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Antitumoral activity of sesquiterpene lactone diacethylpiptocarphol in mice

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ABSTRACT

Ethnopharmacological relevance: Sesquiterpene lactones are organic compounds derived from plants that exhibit anti-inflammatory and antitumor activities being one of the locking mechanisms of action of NF-kB pathway and synthesis of cytokines such as IL-1 and TNF- α.
Aim of the study: The overall objective of the present study was to evaluate the antitumor activity of the sesquiterpene lactone diacethylpiptocarphol (DPC) from *Vernonia scorpioides* (Lam.) Pers. in animal models Ehrlich tumors that has shown antitumor activity.
Materials and methods: The antitumor effects of Intraperitonial administration of DPC (5 mg/kg/day) were evaluated in Balb/c mice on Ehrlich tumors, and further the body weight, the ascitic cells volume measurement, solid tumor measured and TNF-α level was determinate.
Results: Balb/c mice bearing Ehrlich tumors were treated daily with 5 mg/kg/day of the DPC for one week and showed no tumor in the peritoneum after treatment, besides presenting a reduction of TNF-α cytokine. Also the solid tumor reduced size after one week of treatment with DPC.
Conclusions: Sesquiterpene lactone DPC, isolated from *Vernonia scorpioides* showed antitumor activity because it decreased the size of the solid tumor and abolished the ascitic tumor development, and also did not affect the mice body weight, however the treatment reduced the TNF-α level in mice.

1. Introduction

The cancer study leads to the development of new molecules that act specifically in tumor cells by blocking or inhibiting their molecular targets. The anticancer properties of plants have attracted a great deal of interest. Extensive research has been carried out to characterise the molecular mechanisms of anticancer activities as well as their potential use as chemopreventive and chemotherapeutic agents. *Vernonia scorpioides* (*V. scorpioides*) (Lam.) Pers (family, Asteraceae), popularly known as "Enxuga, Erva-de-São-Simão and Piracá" (Correa, 1978), is a Brazilian herb that grows in poor and deforested soils all over the country (Leite et al., 2002). It is a common occurrence of herbaceous plant in the Atlantic Forest (Freire et al., 1996; Lorenzi, 2000). It is a medicinal plant used in Brazil as an anti-inflammatory and antitumor agent, and it is also used to treat some skin conditions (Reitz, 1980), it is used topically by native people to treat a variety of skin conditions, such as allergies, skin parasites, irritations, skin injuries, itching and chronic wounds, including ulcers of the lower limbs (Pagno et al., 2006). Several species of genus Vernonia (Asteraceae) are used in traditional medicine to treat various ailments (Johri and Singh, 1997). Studies focusing on the anti-cancer (Izevbigie et al., 2004), anti-pyretic (Gupta et al., 2003), anti-malarial activities of several Vernonia species have been published (Abosi and Raseroka, 2003; Muregi et al., 2003) and anti-inflammatory (Mazumder et al., 2003; Iwalewa et al., 2003).

Studies of *Vernonia scorpioides* indicated the antitumor activity of these molecules, both *in vitro* and *in vivo* (Khalil et al., 2005; Sweeney et al., 2005; Buskuhl et al., 2009) and also its anti-inflammatory activity (Rauh et al., 2011). Studies with sub-fractions of the crude extract obtained from the leaves of *Vernonia scorpioides* plant showed anti-tumor activities (Kreuger et al., 2009). There are many studies correlating the anti tumoral activity from sesquiterpenes lactones and the deactivation of the NF-kB *via* (Kreuger et al., 2011). NF-kB is a cytoplasmic protein that translocates to the nucleus and regulate many genes whose proteins have proinflammatory and antiapoptotic activities (Zhao et al., 2012). There has been limited research about the cytotoxicity of DPC from *Vernonia scorpioides*; however, our previous

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study demonstrated that the extracts from the aerial parts of this species exhibited cytotoxicity against different cancer cells lines; thus the present study was focused on the antitumor activity of the isolated compound from the aerial parts of this Asteraceae species as well as on the evaluation of its activity on cytokines synthesis. Considering the traditional use of *Vernonia scorpioides* in various ailments include antitumoral activities, we investigated the intraperitoneal and also in solid tumor the effect of the lactone DPC of *Vernonia scorpioides* on Ehrlich tumor models in mice.

2. Material and methods

2.1. Plant material

Aerial components (leaves and flowers) of *Vernonia scorpioides* (Lam.) Pers. (Asteraceae) were collected in November 2014 from wild specimens in a "restinga" forest (a distinct type of coastal tropical and subtropical moist broadleaf forest) in Navegantes (SC), and the identity of the specimens was confirmed by Dra. Ana Claudia Araújo (Universidade do Vale do Itajaí, Santa Catarina, Brazil). Voucher specimens [M. Biavatti 11 (15/03/01)] were deposited at the Herbário Barbosa Rodrigues (Itajaí, Santa Catarina, Brazil).

2.2. Preparation of sesquiterpene lactone diacethylpiptocarphol (DPC)

Approximately four kg of fresh leaves of *Vernonia scorpioides* were extracted with 98% ethanol in the course of two weeks. The extract was then reduced to one third of its original volume, and a liquid-liquid partition was performed with solvents of increasing polarities, yielding the following fractions: Hexane (38.87 g), dichloromethane (5.81 g), ethyl acetate (13.95 g), the aqueous residue was passed through an adsorption resin XAD-4 yielding the XAD-4 fraction (44.10 g).

