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Nrf2-dependent neuroprotective activity of diterpenoids isolated from *Sideritis* spp

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ABSTRACT

Ethnopharmacological relevance: The species of the genus *Sideritis* are extensively used in the Mediterranean area in folk medicine for their digestive, antimicrobial, anti-inflammatory and antioxidant properties, among others. Moreover, *Sideritis* species as tea infusions are popularly known for improving memory function and cognitive ability. Diterpenoids are one of the most abundant and important pharmacological interest of the classes of natural products presented in these medicinal plants.
Aim of the study: To determine for the first time the neuroprotective effects, based on their antioxidant properties, of the three isolated major diterpenoids andalusol, conchitriol and lagascatriol in an oxidative stress model.
Materials and methods: H_2O_2 was used as oxidant inductor and rat adrenal pheochromocytoma PC12 cells as cellular model. Cell viability was measured using MTT and LDH assays, lipid peroxidation was determined by HPLC, GSH and GSSG levels assessed by fluorometric techniques, enzymatic activity and protein expression were determined by spectrofometric techniques and Western blot, respectively.
Results: Pretreatments with the three diterpenoids significantly attenuated H_2O_2-induced changes in mitochondrial integrity and activity (MTT assay), in cell membrane integrity (LDH assay) and in cell morphology. Moreover, these diterpenoids inhibited intracellular ROS production H_2O_2-induced, reduced lipid peroxidation and counteracted GSH/GSSG changes. Furthermore, both activities and protein expression of the endogenous antioxidant enzymes (CAT, SOD, GR, GPx and HO-1) were increased. The Nrf2 pathway was involved, at least in part, in the protective effects of these diterpenoids.
Conclusion: These findings suggest that these natural compounds provide significant antioxidant effects in PC12 under for counteracting the oxidative damage H_2O_2-induced and their potential role as useful agents for the prevention of those oxidative stress-mediated dementia disorders. Andalusol was the most active compound among the studied diterpenoids.

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1. Introduction

Many older people suffer from dementia, a multisystem-related neurodegenerative disorder that can affect the memory, the attention, the language, and the problem solving, and which constitute a growing and important health problem worldwide. Oxidative stress, produced when reactive oxygen species (ROS) surpass the endogenous antioxidant system, is significantly involved in the most common formers of dementia (Mao, 2013). Overproduction of ROS can cause direct cellular damage by promoting protein oxidation, lipid peroxidation and DNA damage, all of which are events involved in the pathophysiology of the dementia (Emerit et al., 2004). Consistent evidences have demonstrated that hydrogen peroxide, the major ROS found in the human body, is involved in the molecular mechanisms by which oxidative stress causes cell death in several types of dementia including Alzheimer's disease (Tabner et al., 2005). Hydrogen peroxide cytotoxicity derives from its conversion into hydroxyl radicals the most harmful free radical by far through Fenton-type reaction (Jellinger, 2009). Hydrogen peroxide readily diffuses across cell membranes, reaching many substrate-targets and extending its cellular damage (Halliwell et al., 2000). Pathological studies on postmortem human brain tissues from patients suffered of dementia have detected excess H_2O_2, supporting the pathological role of this ROS as a cause or a flattering result of neuronal injury (Tabner et al., 2001, 2002). Phytochemical compounds with antioxidants

Abbreviations: CAT, Catalase; DCFH-DA, 2',7'-dichlorofluorescin diacetate; DMSO, Dimethylsulfoxide; GPx, Glutathione peroxidase; GR, Glutathione reductase; GSH, Glutathione; GSSG, Oxidized glutathione; HO-1, hemeoxygenase-1; LDH, Lactate dehydrogenase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; Nrf2, Nuclear factor-erythroid 2; ROS, Reactive oxygen species; SOD, Superoxide dismutase.

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properties are considered as a promising preventive or therapeutic strategy for reducing H_2O_2-mediated oxidative stress (Speciale et al., 2011).

The species of the genus *Sideritis* are extensively used in the Mediterranean area in folk medicine for their digestive, antimicrobial, anti-inflammatory and antioxidant properties, among others. Moreover, *Sideritis* species as tea infusions are popularly known for improving memory function and cognitive ability. Diterpenoids, found in high and great diversity in these medicinal plants, have been reported as one of the main compounds responsible for the activity in folk medicine (Gómez-Serranillos et al., 1997, 1998, 2004; González-Burgos et al., 2011). Andalusol, conchitriol and lagascatriol are common major diterpenoids from *Sideritis* spp. The labdane-type diterpene andalusol, in both *in vitro* and *in vivo* studies, has been found to have marked anti-inflammatory activities (Navarro et al., 1997; de las Heras et al., 1999). *In vitro* immunosuppressive effects have been also described for andalusol (Navarro et al., 2000). The rosane-type diterpene lagascatriol has also shown to have beneficial effects on inflammatory process (de las Heras et al., 2001). To our knowledge, no studies of the possible pharmacological activities of the beyerane-type diterpene conchitriol have been performed.

In view of the evidences mentioned above and in order to further investigate the pharmacological properties of these three major diterpenoids, the present study aimed to evaluate their potential protective effect in an oxidative stress model H_2O_2-induced rat adrenal pheochromocytoma PC12 cells, an *in vitro* cell model extensively used for the study of neuroprotective strategies. The antioxidant properties of these three natural diterpenoids will be assessed and the molecular action mechanisms involved in these protective effects will be elucidated.

