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Protective effect of a marine polyphenol, dieckol against carbon tetrachloride-induced acute liver damage in mouse

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ABSTRACT

In this study, the hepatoprotective effect of dieckol on carbon tetrachloride (CCl_4) induced hepatic damages in ICR mice liver was investigated. Mice were randomly divided into 4 groups such as saline treated (negative control), CCl_4 treated (positive control), CCl_4 + dieckol (5 mg/kg mouse) and CCl_4 + dieckol (25 mg/kg mouse), respectively. The body weights and survival rates of mice, followed by dieckol treatments were significantly increased compared to the positive control. The level of GOT, GPT and MDA in the serum of the dieckol treated groups were reduced dose dependently than the control, significantly. The antioxidant enzymes including CAT, and GSH-px levels were increased significantly compared to the positive control. However, no significant differences were observed on hepatic histophathological analysis in dieckol treated groups dose dependently. Down-regulation of Bax and up-regulation of Bcl-xl protein expressions were observed in liver tissues of the dieckol administered groups. These results suggested that, dieckol can be developed as a therapeutic agent for liver disease by oxidative stress.

1. Introduction

The liver is an organ which plays an important role in the body (Wang et al., 2008), which is functioning extensively as the regulation of blood sugar levels, protein synthesis and detoxification (Bhardwaj et al., 2011). The liver diseases can be caused by toxic substances such as abuse of alcohol, drug and carbon tetrachloride (CCl_4). The CCl_4 has been widely used in the induction of acute liver damage in experiment of mice model (Olorunnisola et al., 2011; Toyin et al., 2008). According to the previous studies CCl_4 assumed to be a typical poison causing oxidative stress (Dolai et al., 2012; Basu, 2003). Acute liver diseases are associated with the causing of CCl_4 and characterized by increasing apoptosis and oxidative stress in the liver. Importantly, the oxidative stress caused by CCl_4 which induces apoptosis and is involved in harmful effects such as cirrhosis and fibrosis in the liver (Palmieri and Sblendorio, 2007). It is known that many human diseases are associated with free radicals and natural antioxidants could be used as free

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radical scavengers (Hamid et al., 2010). Recently both *in vitro* and *in vivo* studies had been examined for the new antioxidants and the effect of hepato-protective substances from natural resources. Previous reports have showed that substances like, resveratrol have both the hepato-protective and antioxidant effect (Chumbhale and Upasani, 2012; Olorunnisola et al., 2011; Rivera et al., 2008).

Seaweeds contain biologically active substances including minerals, polyphenols, polysaccharides and amino acids (Lordan et al., 2011; Yang et al., 2011; Kang et al., 2012). Especially, brown algae are known to have various kind of bioactive compounds including pigments, steroids, phycocolloids and phlorotannins (Shilpi and Nissreen, 2011). In particular, the phlorotannins isolated from brown algae have been exhibited a variety of biological activities such as antioxidant, anti-cancer, anti-inflammation and protective effect against oxidative stress (Li et al., 2011). Among them, dieckol (DK) isolated from brown seaweed *Ecklonia cava*, exhibits various biological activities such as antioxidant (Ahn et al., 2007), ACE inhibitory activity (Wijesinghe et al., 2011), anti-inflammation (Jung et al., 2009) and protective effect against oxidative stress (Lee et al., 2010). Especially, dieckol isolated from *E. cava* showed excellent properties of antioxidant activity. Therefore, the objective of the present study is to evaluate the *in vivo* hepato-protective effect of dieckol isolated from brown seaweed *E. cava* against CCl_4-induced liver damage.

2. Materials and methods

2.1. Materials

Male ICR mice (6 weeks of age; purchased from Joong Ang Lab Animal Co., Seoul, Korea) were used. The thiobarbituric acid-reactive substances (TBARS), superoxide dismutase (SOD), catalase (CAT), reduced glutathione peroxidase (GSH-px) was determined in the liver using a commercial available kit from Sigma Chemical Co. (St. Louis, MO, USA).

All chemicals and reagents used were of analytical and obtained from commercial sources. Antibodies against phosphor-Bax, phosphor-Bcl-xl and β-actin were purchased from Cell signaling Technology (Bedford, MA, USA).

