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Toxicology, genotoxicity, and cytotoxicity of three extracts of *Solanum chrysotrichum*

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ABSTRACT

Ethnopharmacological relevance: Infusions of *Solanum chrysotrichum* (Schldl.) or "sosa" are employed in Traditional Mexican Medicine for the local and systemic treatment of skin and mucosal infections. Different studies have verified its antifungal effectiveness and therapeutic safety in superficial mycosis caused by dermatophytes or yeasts, and have identified a group of spirostanic saponins, denominated SC-2–SC-6, as responsible for the antifungal activity. Of these, SC-2 is the most active molecule. Electron microscopy studies showed that SC-2 disintegrates cell wall and internal membranes of the fungi studied. In order to continue the systematic study of *Solanum chrysotrichum*, the goal of the present study was to evaluate the toxicity, genotoxicity, and cytotoxicity of the three different extracts of *Solanum chrysotrichum*.
Materials and methods: From the dried leaves of *Solanum chrysotrichum*, we obtained the aqueous, hydroalcoholic, and ethanolic extracts. Saponins (SC-2–SC-6) were quantified by High-performance liquid chromatography (HPLC). For the toxicology study, we formed four groups: three experimental groups, treated with each of the extracts at 1-g/kg doses per os (po) during 4 weeks, and a negative control group treated with the vehicle. For the genotoxicity study, we added another group, which was treated with cyclophosphamide for 1 week. The cytotoxicity study was carried out with international methods and employing the nasopharyngeal cancer (KB) and breast cancer (MDA) cell lines.
Results: The three evaluated extracts did not modify either of the behavioral parameters, and on the hepatic-function biochemical tests (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]), did not showed significant increase on comparing to placebo. The genotoxicity parameters did not exhibit differences between the experimental groups and the placebo (control) group. Histologic analysis showed that the three extracts caused amyloidosis and moderate necrosis in liver, and focal tumefaction in kidney, as well as significant, but clinically irrelevant, elevations of creatinine with the aqueous and hydroalcoholic, but not with the ethanolic, extracts. In addition, the aqueous and ethanolic extracts exhibited interesting cytotoxic activity against the KB cell line.
Conclusions: At the doses administered, the ethanolic extract of *Solanum chrysotrichum* showed a slightly toxic effect on liver and kidney, without biochemical or genotoxic repercussions and with cytotoxic activity against the KB cell line.

1. Introduction

The plant species *Solanum chrysotrichum* Schldl. (Solanaceae), popularly known as "sosa", is employed in Traditional Mexican Medicine, particularly in the highland areas of the state of Chiapas, for local and systemic management of skin and mucosal diseases such as athlete's foot or "mazmorra". For oral administration of *Solanum chrysotrichum*, an infusion with 3 g of dried leaves/L of water per day is prepared (Lozoya et al., 1992). This preparation generates 600 mg of dry extract that, administered to a subject weighing 70 kg, is equivalent to approximately 8.6 mg of extract/kg of weight per day.

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Three clinical studies have evidenced the therapeutic effectiveness of herbal medical products developed from the methanolic extract of *Solanum chrysotrichum* in the treatment of superficial mycotic infections, particularly in *Tinea pedis*, *Pytiriasis capitis* (dandruff), and cervicovaginitis due to *Candida* species (Herrera-Arellano et al., 2003, 2004, 2009). The major compounds contained in the active fractions of *Solanum chrysotrichum* correspond to a group of steroidal saponins denominated SC-1–SC-6, with SC-2 presenting the highest antimycotic activity (minimum inhibitory concentration [MIC] = 12.5, 12.5, 100, and 200 μg/mL against *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Aspergillis niger*, and *Candida albicans*, respectively) (Álvarez et al., 2001; Zamilpa et al., 2002). In electronic microscopy studies, it was evidenced that SC-2 affects the morphology and density of the cell wall and the intracellular membranes, particularly of the nucleus and the mitochondria, of the reference strains and clinical isolates of different species of *Candida, Trichophyton*, and *Microsporum*, concluding that SC-2 possesses fungistatic and fungicidal activity depending on the dose applied (Herrera-Arellano et al., 2007; López-Villegas et al., 2009). To continue the systematic study of *Solanum chrysotrichum*, the goal of the present study was to evaluate the possible toxic activity of the aqueous, hydroalcoholic, and methanolic extracts of *Solanum chrysotrichum* in healthy mice, as well as their genotoxicity and cytotoxicity.

2. Methods and materials

2.1. Plant material

Solanum chrysotrichum leaves were harvested from a controlled culture located in the Xochitepec municipality of Morelos State, Mexico, in may-2006. The plant material was authenticated by Abigail Aguilar, M.Sc., Director of the Mexican Institute of Social Security Medical Herbarium (IMSSM), where two reference samples were deposited with key numbers 13,082 and 13,083.

