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Antioxidant and hepatoprotective effects of *A. cerana* honey against acute alcohol-induced liver damage in mice

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ABSTRACT

A. cerana honey, gathered from *Apis cerana Fabricius* (*A. cerana*), has not been fully studied. Samples of honey originating from six geographical regions (mainly in the Qinling Mountains of China) were investigated to determine their antioxidant and hepatoprotective effects against acute alcohol-induced liver damage. The results showed that *A. cerana* honeys from the Qinling Mountains had high total phenolic contents (345.1–502.1 mg GA kg^{-1}), ascorbic acid contents (153.8–368.4 mg kg^{-1}), and strong antioxidant activities in DPPH radical scavenging activity assays (87.5–136.2 IC50 mg mL^{-1}), ferric reducing antioxidant powers (191.8–317.4 mg Trolox kg^{-1}), and ferrous ion-chelating activities (27.5–35.5 mg Na_2EDTA kg^{-1}). Pretreatment with *A. cerana* honey (Qinling Mountains) at 5, 10, or 20 g kg^{-1} twice daily for 12 weeks significantly inhibited serum lipoprotein oxidation and increased serum radical absorbance capacity (ORAC) ($P < 0.05$). Moreover, *A. cerana* honey inhibited acute alcohol-induced increases in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum ($P < 0.05$), reduced the production of hepatic malondialdehyde (MDA) ($P < 0.05$), and promoted superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities ($P < 0.05$). More importantly, it also remarkably inhibited the level of TGF-β1 in the serum and liver ($P < 0.05$). The results of this study indicate that administration of *A. cerana* honey prevents acute alcohol-induced liver damage likely because of its antioxidant properties and ability to prevent oxidative stress.

1. Introduction

Alcoholic liver disease is the most common cause of preventable morbidity and mortality from liver diseases worldwide (Addolorato, Mirijello, Barrio, & Gual, 2016; Mathurin & Bataller, 2015). In the liver, a metabolic hub of the human body, ethanol is oxidized to acetaldehyde by alcohol dehydrogenase and then converted to acetic acid by acetaldehyde dehydrogenase (Liu, 2014; Mello, Ceni, Surrenti, & Galli, 2008). This process generates hepatic cytochrome P4502E1 (CYP2E1) and reactive oxygen species (ROS), leading to increased activity of the microsomal ethanol oxidation system (MEOS) and aggravation of liver damage (Cederbaum, Lu, Wang, & Wu, 2015; Lu & Cederbaum, 2016; Neuman et al., 2015). According to previous studies, the combination of elevated CYP2E1 levels and oxidative stress can cause liver damage (Yang, Wu, & Cederbaum, 2014), so prevention of oxidative stress must be a key mechanism for protecting the liver from alcoholic injury. Notably, acute alcohol consumption is a popular way of drinking alcohol and accounts for the majority of alcohol consumed, and has given rise to typical acute alcoholic liver injury. More and more people are suffering from acute alcoholic liver injury (Stahre, Roeber, Kanny, Brewer, & Zhang, 2014).

Honey is a natural product produced by honey bees from nectar or secretions originally gathered from flowering plants. The main components of honey are saccharides (fructose and glucose), water and some microcomponents, including minerals, proteins, vitamins, organic acids, phenolic compounds and volatile compounds (da Silva, Gauche, Gonzaga, Costa, & Fett, 2016; Solayman et al., 2016). Honey is valuable because of its well-established antimicrobial, anti-cardiovascular, anticancer, and antidiabetic effects (Alvarez-Suarez, Giampieri, & Battino, 2013). In particular, phenolic compounds constitute an important class of biologically active compounds that act as antioxidants and scavenging free radicals (Can et al., 2015; Sousa et al., 2016). Studies in vivo revealed that honey increased serum antioxidant activity by improving the defenses against oxidative stress (Cheng et al., 2014; Cheng, Wu,

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Zheng, & Cao, 2015; Gheldof, Wang, & Engeseth, 2003). As research into honey has increased in depth, it has been reported that honey has potential hepatoprotective effects against chemical-induced (paracetamol and tetrachloride) liver damage, and the protective effects of honey have been demonstrated by a growing number of researchers (Saral et al., 2016; Wang et al., 2015; Yıldız et al., 2013).

Honey made by *Apis cerana Fabricius* (*A. cerana*) is a multifloral honey made from the nectar or secretions from the flowers of multiple honey source plants and is widely distributed in the mountains of China. *A. cerana* honey has been used as a folk medicine for thousands of years before the introduction of *Apis mellifera* into China. Traditionally, *A. cerana* honey is more nutritious than other honey varieties because of its long nectar cycle and the wide variety of nectar source that include some traditional Chinese medicinal materials. A number of books, including *Shen Nong's Herbal Classic* and *Compendium of Materia Medica*, have recorded the use of *A. cerana* honey as a medicine. Recently, fundamental research suggested that *A. cerana* honey's moisture, reducing sugar levels (especially fructose) and major proteins might make *A. cerana* honey a superior a functional food over *Apis mellifera* honey (Joshi, Pechhacker, Willam, & Von Der Ohe, 2000; Mudasar, Shah, Mathivanan, & Mir, 2013; Murphy, 2013; Won, Lee, Ko, Kim, & Rhee, 2008; Won, Li, Kim, & Rhee, 2009). We have confirmed the potential protective effects of honey on chronic alcoholic liver injury that have been previous reported (Cheng et al., 2014), and this study will evaluate, for the first time, the antioxidant activity of *A. cerana* honey from the Qinling Mountains of China and its hepatoprotective effects against acute alcohol-induced liver damage in mice.

