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Hepatoprotective and antioxidant activity of anthocyanins in black rice bran on carbon tetrachloride-induced liver injury in mice

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ABSTRACT

The benefits of anthocyanin-rich black rice bran extract (ARBE) on the liver of carbon tetrachloride (CCl_4)-intoxicated mice was investigated. Based on the *in vivo* experiment, the effect of cyanidin-3-glucoside (Cy-3-G) and peonidin-3-glucoside (Pn-3-G), the predominant anthocyanins in ARBE, on CCl_4 insulted hepatocytes L-02 was further evaluated. Mice treated with ARBE for 7 weeks by oral administration showed reduced aminotransferase activities in serum, accompanied by enhanced superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity, while tiobarbituric acid reactive substances (TBARS), and 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels were significantly decreased compared to CCl_4-intoxicated model group. Histopathological observation showed ARBE administration alleviated the pathological changes in livers of CCl_4-intoxicated mice. Similarly, preincubation of L-02 cells with Cy-3-G or Pn-3-G significantly alleviated CCl_4-induced injury dose-dependently, exhibited higher cell viability, decreased aminotransferase activity and enhanced cellular antioxidant status. Furthermore, Cy-3-G showed much stronger hepatoprotective activity than Pn-3-G at the same concentration. HPLC analysis showed that Cy-3-G accounted for more than 88% of total anthocyanins in ARBE. These results indicate that ARBE is significantly beneficial to liver health, and that Cy-3-G is the predominant anthocyanin in ARBE exerting this effect. The antioxidant activity of anthocyanins is an important mechanism by which ARBE exerts hepatic health benefit.

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Abbreviations: ARBE, anthocyanin-rich black rice bran extract; CCl_4, carbon tetrachloride; Cy-3-G, cyanidin-3-glucoside; Pn-3-G, peonidin-3-glucoside; SOD, superoxide dismutase; GSH-Px, glutathion peroxidase; TBARS, tiobarbituric acid reactive substances; MDA, malondialdehyde; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; DMEM, Dulbecco's modified eagle's medium; FBS, foetal bovine serum; MTT, methylthizol-2-yl-2,5-diphenyl, tetrazolium bromide; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DMSO, dimethyl sulphoxide; BW, body weight

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1. Introduction

The liver has a pivotal role in the metabolism and detoxification of the majority of substances entering human body. Many factors, such as toxic chemicals, excessive consumption of alcohol and virus infections, can cause liver injuries to different extent. Liver diseases have nowadays become one of the main concerns threatening human health at a high prevalence (He et al., 2011; Tanaka et al., 2011). Therefore, both medical experts and general population are paying increasing attention to maintaining liver health and function. Thus, research on natural plant ingredients with potential hepatoprotective activity for functional food and nutraceutical development has been a hot topic in many fields, including nutrition and natural product chemistry.

Black rice is a relatively rare variety of rice mainly cultivated in Southeast Asia. It had been reserved exclusively for emperors as "tribute rice" during ancient Chinese dynasties. As a traditional medicinal food, black rice was recorded to have many health benefits such as invigorating spleen and warming liver in a well-known Chinese ancient pharmacopeia <*Ben Cao Gang Mu*>. Nowadays black rice is frequently appearing on the dining table of common people as a most important ingredient in the healthy diets recommended by dietitians, especially in Asian countries. Many functional foods and beverages with black rice as the raw material have been developed and they are favoured by consumers.