2.2. Preparation of the extracts8/12/13

The leaves were dried under darkness conditions, at room temperature, during 10 days. Three kg of dry material was triturated in an electric mill (Cutting mills, Pulvex, model 95) until obtaining a uniform particle size (2–5 mm), and this was divided into three equal portions of 1 kg each, which were extracted with different solvents.

One portion of dried and ground plant material was extracted with water (5 L) at 60 °C during 2 h; later, the extract was filtered and concentrated to dryness by means of lyophilization in specialized equipment (Heto Drywinner), obtaining a yield of 33.1% (331.3 g). The second portion of plant material was extracted with ethanol (20%, 5 L) at 60 °C during 2 h; the solvent was immediately removed in two stages, initially by distillation at reduced pressure (Rotary Evaporator Büchi-490; Büchi, Switzerland), and was later taken to total dryness by lyophilization, obtaining a yield of 19.4% (194.6 g). The last extract was obtained by exhaustive maceration with ethanol (5 L × 24 h × 3 times), and the solvent was eliminated by reduced pressure, obtaining a yield of 9.1% (91.2 g).

2.3. Calibration curves

Standard solutions were prepared with every compound: SC-2, SC-3, SC-4, SC-5, and SC-6 were previously isolated from the wild plant (Zamilpa et al., 2002). Calibration curves were constructed with dilutions of 100, 200, 400, and 800 μg/mL in methanol. A volume of 70 μL was injected by triplicate and calibration curves were based on the average peak areas of each chromatogram. The calibration curves showed an R^2 of 0.991 for SC-2–SC-4 and an R^2 of 0.996 for SC-5 and SC-6.

2.4. Quantification of SC-2 and of total saponins

Saponin concentration was evaluated in a Merck Hitachi HPLC system with an AS-2000A autosampler, an L-6200A intelligent pump system controller (DAD System Manager software), and an R1-71 Refraction Index Detector, using a LiChrospher® RP-18 (5-mm) column 125 × 4 mm (Merck) at 25 °C. Saponins SC-2, SC-3, and SC-4 were analyzed with a mobile phase consisting of one solvent mixture acetonitrile/water (55:45, v/v) with a 1.2-mL/min flow rate. Saponins SC-5 and SC-6 were analyzed with an isocratic mobile phase acetonitrile/water (33:67, v/v) with a 1.5-mL/min flow rate. Injection volume was 70 μL.

2.5. In vivo toxicological evaluation

2.5.1. Animals

Experimentation with animals was conducted in compliance with the Official Mexican Norm for the care of experimental animals and with internationally accepted principles for the use and care of laboratory animals (Norma Oficial Mexicana NOM-062-ZOO, 1999). Healthy BALB/c strain female mice (weight, 18 ± 2 g) were housed in plastic translucent cages and maintained under standard bioterium conditions (23 ± 2 °C, 12-h:12-h light–dark cycles) and with free access to water and rodent pellet diet (2018S, Harlan Teklad).

2.5.2. Subchronic toxicity

With Local Ethics Committee approval (Registration R-2005-1701-22), 24 mice were randomly grouped (Groups 1–4) into four of eight animals each. Groups 1–3 were orally administered with 1 g/kg body weight of aqueous, hydroalcoholic, and ethanolic extracts of *Solanum chrysotrichum* resuspended in distilled water (0.8 mL/kg) on daily basis for 28 days. Group 4 was treated similarly to those of the extracts except that they received only the vehicle at the same volume. For the genotoxicity study, one extra group was included, considering this as the positive control, to which cyclophosphamide (Sigma-Aldrich; 10 μg/kg, i.p.) was administered daily for 1 week.

Clinical signs of toxicity or alterations in behavior were observed at least once a day throughout the 28 days of dosing based on the Irwin test (Sadraei et al., 2006). On day 29 of the administration period, under deep anesthesia (sodium pentobarbital, 50 mg/kg, intraperitoneally [ip]), a blood sample was collected by retro-orbital puncture to quantify creatinine, as well as the enzymatic activities of the alanine and aspartate aminotransferases (ALT and AST). Later, the animals were sacrificed with an overdose of anesthesia, and liver and kidney were collected and maintained in 10% neutral buffered (pH 7.4) formalin. Tissues were embedded in paraffin and subjected to hematoxylin–eosin (H&E) staining; pathological studies and interpretation were performed by Dr. Laura Romero of the Faculty of Veterinary Medicine and Zootechnics, UNAM, in the Department of Pathology.

2.5.3. Evaluation of hepatotoxicity and nephrotoxicity

Blood samples were collected in 1.5-mL tubes without heparin and centrifuged (955 × *g*, 10 min) to separate the serum. By means of colorimetric techniques, employing Wiener Lab reagents (Argentina), we quantified creatinine, ALT, and AST, following the manufacturer's instructions.

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