Journal of Applied Biomedicine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Applied Biomedicine

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

Monoterpene alpha-terpinene induced hepatic oxidative, cytotoxic and genotoxic damage is associated to caspase activation in rats

Matheus D. Baldissera[a,*], Carine F. Souza[b], Geisa S. Dolci[b], Thirssa H. Grando[a], Michele R. Sagrillo[c], Rodrigo A. Vaucher[c], Sônia C.A. da Luz[a], Sérgio O. Silveira[d], Marta M.M.F. Duarte[d], Thiago Duarte[d], Aleksandro S. da Silva[e], Silvia G. Monteiro[a,*]

[a] Department of Microbiology and Parasitology, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil
[b] Department of Physiology and Pharmacology, UFSM, Santa Maria, RS, Brazil
[c] Laboratory of Cell Culture, Centro Universitário Franciscano, Santa Maria, RS, Brazil
[d] Department of Biochemistry, UFSM, Santa Maria, RS, Brazil
[e] Department of Animal Science, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil

ARTICLE INFO

Article history:
Received 15 December 2016
Accepted 12 January 2017
Available online xxx

Keywords:
Apoptosis
Cell viability
DNA
Monoterpenes

ABSTRACT

The aim of this study was to investigate the occurrence of toxic effects in liver tissue of rats treated with α-terpinene. All treatments were intraperitoneally administered at doses of 0.5, 0.75 and 1.0 mL kg^{-1} during 10 days. Liver samples were collected and assessed by histopathological analysis, caspases −1, −3 and −8 assay, biomarkers of hepatic damage and determination of oxidant/antioxidant status (thiobarbituric acid-reactive substances (TBARS), catalase (CAT), superoxide dismutase (SOD), reactive oxygen species (ROS), glutathione S-transferase (GST) and glutathione peroxidase (GPx)). Additionally, the cytotoxic and genotoxic effects were evaluated by comet assay. An increase was observed on TBARS levels and GPx activity on the hepatic tissue. Instead, CAT and SOD activities decreased in rats treated with a dose of 1.0 mL kg^{-1} of α-terpinene. Concomitantly, ROS levels increased and GST levels decreased in rats treated with α-terpinene at doses of 0.5, 0.75 and 1.0 mL kg^{-1}. Also, there was an increase in frequency of damage, damage index and caspases, while cell viability decreased in rats treated with α-terpinene. Alanine aminotransferase and aspartate aminotransferase increased in rats treated with 1.0 mL kg^{-1} of α-terpinene. Therefore, α-terpinene induces oxidative stress, cytotoxic and genotoxic effects in liver tissue involving the caspases activation.

Introduction

The characterization of plant bioactive molecules is an important research area since many of them present pharmacological or biological activities that provide therapeutic benefits in treating human diseases (Watkins et al., 2015). Natural plant products are extensively used based on the view that they are non-toxic and safe (Asiimve et al., 2014; Dicson et al., 2015). However, a comprehensive analysis of safety issues for the majority of natural products and bioactive molecules has not been fully established, since reports demonstrate toxicity and collateral effects (Park et al., 2010; George, 2011).

Monoterpenes are amongst the main compounds found in essential oils of citrus fruits, vegetables, spices and plants. Monoterpenes are a class of volatile terpenes characterized by strong odors. In the past years, many biological and pharmacological activities were attributed to monoterpenes, such as antioxidant, anti-tumor, antiviral and anti-nociceptive properties, intensifying their use and consumption (Bakkali et al., 2008; Kamatou et al., 2013). Particularly, α-terpinene (1-isopropyl-4-methyl-1,3-cyclohexadine) is a monoterpene found in the essential oils of a large variety of useful and aromatic plants such as *Melaleuca alternifolia* (Baldissera et al., 2014), *Chenopodium ambrosioides* (Brahim et al., 2015) and *Murraya* spp (You et al., 2015). Recently, the repellent effect of α–terpinene against *Tribolium castaneum* has been demonstrated (You et al., 2015), as well as the trypanocidal action against *Trypanosoma evansi* (Baldissera et al., 2016).

* Corresponding authors.
E-mail addresses: matheusd.biomed@yahoo.com.br (M.D. Baldissera), sgmonteiro@uol.com.br (S.G. Monteiro).

http://dx.doi.org/10.1016/j.jab.2017.01.002
1214-021X/

Oxidative stress results from an imbalance between reactive oxygen species (ROS) production and the antioxidant activities such as catalase (CAT), superoxide dismutase (SOD), glutathione-S-transferase (non-enzymatic) (GST) and glutathione peroxidase (GPx) (Schafer and Buettner, 2001). Excess of ROS can damage lipids, fatty acids, proteins and DNA, leading to structural and functional disruption of the cell membrane, inactivation of enzymes and cell death (Galazyn-Sidorczuk et al., 2009; Moshahid Khan et al., 2012). It has been shown that oxidative stress is a central mechanism of cellular damage that affects all tissues, and free radicals generated by some natural compounds are known to cause cellular and DNA damage (Da Silva et al., 2016).