Recent studies have shown that the main difference between black and white rice is that the bran of black rice is highly enriched with phytochemicals, especially anthocyanins (Zhang, Zhang, Zhang, & Liu, 2010). Anthocyanins are flavylium (2-phenylchromenylium) cation derivatives that share 1–3 common hydroxylation at the C-3, C-5, and C-7 positions but have different number and substitution pattern of hydroxyl and/or methoxyl groups on the B-ring. They are the largest subclass of plant flavonoids and are widely distributed in any part of plants imparting vivid colours to flowers, fruits, vegetables, and grains. Besides being used as food pigments, anthocyanins have attracted a great deal of attention from researchers since they exhibit various bioactivities including vision preservation and prevention of Alzheimer's disease among others, based on their potent antioxidant capacity (Miyake et al., 2012; Shih, Chan, Liao, Wang, & Yen, 2010). Moreover, oxidative stress has been reported to be a vitally important mechanism by which various causes induce the occurrence and development of liver diseases (Duygu, Karsen, Aksoy, & Taskin, 2012). Therefore, anthocyanins with strong antioxidant activity are believed to be beneficial to liver health and several studies have reported the hepatoprotective effect of anthocyanin extracts from natural food and plants. Supplementation of red cabbage extract rich in anthocyanins attenuated the hepatic oxidative stress and lipid accumulation induced by high sugar and high fat diet in rats (Sankhari, Thounaojam, Jadeja, Devkar, & Ramachandran, 2012). Anthocyanins from purple-fleshed sweet potato ameliorated dimethylnitrosamine-induced liver fibrosis in rats by increasing the expression of antioxidant enzymes and nuclear factor erythroid 2-related factor 2 and decreasing the expression of inflammatory mediators (Hwang et al., 2011).

Up to now, more than 600 varieties of anthocyanins have been found in nature. The profiles and contents of anthocyanins from different sources are different. There was an apparent discrepancy in structure between the anthocyanins from black rice and those from other sources. The main anthocyanins in purple-fleshed sweet potato and red cabbage were acylated derivatives, including peonidin 3-*O*-(6-*O*-(*E*)-caffeoyl-2-*O*-β-D-glucopyranosyl-β-D-glucopyranoside)-5-*O*-β-D-glucoside and peonidin 3-*O*-(2-*O*-(6-*O*-(*E*)-caffeoyl-β-D-glucopyranosyl)-6-*O*-(*E*)-caffeoyl-β-D-glucopyranoside)-5-*O*-β-D-glucopyranoside among others. (Qiu, Luo, Yao, Ma, & Kong, 2009; Scalzo, Genna, Branca, Chedin, & Chassaigne, 2008). In black rice, however, only non-acylated simple anthocyanins were detected (Zhang, Zhang, Zhang, & Liu, 2010). Although acylated anthocyanins were considered more stable than non-acylated ones (Sadilova, Carle, & Stintzing, 2007), non-acylated anthocyanins were reported to have higher bioavailability (Charron et al., 2009). Furthermore, previous research has shown that aglycone structure and the attached sugar moiety have significant effect on the activity of anthocyanins (Rahman, Ichiyanagi, Komiyama, Hatano, & Konishi, 2006). Therefore, despite the existing results about the hepatoprotective activity of anthocyanins from some materials, it can not be deduced that anthocyanins from all sources have hepatic health benefits without scientific confirmation. As for black rice, there is, to the best of our knowledge, only one study involved in the hepatoprotective effect on alcohol-intoxicated rats (Hou, Qin, & Ren, 2010). However, since only a small portion (22.5%) of black rice extract was anthocyanins in this study, available evidence was not adequate to draw an affirmative conclusion about the protective effect of black rice anthocyanins on liver injury.

Therefore, the hepatoprotective effect of anthocyanin-rich black rice bran extract (ARBE) on carbon tetrachloride (CCl_4)-intoxicated mice was investigated in the present study. Based on the *in vivo* study, the protective activity of cyanidin-3-glucoside (Cy-3-G) and peonidin-3-glucoside (Pn-3-G), the predominant anthocyanin components in ARBE, on CCl_4 insulted hepatocytes L-02 was further evaluated. The objective of this study was (1) to investigate the hepatoprotective effect of ARBE on CCl_4-induced mice; (2) to confirm the predominant anthocyanins providing hepatoprotection in ARBE; (3) to demonstrate the relationship between the hepatoprotective activity and antioxidant activity of anthocyanins in ARBE.

2. Material and methods

2.1. Chemicals

Authentic standards of cyanidin-3-glucoside (Cy-3-G), peonidin-3-glucoside (Pn-3-G), were obtained from Polyphenols AS (Sandnes, Norway). Dulbecco's modified Eagle's medium (DMEM), foetal bovine serum (FBS), glutamine, penicillin, and streptomycin were purchased from GIBCO BRL Co. (Grand

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