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Cranberry anthocyanin extract prolongs lifespan of fruit flies

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ABSTRACT

Cranberry is an excellent source of dietary antioxidants. The present study investigated the effect of cranberry anthocyanin (CrA) extract on the lifespan of fruit flies with focus on its interaction with aging-related genes including superoxide dismutase (SOD), catalase (CAT), methuselah (MTH), insulin receptor (InR), target of rapamycin (TOR), hemipterus (Hep), and phosphoenolpyruvate carboxykinase (PEPCK). Results showed that diet containing 20 mg/mL CrA could significantly prolong the mean lifespan of fruit flies by 10% compared with the control diet. This was accompanied by up-regulation of SOD1 and down-regulation of MTH, InR, TOR and PEPCK. The stress resistance test demonstrated that CrA could reduce the mortality rate induced by H_2O_2 but not by paraquat. It was therefore concluded that the lifespan-prolonging activity of CrA was most likely mediated by modulating the genes of SOD1, MTH, InR, TOR and PEPCK.

1. Introduction

Free radical theory has been considered as one of the major aging theories even though it is still under debate (Peng et al., 2014; Speakman and Selman, 2011). It states that organisms become aged partially due to accumulation of oxidative damages caused by reactive oxygen species (ROS), namely hydroxyl radicals, superoxide anions and hydrogen peroxide. ROS are the byproducts of normal cellular metabolism of oxygen. Endogenous antioxidant enzymes in cells, including catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx), serve as a first line of defense against ROS, while exogenous antioxidants from diets such as plant flavonoids and vitamins function as a second line of defense in terminating the propagation of ROS reactions (Cutler, 1991; Willis et al., 2009).

Recent research has demonstrated that metabolic signaling pathways can regulate aging and lifespan. Insulin receptors (InRs) play a key role in insulin/insulin peptide signaling (IIS) pathway. It has been reported that independent mutation of InR leads to reducing insulin signaling and extending lifespan in fruit flies (Tatar et al., 2001). Target of rapamycin (TOR) is a central controller of cell growth and influences aging (Wullschleger et al., 2006). Inhibition of TOR extends lifespan in both *Drosophila melanogaster* and *Caenorhabditis elegans* (Jia et al., 2004; Kapahi et al., 2004). Phosphoenolpyruvate carboxykinase (PEPCK) encodes a key enzyme that is involved in glucose and lipid metabolism (Chakravarty et al., 2005). Nectarine and plant extracts from Açai palm were reported to extend the lifespan of fruit flies partially due to down-regulation of PEPCK gene (Boyd et al., 2011; Sun et al., 2010). The Jun kinase (JNK) signaling pathway indirectly mediates the oxidative stress response in the cell and extends lifespan. Fruit flies with mutation in hemipterus (Hep), a *Drosophila* encoding gene of June kinase kinase (JNKK), were found more sensitive to oxidative stress and had a shortened lifespan (Wang et al., 2003). Age-related reduction in 26S proteasome activity is a key factor in the onset of neurodegenerative diseases. As one of the components in 26S proteasome, knocking down Rpn11 causes the accumulation of ubiquitinated proteins, and reduces 26S proteasome activity, and thus shortens lifespan (Tonoki et al., 2009). In contrast, over-expressing Rpn11 could reduce the age-related accumulation of ubiquitinated proteins, thus extending survival time of fruit flies (Tonoki et al., 2009). *Methuselah* (MTH) gene has been long recognized as one of the longevity-determined genes. It was reported that MTH mutant flies could live 35% longer than the wild strain and exhibited stronger resistance to exogenous oxidative stress (Lin et al., 1998). A recent study found that the wild-derived MTH *Drosophila* alleles such as, BF54/MTH$^{\Delta 6}$, BF54/MTH^{R3}, and S97/MTH^{R3} had a lifespan longer compared with S97/MTH$^{\Delta 6}$ (Paaby and Schmidt, 2008).

Interest in the relationship between diet and aging is increasing. Previous research have evidenced that both calorie restriction and some natural compounds in diet are able to extend the lifespan and delay the occurrence of age-related diseases in various aging models

Abbreviations: Cat, catalase; CrA, cranberry anthocyanins; DEPC, diethylpyocarbonate; dNTP, deoxy nucleotide triphosphate; Hep, hemipterous; GPx, glutathione peroxidase; InR, insulin receptors; JNK, C-jun N-terminal kinase; JNKK, C-jun N-terminal kinase kinase; MTH, methuselah; OR, oregon-R-C; PCR, polymerase chain reaction; PEPCK, phosphoenolpyruvate carboxykinase; ROS, reactive oxygen species; SOD, superoxide dismutase; SOD1, copper–zinc superoxide dismutase; SOD2, manganese superoxide dismutase; TOR, target of rapamycin.

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(Partridge et al., 2005; Peng et al., 2014). In this connection, fruit fly is one of the most commonly used models to investigate the effects of genetic modification and dietary intervention on aging (Lee et al., 2015). Cranberry has been regarded as a health fruit for its antioxidant, antimicrobial, anti-inflammatory, anti-cancer and anti-atherogenic properties (Duthie et al., 2006; Howell, 2007). Recent studies have found that cranberry extract could prolong the lifespan and increase heat shock tolerance of *C. elegans* or *D. melanogaster* (Guha et al., 2013, 2014; Wang et al., 2013). Given that anthocyanins are the most prevalent antioxidant polyphenols found in cranberry fruits, the present study was carried out to investigate the anti-aging activity of cranberry anthocyanin (CrA) extract with focus on its interaction with gene expressions of SOD1, SOD2, CAT, Rpn11, MTH, InR, TOR, Hep and PEPCK in *D. melanogaster*.

