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Short communication

EPs7630® from *Pelargonium sidoides* increases stress resistance in *Caenorhabditis elegans* probably *via* the DAF-16/FOXO pathway

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ABSTRACT

EPs7630® a water alcohol extract of the roots from *Pelargonium sidoides* contains several secondary metabolites including highly oxygenated coumarins, various phenolics and polyphenols. Using the DPPH• assay to measure antioxidant activity a free radical scavenging activity of 14.7 ± 0.85 μg/ml (IC_{50}) was determined. As an *in vivo* model *Caenorhabditis elegans* was applied to study the effect of EPs7630® on stress resistance. EPs7630® treatment reduces intracellular *hsp-16.2::GFP* expression (induced by the pro-oxidant juglone) indicating that the secondary metabolites of EPs7630® are bioavailable and exhibit antioxidant activities *in vivo*. Application of EPs7630® (50 μg/ml) to the transgenic mutant TJ356 induced the migration of the transcription factor DAF-16 from cytosol to the nucleus, suggesting a prominent role of DAF-16/FOXO in the *daf-2* pathway for stress resistance.

Introduction

Pelargonium sidoides DC is native to the Cape region of South Africa. In traditional medicine of South Africa *P. sidoides* has been applied in the therapy of various diseases including cold, sore throat, and tuberculosis (Bladt, 1977; Van Wyk and Gericke, 2000). *P. sidoides* has also been developed into a registered herbal medicine: A water alcohol root extract, known as Umckaloabo® containing the special extract EPs7630® is a fully licensed medication and one of the most popular herbal remedies in Germany (van Wyk and Wink, 2004). EPs7630® is produced after extraction the milled roots of *P. sidoides* with 11% (w/w) ethanol in water (Schoetz et al., 2008). EPs7630® contains several secondary metabolites including highly oxygenated coumarins, various phenolics and polyphenols. Detailed HPLC analyses of EPs7630® have been published by Schoetz et al. (2008) and Hauer et al. (2010). According to previous studies, EPs7630® showed antibacterial, antiviral, cytoprotective and significant immune modulatory properties *in vitro* and is employed for the treatment of airway infections (Kolodziej et al., 2003; Michaelis et al., 2011; Thale et al., 2011). In general, polyphenols and coumarins have antioxidant properties (van Wyk and Wink, 2004). We were interested whether antioxidant properties are exhibited by EPs7630® and if they might contribute to the general efficacy of EPs7630® seen in clinical studies.

Investigations of possible mechanisms of health disorders are facilitated by the use of the model organism *Caenorhabditis elegans* (a free living nematode). This animal has a large number of advantages such as small size, simplicity of maintenance, short life span and the existence of various transgenic mutants. Additionally, it is possible to translate the resulting knowledge from this tiny model organism into human physiology and disorders, because more than 35% of human genes are shared with *C. elegans* which has more than 21,000 protein coding genes (Hillier et al., 2005).

In the present study, we investigated whether EPs7630® has antioxidant properties *in vitro* and if it can protect *C. elegans in vivo* against oxidative stress induced by the pro-oxidant juglone (a naphthoquinone from *Juglans regia*).

Materials and methods

C. elegans strains and culture conditions

C. elegans strains were acquired from the *Caenorhabditis* Genetic Center (CGC, University of Minnesota, USA) including Wild type N_2, BA17 [*fem-1 (hc17)*] which is fertile at 20 °C and infertile at 25 °C, TK22 [*mev-1 (KN1)*], TJ375 [*hsp-16.2::GFP(gpls1)*] and TJ356 [*daf-16::daf-16-gfp;* rol-6]. For maintenance and growth of *C. elegans* nematode growth medium (NGM) and S-basal medium were used at 20 °C. Synchronized hermaphrodites were treated with 5% sodium hypochlorite and 5 M sodium hydroxide followed by the cultivation of the collected eggs on nematode growth medium covered with *E. coli* OP50 as a nutrient (Stiernagle, 2006).

Chemicals and reagents

EPs7630® was provided by Dr. Willmar Schwabe Pharmaceuticals (Schwabe GmbH, Karlsruhe, Germany). 2,2-Diphenyl-1-picryl-hydrazyl (DPPH•), α-hydroxy-1,