The dichloromethane fraction was applied in a normal phase silica column under a gradient from 20% to 50% acetone in hexanes, yielding a semi-pure fraction (1.89 g). This fraction was further applied to a Sephadex LH-20 separation system conditioned in 50% methanol in acetone, yielding a pure fraction (1.53 g) that was further identified as diacetylpiptocarphol by 1D and 2D NMR and Mass Spectrometry in agreement with previously published data (Catalan et al., 1986; Odonne et al., 2011), and the purity was superior to 90% by HRMS using a QT of detector (Waters Acquity system coupled to a Xevo G2S mass detector). The TIC and PDA view of DCP together with HRMS are presented in Fig. 1 A and B respectively.

2.3. Animals

Male Balb/c mice (8–10 wk of age) were kept under a 12-h light/dark cycle (lights on at 07:00 h) with free access to laboratory chow and tap water. Experiments were performed during the light phase of the cycle. The experimental procedures were previously approved by the Committee on the Ethical Use of Animals (CEUA/UNIVALI 042), where the study was carried out, and were conducted in accordance with Brazilian regulations on animal welfare.

2.4. Ascitic cells from Ehrlich's tumor

The Ehrlich tumor cells were maintained in the ascitic form by passages in syngenic Balb/c mice weekly, with transplantation of 5×10^6 tumor cells intraperitoneally (i.p.). The ascitic fluid was removed by opening the belly and collecting all the fluid using a sterile syringe. Ascitic tumor cell counts were carried out in a Neubauer hemocytometer, using the Trypan blue dye exclusion method. The animals used for the experiment received i.p. 200 µl of a suspension containing 5×10^6 tumor cells, according to a previous study (Matsuzaki et al., 2003).

2.5. Solid tumor

Balb/c mice (18 animals) in which solid tumors were induced in the left using Ehrlich cells. 15 ml of 10^6 ascitic tumor cells were diluted in 50 ml of saline. The 20 ul solutions were injected into the left paw of each mouse. After five days the animals showed solid tumors in the left leg. The animals were divided into three groups to start treatment. The DPC group received injection solution for seven days in the tumor mass containing 5 mg/kg DPC. 5-FU group was given seven days the tumor mass injectable solution containing 20 mg/kg of 5-FU chemotherapy. The control group received 20 ul of saline. After seven-day treatment, the animals were sacrificed and their paws measured using a digital caliper (Starret 727).

2.6. Treatment of animals

The experiments were performed according to Christina et al. (2003), after intraperitoneally implanting the tumor cells, three groups of mice (6 mice per group) were treated with DPC (5 mg/kg) diluted in saline solution 0.9% and treated intraperitoneal (i.p.), the positive control group received 5-fluoro-uracil (5-FU) in saline solution 0.9% (20 mg/kg i.p) and the negative control group received saline solution

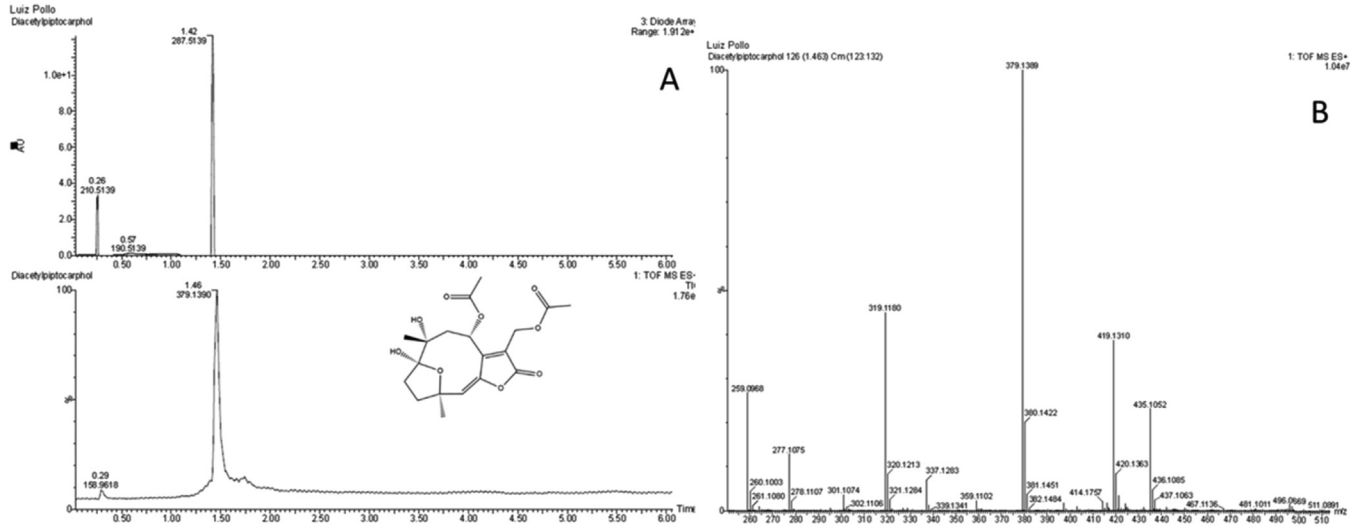

Fig. 1. TIC and PDA view of the isolated DCP (A) and its HRMS (B).