2. Materials and methods

2.1. HPLC analysis of CrA extract

CrA extract containing no vitamin C was obtained from Xi'an Realin Biotechnology Co., Ltd (Xi'an, China). The individual anthocyanins in CrA extract were separated on an Apollo C18 column (250 × 4.6 mm, 5 μm, Grace, Chicago, Illinois, USA) and quantified on a HPLC system with a UV detector at 520 nm. The flow rate was set at 1 mL/min, whereas the gradient mobile phase consisted of 5% acetic acid in water (Solvent A) and methanol (Solvent B). The gradient elution was programmed as following: 0–10 min, 40% B; 10–30 min, 40–45% B; 30–40 min, 45% B; and 40–50 min, 45–40% B. The peaks were identified according to the retention time and UV spectrum of authentic standards. HPLC analysis showed that the CrA extract used in the present study mainly contained cyanidin 3-galactoside (4.25%), cyanidin 3-arabinoside (4.75%), peonidin 3-galactoside (36.36%) and peonidin 3-arabinoside (4.72%) (Fig. 1).

2.2. Fruit fly strain and diets

The wild type fly strain Oregon-R-C (OR) was obtained from Bloomington Drosophila Stock Center (Department of Biology, Indiana University, Bloomington, IN, USA). The control diet was prepared as we previously described (Peng et al., 2009). In brief, 1000 mL of diet contained 105 g cornmeal, 105 g glucose, 21 g yeast and 13 g agar. Ethyl 4-hydroxybenzoate (0.4%) was added in diet to prevent mold growth. Two experimental diets were similarly prepared except for adding 5 (CrA5) or 20 mg (CrA20) of cranberry anthocyanin extract per milliliter diet. For rearing the stocks, 15 mL of the control diet was poured and set into a vial, while for the experimental flies, 5 mL of the control or experimental diets was prepared per vial. All the flies in each vial were incubated at a controlled incubator maintaining at 25 ± 1 °C with 60–70% humidity. In this study, only male flies were used because hormone level could regulate *Drosophila* aging and there was less hormonal effect in the male than that in the female flies (Yamamoto et al., 2013).

2.3. Effects of CrA extract on longevity

Three-day old male flies were randomly divided into three groups with 200 flies each, and reared in 10 vials (20 flies per vial). The control group was maintained on the control diet, while the other two groups were raised on CrA5 and CrA20 diets, respectively. Every 2–3 days, the dead flies were counted and the remaining ones were transferred to a new vial containing the same diet. The feeding lasted 81 days. Another set of fruit flies were maintained as described above and were killed at days 0, 15, 25, 35, 45 and 55 to quantify the expression of SOD, CAT, Rpn11, MTH, InR, TOR, Hep and PEPCK.

2.4. Measurement of body weights

Changes in body weights were used as an indicator of the food intake. To eliminate the possibility that the lifespan extension in survival assay might be due to effect of the dietary restriction, the body weights were recorded on days 0, 15, 25, 35, 45 and 55. Briefly, at selected days, 200 male flies in each group were anesthetized by carbon dioxide and then weighed on a balance. The mean body weights of the flies in each group were calculated.

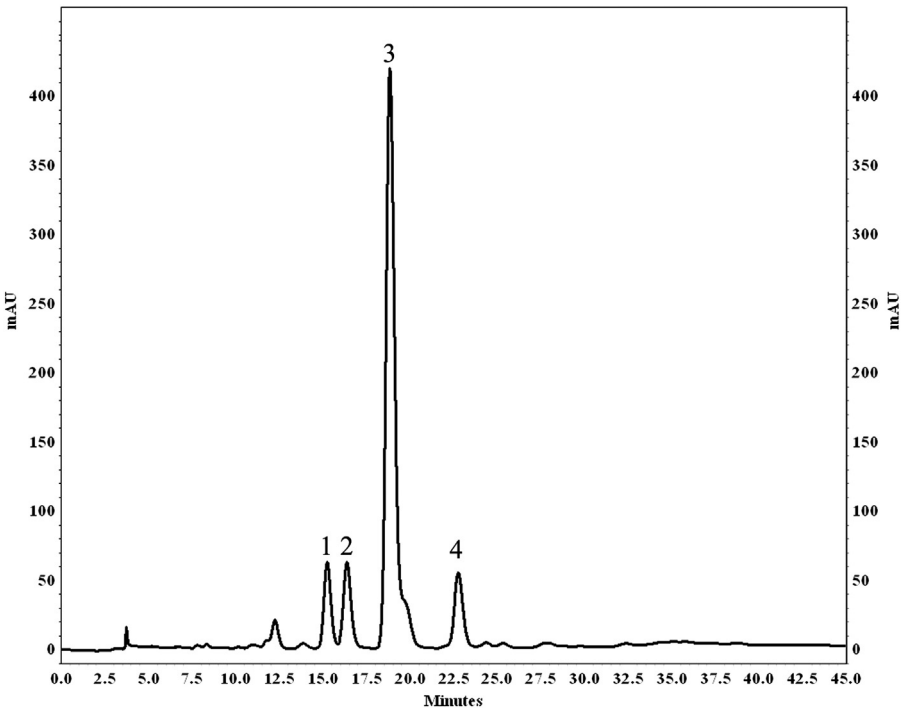

Fig. 1. HPLC chromatogram of cranberry anthocyanin (CrA) extract. Peaks: 1, cyanidin 3-galactoside; 2, cyanidin 3-arabinoside; 3, peonidin 3-galactoside; and 4, peonidin 3-arabinoside.