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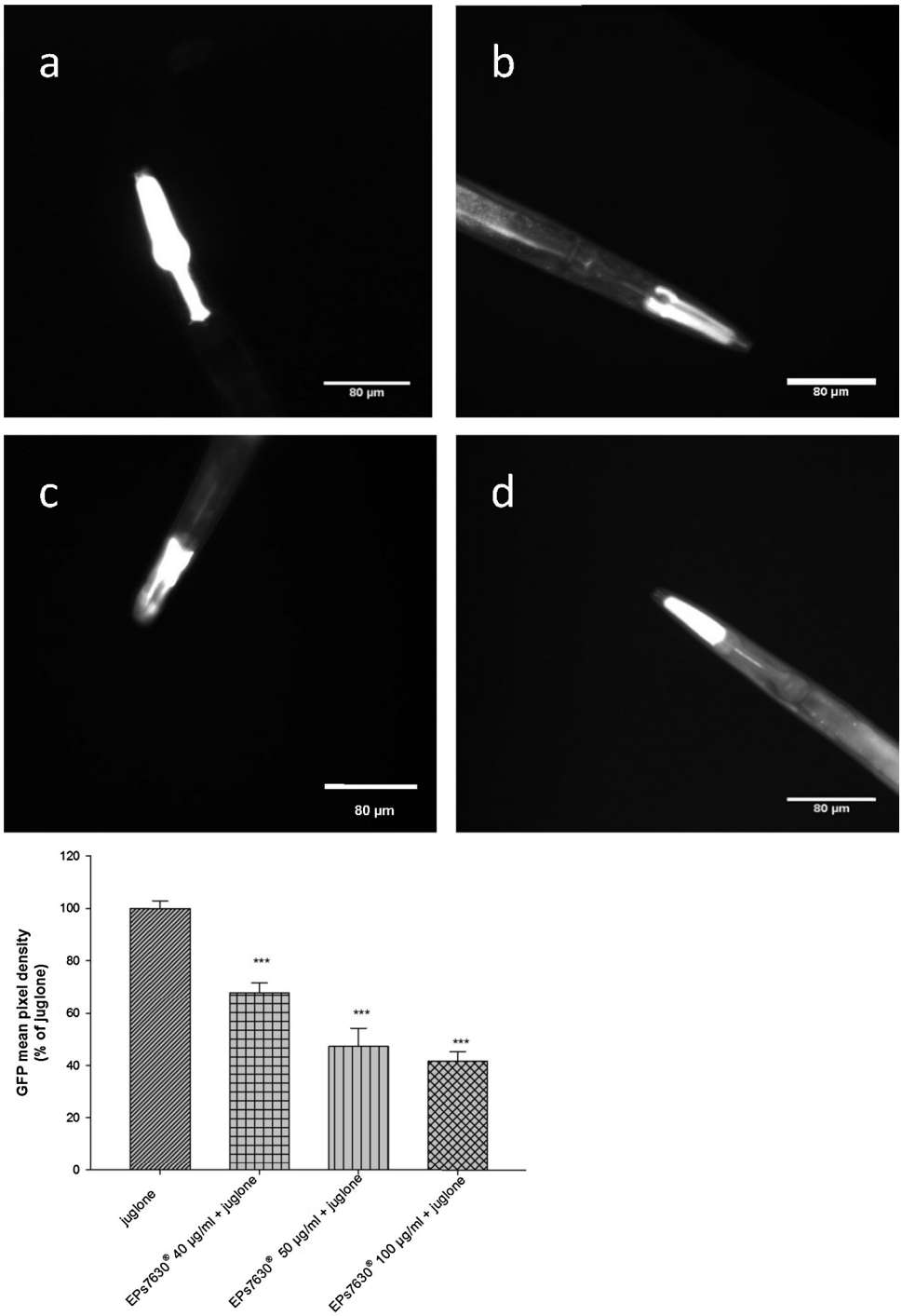

Fig. 1. Effects of EPs7630® on GFP expression in TJ373 *hsp-16.2::GFP* (*gpls1*) strain. Data were obtained from three independent experiments. Values are presented as mean ± SE. ***$p < 0.001$. (a) Treatment with juglone, (b) 40 µg/ml EPs7630® + juglone, (c) 50 µg/ml EPs7630® + juglone, (d) 100 µg/ml EPs7630® + juglone.

4-naphthoquinone (juglone), and epigallocatechin gallate (EGCG) ≥95% were obtained from Sigma (Sigma–Aldrich GmbH, Steinheim, Germany). Sodium azide was purchased from AppliChem (AppliChem GmbH, Darmstadt, Germany).

In vitro studies

DPPH• method

To study the free radical scavenging activity of EPs7630®, the DPPH• assay was carried out following the modified method described by Blois (Brand-Williams et al., 1995). The polyphenol EGCG from green tea was used as a positive control.

In vivo studies

Quantification of hsp-16.2::GFP expression

To measure the expression of *hsp-16.2*/*GFP* under oxidative stress, the TJ375 (*hsp-16.2*::*GFP*) strain in which the *hsp-16.2* promoter is coupled to the gene encoding the green fluorescence protein (GFP) was employed. GFP expression in the pharynx of this strain is readily noticeable after exposure to oxidative stress. L1