Acta Histochemica xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.com/locate/acthis

Sasa quelpaertensis leaves ameliorate alcohol-induced liver injury by attenuating oxidative stress in HepG2 cells and mice

Kalahe Hewage Iresha Nadeeka Madushani Herath[a], So Jin Bing[b], Jinhee Cho[b], Areum Kim[a], Gyeonghun Kim[b], Ju-Sung Kim[c], Jae-Bum Kim[d], Yang Hoi Doh[e], Youngheun Jee[a,b,*]

[a] Interdisciplinary Graduate Program in Advanced Convergence Technology & Science, Jeju National University, Jeju 63243, Republic of Korea
[b] Department of Veterinary Medicine and Veterinary Medical Research Institute, Jeju National University, Jeju 63243, Republic of Korea
[c] Majors in Plant Resource and Environment, College of Agriculture and Life Sciences, SARI, Jeju National University, Jeju 63243, Republic of Korea
[d] Department of Systems Management Engineering, Sungkyunkwan University, Suwon 440-746, Republic of Korea
[e] Department of Electronic Engineering, Jeju National University, Jeju 63243, Republic of Korea

ARTICLE INFO

Keywords:
Eactive oxygen species
Antioxidant enzymes
CYP2E1
Hepatoprotective effect

ABSTRACT

Oxidative stress plays a crucial role in the progression of alcoholic liver diseases and substances of antioxidant property are of special interest for therapeutic purposes. We investigated the hepatoprotective effect of leaf extracts of *Sasa quelpaertensis*, an edible bamboo mainly cultivated in Jeju Island, South Korea. We examined the cytotoxicity of different extracts (distilled water, 20–80% EtOH) of *S. quelpaertensis* on HepG2 cells and their hepatoprotective effect on HepG2 cells stimulated by ethanol (800 mM, 24 h). Furthermore, we measured reactive oxygen species (ROS) production, ethanol toxicity induced cell death, and the activity of antioxidant enzymes. In *in vivo* experiments, liver damage was induced by oral administration of 5 g/kg ethanol with or without potent ethanol extract of *S. quelpaertensis* (10 or 100 mg/kg) 12 h interval for a total of 3 doses. Only 80% ethanol extract of *S. quelpaertensis* (SQEE80) exhibited cytoprotective effect on HepG2 cells against alcohol-induced toxicity. SQEE80 treatment (250, 500 μg/mL) in ethanol exposed HepG2 cells showed significant attenuation of ROS production and ethanol toxicity induced cell death. Furthermore, SQEE80 markedly increased the activity of antioxidant enzyme glutathione peroxidase 1 in ethanol exposed HepG2 cells compared to ethanol stimulated cells. In *in vivo* experiments, SQEE80 treatment evidently suppressed the alcohol-induced histopathological changes in liver, serum ethanol content, and expression of cytochrome P450 2E1. Furthermore, SQEE80 significantly reversed the reduction of glutathione level in the ethanol challenged liver. Taken together, we suggest the possibility of developing SQEE80 as a natural hepatoprotective substance in attenuating alcohol-induced oxidative stress.

1. Introduction

Alcohol is a primitive addictive factor which causes several social and health problems (Galicia-Moreno and Gutierrez-Reyes, 2014). Although moderate alcohol consumption is recognized for its health benefits such as protection from heart stroke, diabetes, and hypertension (Berger et al., 1999; Sacco et al., 1999), excessive and prolonged alcohol consumption causes alcohol liver diseases (ALD) which are mainly responsible for chronic liver disease, fibrosis cirrhosis and hepatic carcinoma, which can be summed-up with premature mortality elevation and life expectancy shortening (Coelho et al., 2013; Gao and Bataller, 2011).

In the progression of ALD, reactive oxygen species (ROS) are known to play a crucial role (Song et al., 2006). Cytochrome P450 2E1 (CYP2E1), one of the three main enzymes involved in alcohol metabolism, metabolizes ethanol to more reactive products elevating ROS generation and is a major target in current therapeutic medicine against alcohol toxicity (Knockaert et al., 2011; Lu and Cederbaum, 2008; Tanaka et al., 1997). CYP2E1 in mitochondria also contributes to the overproduction of mitochondrial ROS and depletes glutathione (GSH) in ethanol exposure (Knockaert et al., 2011). Since ROS are produced in the ethanol metabolic process, cells have developed several protective measures against harmful effects of ROS and maintain the balance between free radicals and antioxidant function (Wu and Cederbaum, 2003). Acute or chronic ethanol exposure is responsible for excessive ROS production in hepatocyte alcohol metabolism and then for the lipid peroxidation, antioxidant depletion, DNA damage, oxidative damage of cellular protein and membrane lipids, hepatocyte injury, which

