms *Lonicera alba, Chiococca racemosa*, *Chiococca brachiata*) roots were collected in the remains of the Atlantic rain forest located in the municipality of Nova Friburgo, Rio de Janeiro, Brazil, between March and October 2001. *Chiococca alba* was identified by Dr. Sebastião Neto, from the Botanical Garden of Rio de Janeiro, Rio de Janeiro, Brazil, and a voucher specimen was deposited (reference number RB 395399) in the Botanical Garden Herbarium.

2.2. Preparation of plant extract

Pulverized dried roots of *Chiococca alba* (2 kg) were initially macerated with 6 l of ethanol for 72 h, and subsequently, Soxhlet-extracted with 2 l of ethanol. The plant ethanol extracts was then concentrated in a rotating evaporator under reduced pressure at 50 °C until all solvent was evaporated. Extraction yield was 86.4 g of ethanol extract residue (EE) per 1000 g of powdered dried roots. EE was kept in a glass dryer with silica gel and protected from light until further use.

2.3. Chemical characterization of the extract

2.3.1. Gas chromatography coupled to mass spectrometry (GC–MSD) analysis

Part of EE (20 g) was fractionated by liquid–liquid partition with hexane (2.1 g), dichloromethane (1.2 g), ethyl acetate (5.7 g), 1-butanol (3.1 g) and water (8 g). The hexane fraction was submitted to silica gel column (80 cm × 1.5 cm) chromatography using hexane/ethyl acetate/methanol gradient to yield 104 fractions. Fractions 39–50 (eluted with hexane/ethyl acetate) were combined, methylated with diazomethane and analysed by using a GC–MSD instrument (Shimadzu model QP5000) equipped with a DB-1 column (30 m × 25 mm, with film of 0.25 μm (J&W Scientific, Rancho Cordova, CA, USA). Gas chromatography conditions were as follows: oven temperature initially, 50 °C rising to 270 °C (5 °C/min); split ratio of 30:1; injector temperature 270 °C; interface 230 °C. Mass spectra were recorded at 70 eV. Identification of constituents was made by comparing fragmentation patterns of sample compounds with those found in the literature (McLafferty and Stauffer, 1989).

2.3.2. High performance liquid chromatograph (HPLC) analysis

One milligram of EE was dissolved in methanol:water (30:70, v/v) and analysed by using a HPLC instrument (Shimadzu) equipped with ultra violet (SPD-10A) and diode array detection (SPD-M10A). Liquid chromatography conditions were as follows: reverse phase octadecylsilane-functionalized silica gel column, 250 mm × 4.6 mm, particle size of 5 μm (LiChrosorb, Merck, Darmstadt, Germany). Mobile phase, linear gradient of methanol:water and flow rate of 1 ml/min. Gradient began with methanol 30% for 10 min (isocratic), 30–40% (10–20 min) and 40–100% methanol (20–60 min).

2.4. Mutagenicity testing

The *Salmonella typhimurium/microsome* assay (tester strains TA97a, TA98, TA100 and TA1535) was performed by the preincubation method, without and with addition of an extrinsic metabolic activation system (S9 mixture), essentially as described by Maron and Ames (1983). All tester strain cultures were prepared from permanents kept in liquid nitrogen. In culture tubes, 100 μl of an overnight grown culture (containing approximately, 1×10^9 to 2×10^9 bacteria ml^{-1}), 100 μl of EE diluted in analytical grade ethanol (70%, v/v), the negative (ethanol 70%) or the positive control substance and 500 μl of phosphate buffer or S9 mixture were pre-incubated at 37 °C with shaking for 20 min. Two milliliters of molten top agar was added to each pre-incubation tube the content of which was then poured onto a plate with minimum glucose medium. Sodium azide (SA 1 μg/plate), 4-nitroquinoline-*N*-oxide (4-NQNO, 1 μg/plate), 2-nitrofluorene (2-NF, 1 μg/plate), 2-aminofluorene (2-AF, 10 μg/plate) and 2-aminoanthracene (2-AA, 1 or 0.5 μg/plate) were employed as positive control substances. SA was dissolved in distilled water, while dimethylsulfoxide (DMSO) was the solvent employed for the remaining mutagens. The S9 mixture was prepared by using lyophilized