2. Materials and methods

2.1. Reagents and standards

The reagents used were Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), horse serum (HS) from Invitrogen (Carlsbarg, CA, USA). CAT (1:1000), actine (1:5000), SOD (1:1000), hydrogen peroxide, dimethyl sulfoxide (DMSO), 2,7-dichlorofluorescein diacetate (DCFH-DA), 3-(3,4-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) were from Sigma-Aldrich (St Louis, MO, USA). GR and GPx (1:2000) were from Abcam (Cambridge, UK). Nrf2 (1:500), HO-1 (1:1000), goat anti-rabbit (1:500) were supplied by Santa Cruz Biotechnology (Santa Cruz, California, USA).

The diterpenoid standards (conchitriol, andalusol, lagascatriol) were isolated from different species of the genus *Sideritis* (Martín Panizo et al., 1972, 1974; von Carstenn-Lichterfelde et al., 1974; López et al., 1977) and were kindly provided by CSIC (Madrid, Spain). The purity of the isolated compounds was checked by HPLC (Gómez-Serranillos et al., 1997, 1998, 2004). Chemical structure of diterpenoids is shown in Fig. 1A.

2.2. Cell culture and treatment

The rat adrenal pheochromocytoma PC12 cell line was obtained from ATCC (American Type Culture Collection, Manassas, USA,

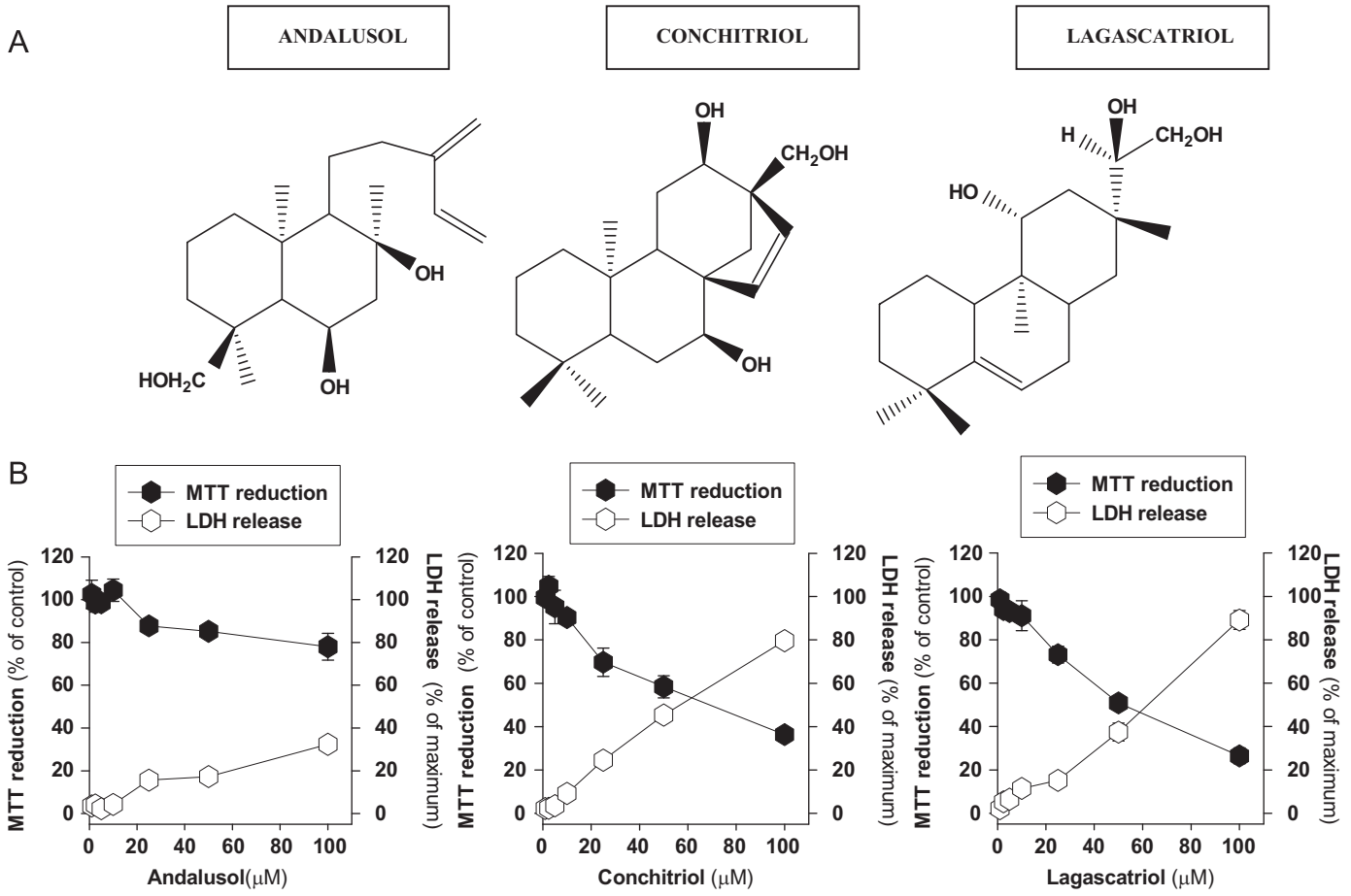

Fig. 1. (A) The chemical structures of andalusol, conchitriol and lagascatriol. (B) Effects of diterpenes on cell viability. PC12 cells were incubated with a range of concentrations from 1 to 100 μM for 24 h, and the cell viability was measured by the MTT and LDH assays.

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