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Original article

Polydatin alleviates alcohol-induced acute liver injury in mice: Relevance of matrix metalloproteinases (MMPs) and hepatic antioxidants

Meghana Koneru[a], Bidya Dhar Sahu[a], Sagarika Gudem[a], Madhusudana Kuncha[a], Halley Gora Ravuri[a], Jerald Mahesh Kumar[b], Eswar Kumar Kilari[c], Ramakrishna Sistla[a,*]

[a] Medicinal Chemistry and Pharmacology Division, CSIR-Indian Institute of Chemical Technology (IICT), Hyderabad-500 007, India
[b] Animal House Facility, CSIR-Centre for Cellular and Molecular Biology (CCMB), Hyderabad 500 007, India
[c] Department of Pharmacology, A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530003, India

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ABSTRACT

Background: Alcohol, a most commonly consumed beverage, is the foremost cause of liver injury throughout the world. Polydatin, a stilbenoid glucoside, was known to possess antioxidant and anti-inflammatory properties and is being investigated for use in various disorders.
Purpose: The present study was intended at investigating the hepatoprotective efficacy of polydatin against acute-alcohol induced liver injury model in mice.
Study design: C57BL/6 mice were fed with five doses of 50% ethyl alcohol (10 ml/kg body weight) to induce acute liver injury. Effect of polydatin against alcohol induced hepatic injury was investigated by giving 50 or 100 mg/kg polydatin, orally, for 8 days.
Methods: Serum markers of liver injury, morphology, histology and fibrosis of liver tissue, levels of enzymatic and non-enzymatic antioxidants and the mitochondrial respiratory enzyme activities in liver tissue were investigated. The activities and the protein expression of matrix metalloproteinases (MMP-2 and -9), the expression of NF-κB in the liver tissue were also studied.
Results: Polydatin pre-treatment significantly alleviated the alcohol induced hepatic injury by reducing the serum liver injury markers, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), attenuating oxidative stress and restoring antioxidant balance in the hepatic tissue. Simultaneously, polydatin pre-treatment also prevented alcohol induced mitochondrial damage and refurbished the matrix metalloproteinases levels of the hepatic tissue.
Conclusion: The findings of the present study suggest that polydatin may have a potential benefit in preventing alcohol-induced acute hepatic injury.

Introduction

Alcohol-induced liver injury remains to be the leading cause for hepatic damage throughout the world (Tang et al., 2014a). The pathogenesis of alcohol induced hepatotoxicity is widely explored and established. Oxidation of ethanol by microsomal oxidizing enzyme cytochrome P450 2E1 (CYP2E1) and alcohol dehydrogenase results in the formation and accumulation of reactive oxygen species (ROS) in the liver (Jang et al., 2014). An unwarranted increase in the free radicals reduces the antioxidant enzyme levels and simultaneously enhances lipid peroxidation leading to the disintegration of the hepatocyte cell membrane (Yang et al., 2013). Furthermore, accumulation of ROS in the mitochondrial membrane depletes the mitochondrial complexes and causes mitochondrial dysfunction (Jaeschke et al., 2002). Acetaldehyde, the key metabolite of alcohol, along with ROS, stimulates the secretion of matrix metalloproteinases (MMPs), facilitating the degradation of extracellular matrix (ECM) components and simultaneously distorts the hepatic tissue architecture (Banerjee et al., 2013). An increase in the hepatic ROS also cause the activation of nuclear factor-kappa B (NF-κB), a nuclear transcription factor, which causes various cel-

Abbreviations: PD, polydatin; Sly, silymarin; Alc, alcohol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ROS, reactive oxygen species; CYP2E1, cytochrome P450, 2E1; HO-1, Heme-oxygenase 1; MMPs, matrix metalloproteinases; TIMP, tissue inhibitor of metalloproteinase; NF-κB, nuclear factor-kappa B.

* Correspondence author.
E-mail address: sistla@iict.res.in (R. Sistla).

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lular transformations including initiation of inflammation (RomaŃ et al., 1999).

Though the etiology of alcohol induced liver disease is well established, effective therapies aimed at preventing its progression were not much studied and still require further attention. Investigation of new chemical entities for the prevention of alcohol induced hepatocellular damage would be highly beneficial to the society. Polydatin (3, 4′, 5-trihydroxystibene-3-β-mono-D-glucoside), a stilbenoid glucoside, usually isolated from the roots of *Polygonum cuspidatum*, can also be obtained from many dietary supplements like grapes, peanuts, cocoa products, and hop flowers (*Humulus lupulus*) (Liu et al., 2015a). Several pharmacological investigations revealed that polydatin, through its antioxidant, anti-inflammatory and anti-apoptotic properties, is effectively used in treating health related disorders like diabetes, cardiac disabilities, learning and memory disorders, atherosclerosis, various carcinomas. It is also indicated in several liver ailments owing to its hepatoprotective properties (Du et al., 2013; Zhang et al., 2012). Though several pharmacological activities of polydatin were studied, the effect of polydatin towards alcohol induced liver injury is not profoundly explored (Pace et al., 2015). Based on the various promising pharmacological properties of polydatin, we aimed to study its protective effect against alcohol induced acute liver injury and investigate the possible underlying mechanisms.

