SUPPLEMENTARY MATERIAL

Isofuranodiene, the Main Volatile Constituent of wild celery (*Smyrniolum olusatrum* L.), Protects D-Galactosamin/Lipopolysacchride Induced Liver Injury in Rats

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Abstract

Isofuranodiene is a natural sesquiterpene rich in *Smyrniolum olusatrum*, a forgotten culinary herb which was marginalised after the domestication of the improved form of celery. Our recent data showed that isofuranodiene inhibited the proliferation and induced apoptosis in cancer cells. In this study, we investigated its protective effect on D-galactosamine/lipopolysacchride (GalN/LPS) induced liver injury in rats. Oral administration of isofuranodiene (20 and 50 mg/kg) dramatically inhibited GalN/LPS-induced serum elevation of aspartate aminotransferase (AST), alanine aminotransferase (ALT), malondialdehyde (MDA) levels, and significantly ameliorated liver injury as evidenced by the histological improvement in H&E staining. Furthermore, isofuranodiene treatment significantly inhibited GalN/LPS-induced mRNA expression of IL-1 β , IL-6, and inducible nitric oxide synthase (iNOS) in liver tissues. The results from this study showed that isofuranodiene protect GalN/LPS-induced liver injury in rats and suggested that it may be a potential functional food ingredient for the prevention and treatment of liver diseases.

Materials and methods

Chemicals

Isofuranodiene (C₁₅H₂₀O, crystals, purity 99% as determined by HPLC) was isolated from the essential oil of flowers of *S. olusatrum* and purified by recrystallization with *n*-hexane as our previous report (Buccioni and Dal Ben 2014, Quassinti et al. 2014). The molecular structure was confirmed by comparison of ¹H and ¹³C-NMR data obtained on a Varian Mercury plus 400 Spectrometer, using CDCl₃ as solvent and the solvent signals as internal references, with those reported in the literature (Maggi et al., 2012). LPS and D-galactosamine were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Kits for aspartate aminotransferase (AST), alanine aminotransferase (ALT), and malondialdehyde (MDA) were purchased from Nanjing Jiancheng Science and Technology Co., Ltd (Nanjing, China). Trizol was obtained from Invitrogen (Carlsbad, CA, USA). High Capacity RT Kit was purchased from Takara Biotechnology Co., Ltd. (Dalian, China). Power SYBR Master Mix was purchased from Shanghai Generay Biotech Co., Ltd (Shanghai, China).

Animals

Male Sprague-Dawley rats (220±20 g) were purchased from the Animal Center of Institute of Surgery Research of the Third Military Medical University (Chongqing, China) and were housed in animal facilities of Zunyi Medical College. Animals were housed free access to rodent feed and tap water at 20~22 °C. Experiments were under the Chinese Guidance of Humane Use of Laboratory Animals.

Experimental design

Thirty-two rats were randomly divided into four groups: the control group, the model group, the low (20 mg/Kg) and high (50 mg/Kg) doses of isofuranodiene groups. Isofuranodiene dissolved in CMC-Na was administrated by ig once a day for three

consecutive days. One hour after the last administration on the third day, rats were treated with GalN/LPS (500 mg/50 µg/kg) by intraperitoneal injection. The control group was administrated with the same volume of CMC-Na. Eight hours after GalN/LPS challenge, the rats were sacrificed and the serum and liver tissues were collected and store at -80°C before use.

Determine serum AST, ALT and MDA

The serum levels of AST, ALT, and MDA were measured with commercial kits following the manufacturer's instructions.

Histological observations

The liver tissues were stained by conventional hematoxylin-eosin (H&E) staining and the sections were observed under an optical microscope to evaluate the morphological changes.

mRNA expression of IL1-β, IL6, and iNOS in liver tissues

Approximate 50-100 mg liver tissues were collected and the total RNA was extracted with a commercial kit according to the manufacturer's instructions. The cDNA was synthesized with High Capacity RT Kit. The mRNA expression levels were determined by Power SYBR Master Mix using primers from Generay Biotech Co., Ltd (Shanghai, China) listed in Table 1. β-actin was used as the internal standard.

Statistical analysis

Data were presented as mean ± SD. Statistics were analysed with one-way ANOVA followed by Tukey's post hoc test. Statistical difference was considered when *P*-value <0.05.

Reference

Buccioni M, Dal Ben D. 2014. Antiproliferative evaluation of isofuranodiene on breast and prostate cancer cell lines.2014:264829.
Quassinti L, Maggi F, Barboni L, Ricciutelli M, Cortese M, Papa F, Garulli C, Kalogris C, Vittori S, Bramucci M. 2014. Wild celery (*Smyrniolum olusatrum* L.) oil

and isofuranodiene induce apoptosis in human colon carcinoma cells. TheScientificWorldJournal. Sep;97:133-141. Epub 2014/06/27.

Figure S1

Figure S1. Effect of isofuranodiene on mRNA expression of IL-6 (A), IL-1 β (B), and iNOS (C) in GalN/LPS challenged rats. ** $p < 0.01$, * $p < 0.05$ compared with the Mod group. Con, control; Mod, model group.

Figure S2

Figure S2. Isofuranodiene protect GalN/LPS induced liver injury in rats. Typical images were chosen from each experimental group (original magnification $\times 200$). (A), Control group; (B), GalN/LPS treated group; (C), isofuranodiene (20 mg/kg) and (D), (50 mg/kg) treated groups.

Table S1. The primers for RT-PCR.

Name	5'-3'	5'-3'
IL-1 β	GTGGTATTCTCCATGAGCTTTGTA	CCATCTTCTTCTTTGGGTATTGTT
IL-6	AGACTTCACAGAGGATACCACCCAC	CAATCAGAATTGCCATTGCACAA
iNOS	AACAGTGGCAACATCAGGTCGG	TGAACTCCAAGGTGGCAGGATC
β -actin	TGACCGAGCGTGGCTACAG	GGGCAACATAGCACAGCTTCT