Journal of Ethnopharmacology 215 (2018) 1–13

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Baccharis trimera protects against ethanol induced hepatotoxicity *in vitro* and *in vivo*

Ana Carolina Silveira Rabelo[a], Karine de Pádua Lúcio[a], Carolina Morais Araújo[a], Glaucy Rodrigues de Araújo[a], Pedro Henrique de Amorim Miranda[a], Ana Claudia Alvarenga Carneiro[c], Érica Milena de Castro Ribeiro[c], Breno de Melo Silva[c], Wanderson Geraldo de Lima[b], Daniela Caldeira Costa[a,*]

[a] *Laboratory of Metabolic Biochemistry (LBM), Department of Biological Sciences (DECBI), Center of Research in Biological Sciences (NUPEB), Federal University of Ouro Preto (UFOP), Ouro Preto, MG 35400-000, Brazil*
[b] *Laboratory of Morphopathology (LMP), Department of Biological Sciences (DECBI), Center of Research in Biological Sciences (NUPEB), Federal University of Ouro Preto (UFOP), Ouro Preto, MG 35400-000, Brazil*
[c] *Laboratory of Biology and Biotechnology of Microorganisms, Department of Biological Sciences (DECBI), Center of Research in Biological Sciences (NUPEB), Federal University of Ouro Preto (UFOP), Ouro Preto, MG 35400-000, Brazil*

ARTICLE INFO

Chemical compounds studied in this article:
Ethanol (PubChem CID): 702
Quercetin (PubChem CID): 5280343
Ascorbic acid (PubChem CID): 54670067
Trolox (PubChem CID): 40634

Keywords:
Ethanol
Hepatotoxicity
Oxidative stress
Baccharis trimera
Carqueja

ABSTRACT

Ethnopharmacological relevance: *Baccharis trimera* has been traditionally used in Brazil to treat liver diseases.
Aim of the study: To evaluate the protective effect of *Baccharis trimera* in an ethanol induced hepatotoxicity model.
Materials and methods: The antioxidant capacity was evaluated in vitro by the ability to scavenged the DPPH radical, by the quantification of ROS, NO and the transcription factor Nrf2. Hepatotoxicity was induced in animals by administration of absolute ethanol for 2 days (acute) or with ethanol diluted for 28 days (chronic). The biochemical parameters of hepatic function (ALT and AST), renal function (urea and creatinine) and lipid profile (total cholesterol, triglycerides and HDL) were evaluated. In addition to antioxidant defense (SOD, catalase, glutathione), oxidative damage markers (TBARS and carbonylated protein), MMP-2 activity and liver histology.
Results: *Baccharis trimera* promoted a decrease in ROS and NO, and at low concentrations promoted increased transcription of Nrf2. In the acute experiment it promoted increase of HDL, in the activity of SOD and GPx, besides diminishing TBARS and microesteatosis. Already in the chronic experiment *B. trimera* improved the hepatic and renal profile, decreased triglycerides and MMP-2 activity, in addition to diminishing microesteatosis.
Conclusion: We believe that *B. trimera* action is possibly more associated with direct neutralizing effects or inhibition of reactive species production pathways rather than the modulation of the antioxidant enzymes activity. Thus it is possible to infer that the biological effects triggered by adaptive responses are complex and multifactorial depending on the dose, the time and the compounds used.

1. Introduction

Alcohol is a psychoactive substance and its abusive consumption is associated with various health problems worldwide (Lívero and Acco, 2016). The liver is responsible for metabolizing the alcohol and its abusive consumption is associated with hepatotoxicity. One of the main mechanisms responsible for ethanol-induced hepatotoxicity is the oxidative stress (Han et al., 2016; Xu et al., 2003; Caro and Cederbaum, 2004). The ethanol is metabolized to acetaldehyde mainly by alcohol dehydrogenase and subsequently oxidized to acetate by acetaldehyde dehydrogenase (Brocardo et al., 2011). However when ethanol is consumed in excess, it is predominantly metabolized to acetaldehyde via cytochrome P450 (CYP2E1) (Beier and McClain, 2010) that is involved in the generation of reactive oxygen species (ROS) (Cederbaum, 2015; Abdelmegeed et al., 2013). Thus, the increase of ROS associated with impaired antioxidant capacity leads to an oxidative stress situation. Recent studies have suggested that oxidative stress induced by ethanol has an important role in the pathogenesis and progression of alcoholic liver disease (Song et al., 2006; Senthil-Kumar et al., 2012; Jin et al., 2013). In this regard, the antioxidants would have a major role in the