2. Materials and methods

2.1. Materials and samples

Methanol (CH_3OH, HPLC-grade) was obtained from Merck (Darmstadt, Germany), while all other reagents used in the current study, such as 2,6-DCPIP (2,6-dichlorophenolindophenol), and Folin-Ciocalteu reagent were purchased from Tianjin Kemiou Chemical Reagent Co. (Tianjin, China). DPPH (1,1-diphenyl-2-picrylhydrazyl), ferrozine (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid monosodium salt), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman carboxylic acid), TPTZ (2,4,6-tri(2-pyridyl)-s-triazine), and AAPH (2,2′-azobis(2-amidino-propane) dihydrochloride), as well as chemical standards were purchased from Sigma-Aldrich (Steinheim, Germany), and the purity of each compound was > 98% based on HPLC analysis.

2.2. Honey samples

Honey samples were collected directly from honeycombs throughout China in 2015. All samples were stored at 4 °C prior to analysis. The geographical origins of honey samples were mainly from four regions (S1: Shaanxi Fengxian; S2: Shaanxi Zhouzhi; S3: Shaanxi Foping; and S4: Shaanxi Zhenping) in the Qinling Mountains of China, and two samples were from Chongqing (S5) and Hubei (S6). Six samples from each region were collected for analysis. The distribution of samples is displayed in Fig. 1, and the physicochemical characteristics are described in Table 1. To study the hepatoprotective activity of *A. cerana* honey from the Qinling Mountain area, honey samples (S7) used in mice treatments were a mixture of equal amounts of samples from the three areas (S1, S2, and S4) of the Qinling Mountain that showed the highest antioxidant activities in vitro (shown in Sections 3.1 and 3.2). All physicochemical characteristic analysis was carried out according to the method set out by the Association of Official Analytical Chemists (Kelrich, K. ur., 1990).

Each 10 g honey sample was dissolved in acidic distilled water (pH = 2), and was absorbed on to a XAD-2 resin. The mixture was eluted with distilled water and methanol at room temperature, and then, the methanol elution was evaporated under vacuum. Finally, the volume of the concentrate was adjusted to 4 mL with methanol, and it was filtered through a 0.45 μm membrane filter prior to chromatographic analysis.

2.3. HPLC conditions

Individual phenolic compounds were identified on an Agilent 1100 separation module (Agilent Technologies Deutschland, Waldbronn, Germany) connected to a diode-array detector with an SB-C18 column (Zorbax, 250 mm × 4.6 mm, 5.0 μm) at a flow rate of 1.0 mL min^{-1}. The mobile phase was A (methanol) and C (0.15% (v/v) acetic acid-water solution) using the following gradient elution: 0–10 min, 5–15% A; 10–20 min, 15% A; 20–25 min, 15–17% A; 25–30 min, 17–30% A; 30–50 min, 30–40% A; 50–60 min, 40–55% A; 60–70 min, 55–70% A; 70–75 min, 70% A. The injection volume was 10 μL, the column oven temperature was maintained at 30 °C, and the detection wavelength was fixed at 280 nm.

A standard working solution for calibration curves was prepared by diluting the mixture standard solution (containing chemical standards) with methanol to give a series of solutions with suitable concentrations. All calibration curves obtained by the HPLC method showed a good linear regression with the measured coefficients (R^2) and adequate coverage.

2.4. Antioxidant analyses

2.4.1. Total phenolic content (TPC)

TPC was estimated using the Folin-Ciocalteu method with a slight modification (Singleton, Orthofer, & Lamuela-Raventós, 1999). A solution of *A. cerana* honey (0.4 mL, 2 mg mL^{-1}) was mixed with 1.0 mL of Folin-Ciocalteu reagent for 5 min. Sodium carbonate solution (5.0 mL, 1 M) was added to the mixture under shaking. An hour later, the absorbance was measured at 760 nm. We prepared a standard curve using gallic acid (GA), and TPC is expressed in terms of GA equivalents per kilogram of honey (mg GA kg^{-1}).

2.4.2. Ascorbic acid content (ASAC)

The ascorbic acid content was measured as previously described (Khalil et al., 2012). Briefly, honey samples (100 mg) were extracted with 1% metaphosphoric acid (10 mL) for 45 min and filtered, the filtrate (1 mL) was then mixed with 2,6-DCPIP (7.5 mM, 9 mL), and the absorbance of the mixture was measured at 515 nm. ASAC is expressed in terms of mg of ascorbic acid per kg of honey (mg kg^{-1}).

2.4.3. DPPH radical scavenging activity

This assay was carried out using the Brand-Williams method (Brand-Williams, Cuvelier, & Berset, 1995). The honey samples were dissolved in methanol (0.1 g mL^{-1}), and different volumes of each sample were mixed with a solution of DPPH in methanol (0.1 mM, 4.0 mL). The reaction mixtures were left for 30 min at room temperature in the dark, and the absorbances were measured spectrophotometrically at 517 nm with methanol as the blank. The percent inhibition of DPPH radical scavenging activity (RSA) by *A. cerana* honey was calculated as follows:

$$RSA\% = (Absorbance_{control} - Absorbance_{sample})/A_{control} \times 100$$

where $Absorbance_{control}$ is the absorbance of the control (blank, without sample) and $Absorbance_{sample}$ is the absorbance of *A. cerana* honey. The IC 50 values (50% inhibiting concentration of *A. cerana* honey) are presented later in the text.

2.4.4. Ferric reducing antioxidant power (FRAP)

The FRAP assay is an evaluation of the reduction of ferric species to ferrous species at low pH (Benzie & Strain, 1996). *A. cerana* honey (0.5 mL at 0.2 mg mL^{-1}) was mixed with 10 mM TPTZ with 20 mM $FeCl_3$ in 300 mM acetate buffer (pH 3.6). The absorbance of the ferrous solution at 593 nm was measured, and a standard curve was prepared