Material and methods

Chemicals, kits and antibodies

Ethanol, absolute (99.9%) was obtained from Changshu Yangyuan chemicals, China. Polydatin (purity: ≥95%), silymarin (purity: ≥98%), reduced glutathione (GSH), 5, 5-dithio-bis (2-nitrobenzoic acid) (DTNB), oxidized glutathione (GSSG) and 2-thiobarbituric acid (TBA) were purchased from Sigma-Aldrich, USA. Antibodies against MMP-2, MMP-9 and TIMP-1 were purchased from Santa Cruz Biotechnology Inc., USA. NF-κB (p65), heme oxygenase-1 (HO-1) and β-actin antibodies were purchased from Cell Signalling Technology Ltd., USA. All other reagents used were of analytical grade.

Experimental animals

C57BL/6 male mice of 20–25 g were acquainted to the laboratory conditions before the start of the study in the BIOSAFE, an animal quarantine facility of the institute (Registration No: 97/GO/RBi/S/1999/CPCSEA). All animals were given standard pellet diet and fresh drinking water *ad libitum*. The animals were maintained under 12 h/12 h of light/dark cycle, 24 ± 3 °C temperature and 55 ± 15% relative humidity throughout the experiment. All the animal study protocols were performed following the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines under the supervision of Institutional Animal Ethics Committee (IAEC) of the CSIR-Indian Institute of Chemical Technology (IICT), Hyderabad, India (Approval No: IICT/14/2015).

Experimental design

All the experimental animals were randomly assigned to five groups containing eight animals in each group. The animals of group-I (Vehicle control, Control) and group-II (Alcohol control, Alc) were administered with a 2% gum acacia suspension orally (p.o.) for a period of 8 days. All animals of group-III (Positive control; Silymarin + Alcohol, Alc + Sly) were given silymarin (25 mg/kg) in 2% gum acacia suspension orally (p.o.) for 8 days. Mice of group-IV (Polydatin-50 + Alcohol, Alc + PD 50) and group-V (Polydatin-100 + Alcohol, Alc + PD 100) were given 50 and 100 mg/kg of polydatin respectively in 2% gum acacia suspension orally (p.o.) for 8 days. Starting on day 6, mice of group-I were intragastrically administered with 5 doses of 10 ml/kg of water at an interval of 12 h. Mice of all the other groups (Alc, Alc + Sly, Alc + PD-50, Alc + PD-100) were intragastrically administered with 5 doses of 10 ml/kg of 50% ethanol at 12 h interval. The dose of alcohol was selected based on the previously established protocol (Koneru et al., 2016).

On day 8, blood was withdrawn from the retro orbital plexus and the animals were euthanized through CO2 asphyxiation. The change in body weight (%) was measured by comparing the end day body weight with the body weight on the day of alcohol administration. Serum was separated and used for the estimation of biochemical parameters. Liver tissue was dissected, washed, weighed and stored at -80 °C for further analyses.

Estimation of biochemical parameters

The serum obtained from the experimental animals was analysed in an auto analyser (Siemens, Dimension Xpandplus, USA) for the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using respective kits (Siemens Diagnostics, USA).

Estimation of cellular antioxidant levels

The levels of various antioxidants like catalase (CAT) (Aebi, 1974), superoxide dismutase (SOD) (SOD assay kit, Sigma-Aldrich Chemical Company, USA), reduced glutathione (GSH) (Ellman, 1959), glutathione S-transferase (GST) (Habig et al., 1974), glutathione reductase (GR) (Carlberg and Mannervik, 1975), vitamin C (Omaye et al., 1979) and NAD (P) H: quinine oxidoreductase-1 (NQO1) (Zhu et al., 2005) were assessed as markers of the oxidative stress by following previously reported protocols. The total protein content was estimated using Bicinchoninic acid (BCA) assay kit (Pierce Biotechnology, Rockford, USA) and by employing BSA as standard.

Estimation of lipid peroxidation, protein carbonyl content and nitrites

The degree of lipid peroxidation was measured as thiobarbituric acid reactive substances (TBARS) by a previously reported method (Ohkawa et al., 1979). Simultaneously, the amount of protein carbonyl content (Dalle-Donne et al., 2003) and nitrite levels (Sahu et al., 2011a) were estimated in the hepatic tissue according to a previously established methods.

Isolation of hepatic microsomes and estimation of CYP2E1 activity

The microsomal fraction of the hepatic tissue was isolated and this microsomal rich fraction was used to estimate the enzymatic activity of CYP2E1 by following a previously reported protocol (Cannady et al., 2003).

Isolation of mitochondria and estimation of mitochondrial respiratory enzymes

The hepatic fractions rich in mitochondria were isolated using a previously reported method (Johnson and Lardy, 1967). The levels of various mitochondrial respiratory enzymes NADH dehydrogenase (King and Howard, 1967), Succinate dehydrogenase (King, 1967), cytochrome c oxidase (cytochrome c oxidase assay kit, Sigma Aldrich Co., MO, USA) and mitochondrial redox activity (MTT assay) (Sahu et al., 2014b) were measured as per previously reported protocols.