* Corresponding author at: Department of Veterinary Medicine and Veterinary Medical Research Institute, Jeju National University, 102 Jejudaehakno, Jeju 63243, Republic of Korea.
E-mail address: yhjee@jejunu.ac.kr (Y. Jee).

https://doi.org/10.1016/j.acthis.2018.05.011
Received 9 October 2017; Received in revised form 18 April 2018; Accepted 14 May 2018

eventually results in cell death (Hemnani and Parihar, 1998; Jaeschke et al., 2002; Wu and Cederbaum, 2003). Hence, maintaining the balance between ROS-triggered apoptotic effect and systemic response against it is crucial in regulating the progression of ALD (Wang, 2014). Drugs like corticosteroids, antiviral, and immunosuppressant are currently prescribed for liver diseases (Abou Seif, 2014). However, such therapeutic agents are inadequate to recover livers from diseases such as fatty liver, cirrhosis, and hepatitis; they often inflict hepatic damages in prolonged use (Abou Seif, 2014). Hence, developing natural product based therapeutic agents is of high interest in managing ALD.

Sasa quelpaertensis is a native Korean dwarf bamboo known to inhabit only at mount Halla, Jeju Island, Korea (Kim et al., 2014b). Its leaf extracts contain a mixture of amino acids, polysaccharides, and more importantly polyphenols such as p-coumaric acid and tricin (Kim et al., 2013a, 2015a,b) and are reported to show beneficial biological properties such as anti-inflammatory, antioxidant, lipid metabolism, antiviral, and anticancer activities (Kim et al., 2013a,b, 2014a,b). It is well known that the more phenolic and flavonoid constituent extracts have, the more potent their antioxidant activity is (Khorasani Esmaeili et al., 2015). Health promoting efficacy of *S. quelpaertensis* leaf extracts has been further examined in more practical contexts recently: they are shown to exhibit protection against radiation induced oxidative stress in mice (Kim et al., 2015a,b) and to attenuate oleic-induced lipid metabolism in HepG2 cells (Kim et al., 2013a).

Interestingly, it turns out that different concentrations of ethanol, an efficient solvent for *S. quelpaertensis* yielding high content of polyphenolic compounds in the extract (Kim et al., 2013b), in solvent medium produce leaf extracts of widely varying potency. For instance, 70% ethanol extract of *S. quelpaertensis* is identified for modulating antioxidant enzymes in dextran sulfate sodium-induced mouse colitis (Yeom and Kim, 2015). Various fractions (chloroform and ethyl acetate) of 80% ethanol extracts also showed high content of phenolic acids and flavonoids predominantly p-coumaric acid, ruitin and myricetin (unpublished data).

In this study, we evaluated different extracts of *S. quelpaertensis* (Distilled water (DW), 20%, 40%, 60%, and 80% EtOH extracts) for their cytoprotective effect against alcohol-induced cytotoxicity. Identifying 80% ethanol extract of *S. quelpaertensis* leaves (SQEE80) the most effective against alcohol-induced toxicity, we investigated its hepatoprotective effect against alcohol-induced oxidative stress and antioxidant enzyme expression using HepG2 cells followed by assays using mouse models. In mouse models, we induced liver injury through oral administration of ethanol with or without SQEE80 and investigated the toxicity of ethanol on liver and hepatoprotective effect of SQEE80 on ethanol toxicity.

2. Materials and methods

2.1. Bamboo extraction preparation

S. quelpaertensis leaves purchased from Jeju Plant Resource Lab in 2015 at dry condition were pulverized into 1 mm fine powder to increase the surface area. Then, coarse powder of (5 g) *S. quelpaertensis* leaf extract (SQE) was obtained by dissolving the pulverized fine powder in 100 mL of distilled water (SQEDW), 20% (v/v) EtOH (SQEE20), 40% (v/v) EtOH (SQEE40), 60% (v/v) EtOH (SQEE60), and 80% (v/v) EtOH (SQEE80), respectively, in ultrasonic bath (Power sonic 520, Hwashin Co., Korea) for 90 min. The extraction process was carried out in triplicate to maximize the yield. The solution was filtered by using Whatman No. 2 filtering papers (Whatman International Limited, Kent, England) and concentrated to dryness by using rotary vacuum evaporator (Hei-VAP Precision, Heidolph, Germany). Dried samples were stored under −20 °C until usage.

