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Original Article

Hepatoprotective effects and antioxidant, antityrosinase activities of phloretin and phloretin isonicotinyl hydrazone

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Abstract

Background: Acute liver damage is primarily induced by one of several causes, among them viral exposure, alcohol consumption, and drug and immune system issues. Agents with the ability to inhibit tyrosinase and protect against DNA damage caused by reactive oxygen species (ROS) may be therapeutically useful for the prevention or treatment of ROS-related diseases.
Methods: This investigation examined the hepatoprotective effects of phloretin and phloretin isonicotinyl hydrazone (PIH) on D-galactosamine (D-GalN)-induced acute liver damage in Kunming mice, as well as the possible mechanisms. The serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (γ-GT), alkaline phosphatase (ALP), and total bilirubin (TB) as well as the histopathological changes in mouse liver sections were determined. The antioxidant effects of phloretin, quercetin, and PIH on lipid peroxidation in rat liver mitochondria *in vitro*, 1,1-diphenyl-2-picrylhydrazyl (DPPH) or 2,2-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) free radical scavenging activity *in vitro*, and supercoiled pBR322 plasmid DNA were confirmed. The experiment also examined the antityrosinase activity, inhibition type, and inhibition constant of phloretin and PIH.
Results: Phloretin, quercetin, or PIH significantly prevented the increase in serum ALT, AST, γ-GT, ALP, and TB in acute liver damage induced by D-GalN, and produced a marked reduction in the histopathological hepatic lesions. Phloretin, quercetin, or PIH also exhibited antioxidant effects on lipid peroxidation in rat liver mitochondria *in vitro*, DPPH or ABTS free radical scavenging activity *in vitro*, and supercoiled pBR322 plasmid DNA. Phloretin, quercetin, or PIH also exhibited good antityrosinase activity.
Conclusion: To the best of our knowledge, this was the first study of the hepatoprotective effects of phloretin and PIH on D-GalN-induced acute liver damage in Kunming mice as well as the possible mechanisms. This was also the first study of the lipid peroxidation inhibition activity of phloretin and PIH in liver mitochondria induced by the Fe^{2+}/vitamin C (Vc) system *in vitro*, the protective effects on supercoiled pBR322 plasmid DNA, and the antityrosinase activity of phloretin and PIH.

Keywords: acute liver damage; antioxidant activity; antityrosinase activity; D-galactosamine; phloretin; phloretin isonicotinyl hydrazone

1. Introduction

Acute liver damage is mainly induced by a virus, alcohol, drugs, and immune system issues. The main treatment measures include administration of antioxidants and suitable support treatment. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase (γ-GT), alkaline phosphatase (ALP), and total bilirubin (TB) are the

Conflicts of interest: The authors declare that there are no conflicts of interest related to the subject matter or materials discussed in this article.

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most common diagnostic level indicators of liver damage. D-Galactosamine (D-GalN) is the interference agent of uridine diphosphate (UDP) in liver cells. UDP-D-GalN can hinder the biosynthesis of nucleic acid, glycoprotein, and glycogen. Finally, D-GalN can cause injury to and necrosis of liver cells, which is a common model used to observe the hepatoprotective effects of drugs.[1,2]

Phloretin is a type of flavonoid that has beneficial anticancer and antioxidant properties, and is primarily derived from various species of apple. Phloretin isonicotinyl hydrazone (PIH) is a new compound synthesized by our team, which has more antioxidant activity than phloretin.[3] Phloretin, whose molecular formula is $C_{15}H_{14}O_5$, is a di-hydrogen-chalcone flavonoid. Phloretin can fade melanin, making the skin whiten, with proven effects superior to kojic acid and arbutin. Additionally, phloretin is commonly used as a new type of whitening agent in cosmetics, and has many biological applications, including antioxidant activity.[4–8]

This experiment examined the hepatoprotective effects of phloretin and PIH on D-GalN-induced acute liver damage in Kunming mice as well as the possible mechanisms. The serum levels of ALT, AST, γ-GT, ALP, and TB and histopathological changes in mouse liver sections were determined. The antioxidant effects of phloretin, quercetin, or PIH on lipid peroxidation in rat liver mitochondria *in vitro*, 1,1-diphenyl-2-picrylhydrazyl (DPPH) or 2,2-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) free radical scavenging activity *in vitro*, and supercoiled pBR322 plasmid DNA were also determined. The experiment also examined the antityrosinase activity, inhibition type, and inhibition constant of phloretin, quercetin, or PIH.