2.2. Isolation of dieckol from Ecklonia cava

The powdered *E. cava* was extracted with 80% aqueous EtOH, and was evaporated under vacuum. The EtOH extract was then partitioned with EtOAc. The EtOAc extract was fractionated *via* silica column chromatography with the stepwise evolution of $CHCl_3$–MeOH mixture (100:1–1:1) to generate and separate the active fractions. The combined active fraction was then further subjected to a Sephadex LH-20 column (GE Healthcare, USA) saturated with 80% MeOH, and finally purified *via* reverse-phase HPLC (ThermoFisher Scientific, USA) using a Waters HPLC system equipped with a Waters 996 photodiode array detector and C18 column (J'sphere ODS-H80, 150 × 20 mm, 4 μm, YMC Co.) by stepwise elution with methanol–water gradient (UV range: 230 nm, flow rate: 0.8 ml/min). The purified compound, dieckol was confirmed by comparing their LC/MS, ^{1}H NMR data to the literature report (Li et al., 2009)

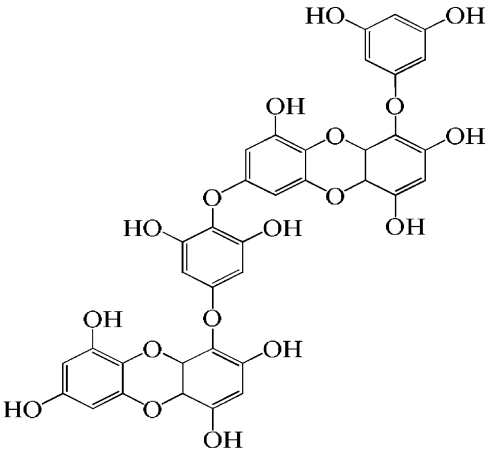

Fig. 1 – Chemical structure of dieckol isolated from *E. cava*.

Dieckol: LC/MS data (M^+, *m/z*: 742.0 Calcd. For $C_{36}H_{22}O_{18}$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.71(1H, s, OH-9), 9.61 (1H, s, OH-9″), 9.51 (1H, s, OH-4″), 9.46 (1H, s, OH-4), 9.36 (2H, s, OH-3″, 5″), 9.28 (1H, s, OH-2″), 9.23 (1H, s, OH-2), 9.22 (1H, s, OH-7″), 9.15 (2H, s, OH-3′, 5′) 6.17 (1H, s, H-3″), 6.14 (1H, s, H-3), 6.02 (1H, d, *J* = 2.7 Hz, H-8), 5.98 (1H, d, *J* = 2.7 Hz, H-8″), 5.95 (1H, s, H-2‴, 6‴), 5.82 (1H, d, *J* = 2.7 Hz, H-6), 5.81 (1H, d, *J* = 2.7 Hz, H-6″), 5.80 (1H, t, *J* = 2.0 Hz, H-4′), 5.78 (2H, d, *J* = 2.0 Hz, H-2′, 6′).

The purity of dieckol (Fig. 1) was >95%, based on the peak area of all components absorbed at each specific wavelength in HPLC analysis. Dieckol was dissolved in DMSO and was used for experiments adjusting the final concentration of DMSO in the culture medium to <0.01%.

2.3. Animals

Male ICR mice, weighing 25–30 g, were acclimated to temperature (22 °C) and humidity (55%) controlled rooms with a 12-h light/dark cycle for 1 week prior to use. Male ICR mice of Six-week-old were randomly divided into 4 groups. A negative control is described as saline oral administrated mice (saline, 200 μl), for 6 days daily during the experimental period. A positive control group is described as saline oral administrated mice (saline, 200 μl) for 6 days, followed by CCl_4 oral administrated (0.5 mg/kg, mouse) on 6th day. In addition, dieckol (5 mg/kg, mouse) oral administration for 6 days followed by CCl_4 oral administration (0.5 mg/kg, mouse) on 6th day and dieckol (25 mg/kg, mouse) oral administration followed by CCl_4 oral administration (0.5 mg/kg, mouse) on 6th day mouse groups were described as experimented two groups, respectively. Each group consisted of 5 mice. During the experimental period, the survival rates were investigated daily. All the animals during 6 days pre-treated by dieckol and CCl_4 oral administrated after 24 h (7th day) were killed. The body weights and survival rates were investigated daily. After 7 days, the mice were anesthetized and blood samples were collected to determine biochemical parameters. The liver (from three animals of each group) immediately fixed in 10% formalin, and then stained with hematoxylin and