2.2. Cell culture

HepG2 cells, human liver epithelial-like monolayer hepatoblastoma cells, were used for *in vitro* assays. Although derived from the liver tissue of a hepatocellular carcinoma patient, HepG2 cells exhibit many characteristic functions of normal human liver hepatocytes and are widely used in liver related studies (Balasubramaniyan et al., 2007; Knasmüller et al., 2004; Louvet and Mathurin, 2015; Reddy et al., 2008). HepG2 cells of initial passage number 100 were obtained from Korean cell line bank (The Korean cell line bank (KCLB) number 88065). Cells were cultured in 75 cm^2 cell culture flasks (SPL Life sciences, Korea) containing Dulbecco's modified eagle medium supplemented with 10% fetal bovine serum and 1% antibiotics (100 U/mL of penicillin and 100 µg/mL of streptomycin, Gibco Life Technologies, NY, USA), and incubated at 37 °C under 5% CO_2 in a humidified chamber. Cells reached > 80% confluence were subcultured at the density of 4×10^6 cells/dish. HepG2 cells of > 95% viability were used in the current study.

2.3. MTT and colony formation assay to measure cytotoxicity and cytoprotective effect of SQE in ethanol exposed HepG2 cells

Thiazolyl blue tetrazolium bromide (MTT) assay was performed to assess the cell viability as previously described (Sun et al., 2011). In brief, HepG2 cells (1×10^4/well) were seeded in 96-well plates in triplicate and allowed for 12 h to adhere to each other at 37 °C under 5% CO_2 in a humidified chamber. Then, cells were treated with varying concentrations (0, 3.9, 7.8, 15.6, 31.2, 62.5, 125, 250, 500, and 1000 µg/mL) of various SQEs (SQEDW, SQEE20, SQEE40, SQEE60, SQEE80), respectively, and incubated in a humidified chamber. Similarly, cells were treated with identical set of concentrations of various SQEs, respectively, with simultaneous exposure to 800 mM ethanol and incubated similarly. After 24 h incubation, 15 µl of MTT (5 mg/mL of stock, Sigma-Aldrich) was added to each well of both batches for 4 h incubation. Then formazan crystals were dissolved in 100 µL/well of solubilization buffer [10% sodium dodecyl sulfate (SDS) and 50% dimethyl formamide (Sigma-Aldrich)] and absorbance of each well was read at 540 nm using an immunosorbent assay (ELISA) plate reader.

In parallel, we performed colony formation assay to assess the clonogenesity of HepG2 cells. HepG2 cells (1×10^3) were seeded in 6-well plates and incubated for 10 days to form normal colonies, and, after an overnight attachment, cells were exposed to varying concentrations of different SQEs with or without EtOH for 24 h. The cells were then washed with DPBS and allowed to grow for 10 days (Wu et al., 2015). Medium was replaced at every three days. Then the cells were fixed with a methanol/acetic acid (3:1) mixture for 5 s at room temperature. The fixed cells were washed with DPBS and stained with 0.4% crystal violet for 30–60 s. Stained cells were washed with distilled water several times, air dried, and the number of colonies was counted by eye. Confirming that SQEE80 was the most effective extract fraction through preliminary experiments, we utilized it throughout the rest of this study.

2.4. DCF-DA assay

Production of intracellular ROS was evaluated using 2,7-dichlorofluorescein diacetate (DCF-DA) assay as previously described with minor modifications (Žegura et al., 2004). In brief, HepG2 cells were seeded at a density of 2.5×10^4/well in 96-well plates in triplicate and allowed to adhere to each other for 12 h in a humidified chamber at 37 °C under 5% CO_2. Cells were then treated with varying concentrations (0, 250, and 500 µg/mL) of SQEE80 with a simultaneous application of 800 mM ethanol in order to induce ethanol cytotoxicity. After 24 h exposure, cells were incubated with 2.5 mM of DCF-DA fluorescent dye for 30 min in dark. Fluorescence intensity at excitation wavelength of 485 nm and emission wavelength of 530 nm was determined using a