Phytomedicine 18 (2011) 170–175

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

The neuroprotective effect of olive leaf extract is related to improved blood–brain barrier permeability and brain edema in rat with experimental focal cerebral ischemia

Fatemeh Mohagheghi [a], Mohammad Reza Bigdeli [a,*], Bahram Rasoulian [b], Payman Hashemi [b,c], Marzyeh Rashidi Pour [d]

[a] Faculty of Biological Sciences, Shahid Beheshti University, G.C. Tehran, Iran
[b] Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran
[c] Department of Chemistry, Faculty of Science, Lorestan University, Khorramabad, Iran
[d] Youth Researchers Club, Islamic Azad University, Khorramabad, Iran

ARTICLE INFO

Keywords:
Neuroprotective
Olive leaf extract
Olea europea
Focal cerebral ischemia
Infarct volume
Brain edema
Blood–brain barrier permeability

ABSTRACT

Recent studies suggest that olive extracts suppress inflammation and reduce stress oxidative injury. We sought to extend these observations in an *in vivo* study of rat cerebral ischemia-reperfusion injury.

Four groups, each of 18 Wister rats, were studied. One (control) group received distilled water, while three treatment groups received oral olive leaf extract (50, 75 and 100 mg/kg/day respectively). After 30 days, blood lipid profiles were determined, before a 60 min period of middle cerebral artery occlusion (MCAO). After 24 h reperfusion, neurological deficit scores, infarct volume, brain edema, and blood–brain barrier permeability were each assessed in subgroups of six animals drawn from each main group.

Olive leaf extract reduced the LDL/HDL ratio in doses 50, 75, and 100 mg/kg/day in comparison to the control group ($P < 0.001$), and offered cerebroprotection from ischemia-reperfusion. For controls vs. doses of 50 mg/kg/day vs. 75 mg/kg/day vs. 100 mg/kg/day, attenuated corrected infarct volumes were $209.79 \pm 33.05\ mm^3$ vs. $164.36 \pm 13.44\ mm^3$ vs. $123.06 \pm 28.83\ mm^3$ vs. $94.71 \pm 33.03\ mm^3$; brain water content of the infarcted hemisphere $82.33 \pm 0.33\%$ vs. $81.33 \pm 0.66\%$ vs. $80.75 \pm 0.6\%$ vs. $80.16 \pm 0.47\%$, and blood–brain barrier permeability of the infarcted hemisphere $11.22 \pm 2.19\ \mu g/g$ vs. $9.56 \pm 1.74\ \mu g/g$ vs. $6.99 \pm 1.48\ \mu g/g$ vs. $5.94 \pm 1.73\ \mu g/g$ tissue ($P < 0.05$ and $P < 0.01$ for measures in doses 75 and 100 mg/kg/day vs. controls respectively).

Oral administration of olive leaf extract reduces infarct volume, brain edema, blood–brain barrier permeability, and improves neurologic deficit scores after transient middle cerebral artery occlusion in rats.

1. Introduction

Brain ischemia induces the release of excitatory amino acids, with subsequent receptor activation leading to calcium influx, metabolic and electrophysiological dysfunction, and oxidative stress (including lipid peroxidation) (Lipton, 1999). Subsequent reperfusion worsens this oxidative stress, potentiating ischemic injury (Traystman et al., 1991). Diets rich in antioxidants might thus offer neuroprotection in cases of ischemic stroke.

The Mediterranean diet is powerfully antioxidant, and a number of international scientific organizations now recommend a modified version (Krauss et al., 2000) to prevent conditions in which oxidative stress may play an etiological role (Violi and Cangemi, 2005). Mediterranean diet contains high consumption of olive products constitute a rich source of polyphenols such as oleuropein and its derivatives, including hydroxytyrosol (HT), which scavenge free radicals and inhibit the chemical oxidation of LDL (Stupans et al., 2002; Visioli et al., 1998a).