* Corresponding author.
E-mail address: dani.caldeiracosta@gmail.com (D.C. Costa).

https://doi.org/10.1016/j.jep.2017.12.043
Received 3 January 2017; Received in revised form 13 December 2017; Accepted 26 December 2017
Available online 28 December 2017

inhibition of reactive species and in the antioxidant enzymes response, decreasing the hepatic injury (Das and Vasudevan, 2006). ROS is neutralized by enzymatic antioxidants, such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione reductase (GR) and nonenzymatic as glutathione, vitamins and dietary antioxidants (22). In this sense, it is known that a major regulator of cellular redox balance is erythroid 2-related factor 2 (Nrf2) (Tang et al., 2014). Under oxidative stress, the Nrf2 translocates into the nucleus and interacts with antioxidant response element (ARE), promoting the antioxidant enzymes expression (Zhang et al., 2013). Even today there are no effective therapies to minimize or reverse the progression of alcoholic liver injury; therefore, the search for new therapies becomes necessary to prevent the disease progression. Medicines plants and their active phytochemicals have attracted the attention of researchers as potential agents against alcoholic liver injury because of their antioxidant potential and the few side effects (Ding et al., 2012). The popular use of medicinal plants has increased exponentially in recent decades; however, there is still little scientific evidence to validate the use of these plants as curative or preventive of different diseases (Stickel and Shouval, 2015). A medicinal plant used in popular culture is *Baccharis trimera*, widely distributed in South America (Paul et al., 2009). In Brazil, this plant is popularly known as carqueja and used to treat diseases such as diabetes, inflammatory processes and liver diseases (Abad and Bermejo, 2007). Various biological effects are attributed to this plant such as hypoglycemic (Oliveira et al., 2011), hepatoprotective (Soicke and Leng-Peschlow, 1987), antioxidant (Pádua et al., 2010) and anti-inflammatory (Paul et al., 2009). More recently, our research group described that the hydroethanolic extract of *B. trimera* inhibits the ROS production by decreasing the protein expression and the PKC enzymatic activity and down-regulation of sub-unit of $p47^{phox}$ NOX phosphorylation (de Araújo et al., 2016), demonstrating the potential of *B. trimera* in modulating of important signaling pathways related to oxidative stress. Based on these evidences and on the fact that there are no drugs available that can reverse hepatic injury and that oxidative stress is involved in aggravation in this injury our goal was to verify the effect of *B. trimera* on protection against ethanol induced hepatotoxicity in vitro and in vivo model.

2. Materials and methods

2.1. Reagents

The DMEM culture, dimethyl sulfoxide reagents (DMSO), 3- (4,5-dimethyazol 2yi) −2,5 diphenyltetrazolium bromide (MTT), gelatin from porcine skin, vitamin C, quercetin, trolox, glutathione reductase kit were purchased from Sigma-Aldrich (St. Louis, MO). Fetal bovine serum (FBS), the kit for quantitation of ROS (Kit Image-iT ™ Live Green Reactive Oxygen Species), quantification of NO (DAF-FM diacetate-4-amino) and the lipofectamine were purchased from Invitrogen. Kits for cholesterol total, triacylglycerides, HDL, creatinine, urea, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) dosage were purchased from Bioclin. Plasmid pRL-TK, pGL37 and Dual Luciferase assay System kit were purchased from Promega.

