SHORT COMMUNICATION

Isofuranodiene, the main volatile constituent of wild celery (Smyrnium olusatrum L.), protects D-galactosamin/lipopolysaccharide-induced liver injury in rats

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Isofuranodiene is a natural sesquiterpene rich occurring in Smyrnium 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/lipopolysaccharide (GalN/LPS)-induced liver injury in SD rats. Oral administration of isofuranodiene (20 and 50 mg/kg) dramatically inhibited GalN/LPS-induced serum elevation of aspartate aminotransferase, alanine aminotransferase and malondialdehyde 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\(\beta\), IL-6 and inducible nitric oxide synthase in liver tissues. The results from this study showed that isofuranodiene protects GalN/LPS-induced liver injury in SD rats and suggested that it may be a potential functional food ingredient for the prevention and treatment of liver diseases.

**Keywords:** isofuranodiene; galactosamine/lipopolysaccharide; hepatoprotective; rats

1. Introduction

Isofuranodiene ([5\(E\),9\(E\)]-3,6,10-trimethyl-4,7,8,11-tetrahydrocyclodeca[b]furan), CAS Registry Number: 57566-47-9, molecular weight: 216.1514) (Figure 1(A)) is a natural furanosesesquiterpene isolated from Arminacean nudibranch Leminda millecra (McPhail et al. 2001), Antarctic gorgonian Dasystenella acanthine (Gavagnin et al. 2003) and rhizomes of Curcuma ochrorhiza

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Its chemical structure was first identified by Rückner et al. (Rückner et al. 1971). However, its biological activities remained unknown for decades. Our recent findings showed that wild celery (*Smyrnium olusatrum* L., Apiaceae) is a rich source of isofuranodiene (Maggi et al. 2012). Wild celery, also known as Alexanders, is a biennial herb used in cuisine by Romans and cultivated all over European kitchen gardens for many centuries owing to the particular myrrh-like aroma. Its use as a vegetable was abandoned in the middle ages when it was replaced by celery (*Apium graveolens* L.) (Mölleken et al. 1998). Besides culinary uses, wild celery was also used as medicinal plant during Greek and Roman ages; the roots were used as antiscorbutic, the juice of the root for its aromatic, appetite stimulant, diuretic and laxative properties, the fruits as stomachic and antiasthmatic and the stems as depurative (Fournier 1947). Wild celery enjoyed a reputation also in Great Britain, especially among seafarers, for ‘clearing’ the blood and preventing scurvy (Allen & Hatfield 2004). Recent studies revealed that flowers and roots of wild celery contain essential oils, with isofuranodiene as the major constituent (Maggi et al. 2014).

Isofuranodiene was found to be a cytotoxic natural product *in vitro*. It dramatically inhibited the proliferation of breast and prostate cancer cell lines (Buccioni et al. 2014) and induced apoptosis in human colon carcinoma cells (Quassinti et al. 2014). Here, we report its hepatoprotective effect in a rat model for the first time.

2. Results and discussion

Isofuranodiene is considered the precursor of furanosesquiterpenes. Our recent data showed that it demonstrated anti-cancer activities *in vitro* cell lines but is relatively low toxic to normal cells.

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**Figure 1.** Effect of isofuranodiene on serum AST (B), ALT (C) and MDA (D) in GalN/LPS-challenged rats. *p < 0.05; **p < 0.01 with respect to control.
Previous studies showed that compounds with similar structure protected GalN/LPS and GalN/TNFα-induced liver injury in mice (Matsuda et al. 1998; Morikawa et al. 2002). Here, we investigated for the first time the liver protective effect of isofuranodiene in the GalN/LPS-induced rat model. As shown in Figure 1, GalN/LPS treatment significantly increased in serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), two common biomarkers for liver injury. Furthermore, the serum level of malondialdehyde (MDA), a biomarker for oxidative stress, was also dramatically increased. Previous reports showed that GalN/LPS treatment induced serum of AST, ALT and liver content of MDA (Wen et al. 2007; Wang et al. 2008). Therefore, the increase of serum AST, ALT and MDA in GalN/LPS-treated SD rats was possibly due to the oxidative injury of the liver. Isofuranodiene treatment dramatically suppressed GalN/LPS-induced serum levels of AST, ALT and MDA in a dose-dependent manner, suggesting that the liver injury was partly improved by isofuranodiene. Our previous results showed that isofuranodiene showed weak in vitro antioxidant potentials in DPPH and ABTS assays but with moderate antioxidant effect in the FRAP assay. These data provide clue that the antioxidant potentials of isofuranodiene might contribute to its hepatoprotective effect but need further studies to be elucidated. The protective effect of isofuranodiene was further confirmed by H&E staining. The liver lobular architectures and cell structures in the control group were normal and integrated. However, GalN/LPS treatment induced serious congestion, broad haemorrhagic necrosis and extensive areas of portal inflammation (arrows) (supplementary data). These morphological alterations were significantly improved by isofuranodiene treatment. Inflammatory cytokines such as IL6, IL-1β and TNF-α play important roles in the GalN/LPS-induced liver injury model (Josephs et al. 2000; Sass et al. 2002; Wu et al. 2014). Increased expression of inducible nitric oxide synthase (iNOS) and NO production was also found in concanavalin A (Sass et al. 2001) and GalN/LPS (Wen et al. 2007; Hijikawa et al. 2008) induced liver injury models. Here, we found that the mRNA expressions of IL6, IL-1β and iNOS increased to tens and even hundreds of folds with respect to those of control group after 8 h of GalN/LPS treatment, while isofuranodiene administration dramatically decreased the expression of IL6, IL-1β and iNOS (supplementary data). These results suggested that the hepatoprotective effect of isofuranodiene in GalN/LPS-induced liver injury might relate to the inhibition of inflammatory cytokines.

3. Conclusion

In conclusion, isofuranodiene, a natural sesquiterpene contained in high concentrations in a forgotten food plant, protects GalN/LPS-induced liver injury in SD rats. These results may support the traditional uses of wild celery as depurative. More importantly, they might promote the understanding of the health benefits of neglected vegetables, leading to the development of functional food ingredients for the prevention and treatment of liver diseases.

Supplementary material

Supplementary material relating to this paper is available online.

Conflicts of interest

The authors have declared no conflicts of interest.

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