HT administration enhances plasma antioxidant capacity and lowers production of pro-inflammatory and prothrombotic mediators in laboratory animals (Fki et al., 2007).

An HT extract prepared from olive mill wastewater reduces neuronal damage induced by chemical oxidative stress (from ferrous salts or sodium nitroprusside administration) (Schaffer et al., 2007).

The olive leaves have a hypoglycemic, hypotensive, vasodilatory and antiarrythmic effect both in vitro and *in vivo* (Lasserre et al., 1983; Duarte et al., 1993; Zarzuelo et al., 1991; Occhiuto et al., 1990; Gonzalez et al., 1992). These effects are attributed

* Corresponding author. Tel.: +98 21 44209681; fax: +98 21 22431664.
E-mail address: bigdelimohammadreza@yahoo.com (M.R. Bigdeli).

0944-7113/$ – see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2010.06.007

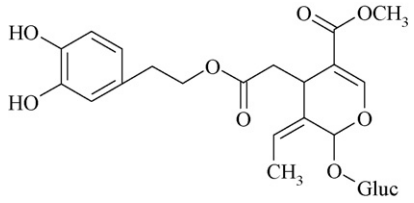

Fig. 1. Chemical structure of Oleuropein.

to oleuropein which has calcium antagonistic activity (Rauwald et al., 1994) and also enhances nitric oxide production by mouse macrophages (Visioli et al., 1998b).

Oleuropein clears superoxide anions and hydroxyl radicals, and inhibits the respiratory burst of neutrophils and related radicals (Chimi et al., 1991; Visioli et al., 1998a).

Oleuropein exhibits anti-ischemic, antioxidative, hypolipidemic effects and therefore provides cardioprotection (Andreadou et al., 2006).

It has been shown that olive leaf extract (OLE) has anti-atherogenesis effects that are related to the suppressed inflammatory response, which is an important mechanism in addition to the decrease of serum lipid levels (Aguilera et al., 2002; Cullinen, 2006; Wang et al., 2008).

In our laboratory, we have recently shown that pretreatment with various dietary doses of virgin olive oil induces ischemic tolerance and confers different degrees of neuroprotection in the rat brain (Mohagheghi et al., 2009, 2010).

In vivo neuroprotective effects of OLE from ischemia-reperfusion injury have yet to be clarified. We thus sought to characterize the impact of dietary OLE on brain infarct volume, brain edema, blood–brain barrier permeability, and neurological dysfunction resulting from transient middle cerebral artery occlusion (MCAO) in rats.

2. Materials and methods

A special preparation of OLE (*Olea europaea*; variety of *Sevillano*) extremely enriched in oleuropein, was provided by Herbal Medicine Institute (Lorestan, Iran). The OLE powder was dissolved in distilled water before use.

2.1. High-performance liquid chromatography (HPLC) procedure

HPLC analysis of the samples was conducted on a Shimadzu (model L-10AD) instrument consisting of two reciprocating pumps, a DGU-14A in-line degasser, a model CT10-10AC oven, a high pressure manual injection valve (20 μl injection loop) and a UV/VIS (model SPD-10A) detector. The software used for the data acquirement and processing was Class-vp v.R 6.1. The analytical column was a 25 cm × 4.6 mm i.d. RP-8 column (Shim-Pack CLC-C8) packed with 5 μm particles and equipped with a 1 cm guard column. A 25 μl HPLC microsyringe (F-LC, SGE, Australia) was used for the sample withdrawal and injection. A variable automatic pipette (ISOLAB, Gmbts) was used for the RP-DLLME procedure. A totally glass Fisons (UK) double distiller was used for preparation of doubly distilled water.