2.2. Plant material and chemical analyses

The aerial parts of *Baccharis trimera* were collected in Ouro Preto city, in Minas Gerais state, Brazil. The specimens were authenticated and deposited at the Herbarium José Badini (UFOP), OUPR 22.127. After identification, the plant's aerial parts were dried in a ventilated oven, pulverized and stored in plastic bottles. To obtain the hydroethanolic extract, approximately 100 g of the plant was extracted with distilled water and 70% ethanol at a ratio of 1:1 for 24 h. The solids were removed by vacuum filtration and the solvent was removed by a rotary evaporator (Pádua et al., 2010). The phytochemical characterization of the *Baccharis trimera* hydroethanolic extract was previously performed by our group in another study, where five flavonoids and nine chlorogenic acids were identified by LC-DAD-ESI-MS (de Araújo et al., 2016).

2.3. In vitro tests

2.3.1. DPPH radical-scavenging activity

The percentage of antioxidant activity of each substance was assessed by DPPH free radical assay, according to Brand-Williams et al. (1995). Hydroetanolic extract was diluted in methanol 80% and dilutions were realized to obtain the concentrations (25–500 μg/mL), in the same way, quercetin was diluted and the concentrations were obtained (25–500 μM). The standard curve was performed with the reference antioxidant Trolox (6-hydroxy-2,5,7,8- tetramethylchrome −2-carboxylic acid). The solution absorbance at 515 nm was determined. Methanol (80%) was used as a blank. The antioxidant activity was determined by the decrease in the DPPH absorbance and the percent inhibition was calculated using the following equation:

$$\%\ \text{antioxidant activity} = (1 - \text{ASample } 515/\text{AControl}515) \times 100$$

2.3.2. Cell culture

Hepatocyte carcinoma (HepG2) cell line was acquired from the Cell Bank from the Federal University of Rio de Janeiro (UFRJ) and was cultured in DMEM medium supplemented with 10% FBS, 1% glucose, 1% glutamine, 100 U/mL penicillin and 100 lg/mL streptomycin in a humidified atmosphere containing 5% CO2 in air at 37 ˚C.

2.3.3. Cell viability assay

Cell viability was determined using colorimetric MTT (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay as described previously (Fotakis and Timbrell, 2006). In brief, HepG2 cells (1×10^5) were cultured in 96 well-plates with or without different hydroethanolic extract concentrations of *B. trimera* (5–600 μg/mL), our positive control-quercetin (5–600 μM) and ethanol (5–800 mM) for 24 h. After incubation, medium was removed and 200 μL of 5 mg/mL MTT solution was added and incubated at 37 ˚C for further 1 h. Subsequently, 100 μL of dimethylsulfoxide (DMSO) was added to dissolve formazan crystals and the absorbance was measured at 570 nm using microplate reader. The cell viability percentage was calculated based in the formula: (absorbance of treated cells/absorbance control) × 100. The control was assigned 100% viability.

2.3.4. ROS and NO production

In brief, $2{,}5 \times 10^4$ of cells were cultured in white 96 well-plates with two different *B. trimera* concentrations (10 and 50 μg/mL) or quercetin (10 and 50 μM). After an incubation of 3 h, the medium was removed and 200 mM of ethanol was added with 50 μM of carboxi-H2DCFDA (for ROS production) or 10 μM of DAF-FM (for NO production). The plate was incubated for 24 h and, then, the medium was removed and 100 μL of HANKS was added. The reading was obtained in VICTOR reader, using the 485 nm for excitation microwave, and 535 nm for emission microwave.

2.3.5. Luciferase reporter assay

In this assay the reporter gene system is used, which encodes proteins that are easily detected, quantified and differentiated from endogenous proteins. For this, a kit Dual Luciferase assay System was used, from Promega. Briefly, 1.5×10^5 HepG2 cells were plated in a 24-well plates and incubated for 24 h. The supernatant was discarded and DMEM High Glucose (HG) medium was added without fetal bovine serum (SFB) and incubated for further 24 h. After the 24-h incubation time, transfection was performed with 100 μL per well of mix (500 ng of lipofectamine, 100 ng of pRL-TK, 400 ng of pPGL37 and medium HG). Six hour incubation was carried out and, then, the supernatant discarded and 500 μL of DMEM with 5% FBS was added. After 1 h, the