It has been suggested that oxidative stress has a critical role in tissue damage (Khan et al., 2011). Oxidative damage, inflicted by excess of ROS, has been proposed as a possible mechanism of action for DNA damage, implicated in much pathologies (Loft and Poulsen, 1996; Wang et al., 2011). The cells and tissues are continuously attacked by ROS, which are produced in large amounts by chemical agents (Moller et al., 1996). The oxidative damage to DNA by ROS results in DNA base modifications and single- double-strand breaks, that if not repaired may result in mutations (Fearon, 1997). Therefore, ROS are not only implicated in the etiology of disease states, but the resulting DNA damage may be a direct contributor to deleterious biological consequences. Normally, the cells in tissues are protected by the antioxidant system in order to maintain redox homeostasis. The enzymes CAT, SOD, GR and GSH play an important role during this process by scavenging ROS or preventing their formation (Veerappan et al., 2004). However, when the excess of ROS impairs antioxidant defenses or exceeds the scavenging ability of the antioxidant defense system, oxidative stress, and injury may be unavoidable.

The relationship between induction of apoptosis and oxidative stress is well established by many authors (Ma et al., 2014; Akal et al., 2014). According to Bai and Meng (2005) the signaling pathways leading to apoptosis involve the sequential activation of cysteine protease known as caspase. The caspase-2, −3, −6, −7, −8, −9 and −10 are involved in the regulation and execution of programed cell death, i.e., apoptosis (Bergeron et al., 1998). These proteases are separated into two general subcategories, based on their entry into the apoptotic cascades, either as initiator (caspases-2, −8, −9 and −10) or as effector (caspases-3, −6 and −7) caspases (Park, 2012). In cellular response to cytotoxic drugs, DNA damage or ROS, the pathway is activated during disruption of mitochondria, which initiates the signaling pathway via caspase-9 activation. The activation of caspases −8 and −9 result in the activation of their major effector caspase-3 (Decordier et al., 2008; Suen et al., 2008).

Bioactive compounds extracted from plants are often perceived as “natural” and therefore free from side effects (Shin et al., 2013). However, oxidative stress and DNA damage is mediated by ROS production that may be caused by these compounds, suggesting the need of a comprehensive analysis of safety issues (Gao et al., 2015). Therefore, the aim of this study was to investigate the occurrence of oxidative stress, cytotoxic and genotoxicity effects, and apoptosis in liver tissue of rats treated with α-terpinene.

Materials and methods

Chemicals

α-terpinene with 85% of purity was purchased from Sigma-Aldrich Corporation (St. Louis, United States).

Animals

The experiment was conducted using 90-days-old Wistar rats (female, outbred strain, heterogenic, conventional, weighing 230 ± 30 g). Animals were kept in a separate animal room, on a 12/12 h light/dark cycle, with lights on at 7:00a.m., at room temperature (22 ± 2 °C), and with free access to food and water. All animals were submitted to a period of 15 days for adaptation. Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Santa Maria, Brazil, under protocol number 2249060515.

Experimental design

The 24 animals were assigned into four groups with six animals each. Animal grouping was set randomly, as follows: the control group received saline solution (vehicle) intraperitoneally; and the other groups received three different doses (0.5, 0.75 and 1.0 mL kg^{-1}) of α-terpinene diluted in saline solution (1:10 v/v) administered intraperitoneally for 10 consecutive days.

Sample collection and preparation of tissue homogenate

Animals were anesthetized with isoflurane in an anesthetic chamber. Blood collection was performed by cardiac puncture, using a syringe and a needle 25 × 7, 10 days after the beginning of the treatment. Blood collected was stored in tubes with EDTA (2 mL) for hematological analysis, and in tubes containing gel tab (6 mL) for obtainment of serum and posterior determination of biochemical parameters. Thereafter, animals were euthanized by decapitation, following recommendations of the Ethics Committee. Subsequently, fragments of liver were collected to assessment of oxidative stress parameters and to cytotoxic, genotoxic, caspases and histology analysis. Protein content in liver homogenate was determined by the method described by Lowry et al. (1951).

Liver was homogenized (1:10 w/v) in a glass potter with Tris-HCl buffer (10 mM, pH 7.4) and centrifuged at 2000 × *g* for 10 min. Aliquots of the supernatants were stored at −20 °C.

Oxidative stress parameters

Thiobarbituric acid reactive substances (TBARS)

As an index of lipid peroxidation, we used TBARS formation during an acid-heating reaction as previously described (Ohkawa et al., 1979) with modifications. For this, 200 μL of homogenized tissue supernatant (1:10 w/v) were mixed with 500 μL of 2.5 M acetic acid pH 3.4, 500 μL of 0.8% thiobarbituric acid, 200 μL of 8.1% sodium dodecyl sulfate (SDS) and 100 μL of Milli-Q water. This mixture was then heated in a boiling water bath for 2 h. A malondialdehyde (MDA) solution was used as reference standard. TBARS were determined by the absorbance at 532 nm and were expressed as malondialdehyde equivalents (nmol MDA/mg of protein).

ROS production

The 2′7′-dichlorofluorescein diacetate (DCF-DA) levels were determined via the DCFH oxidation assay as an index of the peroxide production in the cellular components. This experimental method of analysis is based on the deacetylation of the DCFH-DA probe, and its subsequently oxidation by reactive species into DCF, a highly fluorescent compound (Halliwell and Gutteridge, 2005). The ROS levels in liver homogenate were determined using the DCFH-DA method (Lawler et al., 2003). The supernatant of liver homogenate was added to a medium containing Tris-HCl buffer (10 mM; pH 7.4). The absorbance of the samples was measured by a spectrophotometer at an emission wavelength of 522 nm.

Please cite this article in press as: M.D. Baldissera, et al., Monoterpene alpha-terpinene induced hepatic oxidative, cytotoxic and genotoxic damage is associated to caspase activation in rats, J. App. (2017), http://dx.doi.org/10.1016/j.jab.2017.01.002