2.2. Quantification of some identified phenolic compounds by HPLC

For the HPLC separation and quantitation of oleuropein (Fig. 1), tyrosol, hydoxytyrosol and caffeic acid, a gradient elution with a mixture of solvents A (0.05 mol l^{-1} acetate buffer pH 5.0 in water) and B (acetonitrile) with a flow rate of 1.0 ml min^{-1} was used. The elution program was as follows: 0–8 min, 20–80% B; 8–11 min, 50–70% B; 11–13 min 70% B and 13–15 min, 70–20% B. The chromatograms were acquired at 240 nm (Hashemi et al., 2010). The main phenolic compositions of the olive leaf extract are oleuropein (356 mg/g), tyrosol (3.73 mg/g), hydroxy tyrosol (4.89 mg/g), caffeic acid (49.41 mg/g) of the dry extract (Fig. 2).

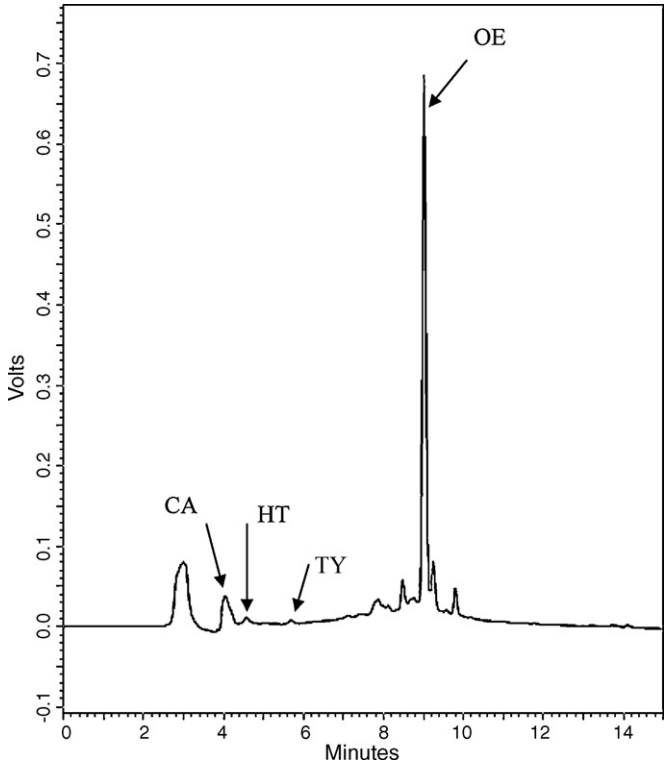

Fig. 2. High-performance liquid chromatogram of olive leaf extract at 240 nm.CA: caffeic acid; HT: hydoxytyrosol; TY: tyrosol; OE: oleuropein.

2.3. Analysis of total phenolic compounds in the extract

The concentration of total phenolic compounds in olive leaf extract was determined using Folin–Ciocalteau assay (Folin and Ciocalteau, 1927) as described by Othman (Othman et al., 2009). Briefly, to 0.5 ml of a 5.5 g/l diluted extract, 2.5 ml of Folin–Ciocalteau reagent (diluted 10 times with water) was added. After about 3 min, 2 ml of Na_2CO_3 (75 g/l) was added and the sample was incubated for 5 min at 50 °C and then cooled. For the control sample, 0.5 ml of distilled water was used. The absorbance was measured at 760 nm. The results were expressed in mg of tyrosol per g of dry matter (De Marco et al., 2007). Total phenolic compounds are 349.4 that expressed as mg tyrosol per g of dry matter.

2.4. Determination of radical scavenging activity of the extract

The radical scavenging activity of the extract was determined as described by Moon and Terao with little modification; based on the scavenging ability to 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable radicals (Moon and Terao, 1998). Briefly, the samples (from 100 to 350 μg/ml) were mixed with 1 ml of 500 μmol/l DPPH solution and 0.8 mL Tris–HCl buffer (pH 7.4) and filled up with ethanol, to a final volume of 2 ml. The reaction mixture was shaken in a room temperature dark room for 40 min. Butylated hydroxytoluene (BHT) was used as a positive control. The control was prepared as the test samples and ethanol was used for the baseline correction. Absorbance at 517 nm was measured on a UV–Vis spectrophotometer (Biochrom Ltd, England). IC_{50} values, which represent the concentration of the