2. Methods

2.1. Animals

Male and female Kunming mice (weighing 20–25 g), Sprague Dawley (SD) rats (weighing 200–220 g), and their food were obtained from the Experimental Animal Center of Jiangxi University of Traditional Chinese Medicine (Jiangxi, China). They were allowed free access to water and food. All the animals were housed in a room maintained at a temperature of $23 \pm 3^{\circ}C$ and a relative humidity of $50 \pm 10\%$, with artificial lighting from 8:00 AM to 8:00 PM for 1 week before and during the experiments. All experiments were performed in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China.

2.2. Chemicals and reagents

Diagnostic kits for ALT, AST, γ-GT, ALP, and TB were obtained from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Also, a C3606 organization mitochondria separation reagent kit was purchased from Biyuntian (Shanghai, China). D-GalN, phloretin, quercetin, DPPH, thiobarbituric acid (TBA), and ABTS were purchased from the Sigma Chemical Company (St. Louis, MO, USA). PIH is a new compound synthesized by our team, whose purity is more than 95%. Sodium dihydrogen phosphate, disodium phosphate, ferrous sulfate, potassium sulfate, and potassium persulfate ($K_2S_2O_8$) were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All other chemicals and solvents were of analytical grade and commercially available.

2.3. Synthesis and characterization of PIH

Phloretin (5.4854 g, 20 mmol) and isoniazid (2.8802 g, 21 mmol) were placed in a triple-neck flask and dissolved with 5 mL of ethanol. Subsequently, 50 mL of toluene and 0.5 g of p-toluene sulfonic acid were added and allowed to react for 48 hours. The synthesis route is shown in Fig. 1A. The liquid was separated three times using 40–70 μm silica gel column chromatography. The elution agents were composed of 20:1 ethyl acetate/methanol. Thin layer chromatography was carried out to detect PIH. The second component was collected and vacuum-dried to a constant weight.

A Hitachi L-2000 high-performance liquid chromatography (HPLC) instrument (Tokyo, Japan) and an Autima reversed-phase chromagraphy column (Tokyo, Japan) were used for the HPLC of PIH. The mobile phase was composed of 45% acetonitrile and 55% water. The volume of each sample was 10 μL and the wavelength used was 310 nm. The samples for the infrared (IR) spectrum were prepared via the potassium bromide method. The IR spectrum of PIH was measured within the scope of 400–4000 cm^{-1} using a Fourier transform infrared spectrometer (Tokyo, Japan). The hydrogen-1 nuclear magnetic resonance (NMR) and Carbon-13 NMR data and the HPLC and IR profiles of PIH were shown in the report.[3] The yield efficiency of PIH was $68 \pm 5.3\%$.

2.4. D-GalN-induced hepatotoxicity model

Mice were randomly divided into seven groups of eight animals (4 males and 4 females) each. In the control group and D-GalN-intoxicated group, animals were given a single dose of distilled water [0.4 mL/20 g, intragastrically (ig)] daily. In the test groups, animals were given phloretin or quercetin or PIH (0.877 or 1.754 mmol/kg, 0.4 mL/20 g, ig) once daily. All administrations were conducted for 7 consecutive days. On the 7th day, all mice except those in the control group were simultaneously given a D-GalN/water mixture (800 mg/kg, 0.2 mL/20 g, intraperitoneally) 1 hour after the last administration, whereas the control group received only distilled water. Then, all animals were fasted for 16 hours and were subsequently tested for the following analysis. Because the molecular weights of these three compounds are different, the same unit mmol was used to determine comparative pharmacodynamics. For example, 0.877 mmol/kg or 1.754 mmol/kg phloretin means 240.3 mg/kg or 480.6 mg/kg, whereas 0.877 mmol/kg or 1.754 mmol/kg quercetin means 265 mg/kg or 530 mg/kg. Because the newly synthesized pure compound PIH was obtained with great difficulty, only 0.877 mmol/kg (344.7 mg/kg) PIH was used.