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Blueberry extract prolongs lifespan of *Drosophila melanogaster*

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ABSTRACT

Blueberry possesses greater antioxidant capacity than most other fruits and vegetables. The present study investigated the lifespan-prolonging activity of blueberry extracts in fruit flies and explored its underlying mechanism. Results revealed that blueberry extracts at 5 mg/ml in diet could significantly extend the mean lifespan of fruit flies by 10%, accompanied by up-regulating gene expression of superoxide dismutase (SOD), catalase (CAT) and Rpn11 and down-regulating Methuselah (MTH) gene. Intensive H_2O_2 and Paraquat challenge tests showed that lifespan was only extended in Oregon-R wild type flies but not in SOD^{n108} or Cat^{n1} mutant strains. Chronic Paraquat exposure shortened the maximum survival time from 73 to 35 days and decreased the climbing ability by 60% while blueberry extracts at 5 mg/ml in diet could significantly increase the survival rate and partially restore the climbing ability with up-regulating SOD, CAT, and Rpn11. Furthermore, gustatory assay demonstrated that those changes were not due to the variation of food intake between the control and the experimental diet containing 5 mg/ml blueberry extracts. It was therefore concluded that the lifespan-prolonging activity of blueberry extracts was at least partially associated with its interactions with MTH, Rpn11, and endogenous antioxidant enzymes SOD and CAT.

1. Introduction

Aging is a complex biological process. Oxidative stress has been shown to increase with aging. It is believed that accumulation of oxidative damages caused by reactive oxygen species (ROS) is one of the major contributors responsible for aging (Harman, 1956). To scavenge excess amount of ROS, antioxidant defenses including endogenous antioxidant system and exogenous antioxidant intake are indispensable. In this regard, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) serve as primary endogenous antioxidants to deactivate ROS in vivo, while exogenous antioxidants, such as vitamin C and E, polyphenols, help to eliminate free radicals by terminating the propagation of ROS reaction (Willis et al., 2009; Matés and Sánchez-Jiménez, 1999).

Mitochondria and longevity-determined genes may play a vital role in aging process. Mitochondrial respiratory capacity has been shown to decline with aging. As the terminal oxidoreductase of mitochondrial electron transport chain (ETC), cytochrome c oxidase (CcO) shows an age-related decline in both invertebrates and vertebrates (Schwarze et al., 1998). CcO deficiency would lead to the reduction of total ETC activity, resulting in increased amount of either superoxide anion radicals or hydrogen peroxide in mitochondria (Sohal et al., 2008). In addition, Rpn11 is one lid component of the multiple subunits making up the 19S regulatory subunit, which is essential for 26S proteasome intact structure and activity. It has been reported that knocking down of Rpn11 results in accumulated ubiquitinated proteins, reduces 26S proteasome activity, and shortens lifespan, whereas overexpressing Rpn11 can reduce age-related accumulation of ubiquitinated proteins, thus extending survival time (Tonoki et al., 2009). Moreover, one of the longevity-determined genes namely *methuselah* (MTH) is of great interest in *Drosophila* as MTH mutant flies can live 35% longer than the wild type strain as well as they exhibit stronger resistance to exogenous oxidative stress (Lin et al., 1998). However, the specific function of MTH and its potential connection with antioxidant defense systems remain unknown.

Fruit fly is one of the models to study aging and age-related diseases (Jafari, 2010). Humans actually share a huge amount of conserved biological pathways and diseases-causing genes with this tiny insect (Reiter et al., 2001; Bauer et al., 2004). Compared with other models, fruit fly is relatively easier to maintain in a large quantity due to their tiny body size and short lifespan. Previous

Abbreviations: CAT, catalase; CcO, cytochrome c oxidase; SOD1, copper–zinc containing superoxide dismutase; MTH, Methuselah; SOD2, manganese containing superoxide dismutase; SOD, superoxide dismutase, ROS, reactive oxygen species; ROS, reactive oxygen species.

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reports have revealed that dietary modification, including calorie restriction and dietary supplementation, can extend lifespan and ameliorate certain age-related diseases (McCay et al., 1935; Lin et al., 2002; Partridge et al., 2005; Lee et al., 2006; Piper and Bartke, 2008).

Blueberry, containing large amounts of polyphenols, possesses a greater antioxidant capacity than most other fruits and vegetables (Prior et al., 1998). It has been reported that consumption of blueberry can retard age-related physiological and functional deficits (Joseph et al., 2005). Krikorian et al. (2010) have recently finished their human trial study evaluating the health benefits of blueberry supplementation, revealing that daily consumption of wild blueberry juice for 12 weeks could improve memory function in older adults with early memory decline. However, larger sample size and more consistent clinical data are lacking to draw a conclusion. As to the experiments conducted in animal models, Galli et al. (2006) claimed that blueberry-supplemented diet could reverse age-related decline in hippocampal heat shock protein (HSP) neuroprotection in rats. Similarly, blueberry is also effective in enhancing cognitive and motor behavior as well as attenuating cognitive declines in object recognition memory in aged rats (Goyarzu et al., 2004). Furthermore, age-related deficits in N-methyl-D-aspartate receptor-dependent long-term potentiation, a cellular substrate for learning and memory, are also reported to be ameliorated by blueberry-enriched diet (Coultrap et al., 2008).

No report to date has studied the effect of blueberry on the lifespan of *Drosophila melanogaster*. The present study was therefore to investigate (i) anti-aging activity of blueberry extracts in fruit flies; (ii) interaction between supplementation of blueberry extracts and gene expressions of endogenous antioxidant enzymes, CcO subunits III and VIb, Rpn11, and MTH in *D. melanogaster*.

2. Materials and methods

2.1. Fly strains

Fly strains chosen in this study included Oregon-R-C (OR), OE^{-}/SM5;Cat^{n1}/TM3 (Cat^{n1}), and SOD^{n108}/TM3 (SOD^{n108}) (Bloomington Drosophila Stock Center, Department of Biology, Indiana University, Bloomington, IN, USA). OR is a wild type fly strain that was used in all experiments unless specified otherwise. SOD^{n108} is a mutant strain with one pair of single SOD gene on 3L chromosome being knocked out while Cat^{n1} is a mutant strain with CAT gene on chromosome 3L being knocked out by a point mutation.

2.2. Diet

The basal diet was prepared according to the standard formulation described previously (Li et al., 2007; Peng et al., 2009). In brief, 1000 ml diet contained 105 g cornmeal, 105 g glucose, 21 g yeast, and 13 g agar. Ethyl-4-hydroxybenzoate (0.4%) was added into diet as an anti-mold agent. Two experimental diets were prepared by adding blueberry extracts powder at 2 mg (Bbe) or 5 mg (BBE) in the control diet per milliliter. For rearing the stocks, 15 ml of the basal diet was poured and set into a vial. For the experimental flies, 5 ml of the basal or experimental diets were prepared per vial.

2.3. HPLC analysis of blueberry extracts

Blueberry extract was obtained from Tianjin Jianfeng Natural Product Co., Ltd, Tianjin, China. The extraction process is shown in the Supplementary Information. In brief, individual components in blueberry extract were separated on a C-18 (250×4.6 mm) column and quantified on a HPLC system with a UV detector at 520 nm. The column temperature and flow rate were set at 30 °C and 0.8 ml/min respectively. The gradient mobile phase consisted of 0.5% H_3PO_4 (solvent A) and H_2O:acetonitrile:acetic acid:phosphoric acid (50:48:5:1:0.5, solvent B). The ratio of A to B was programmed 4:1 to 2:3 in 26 min and then back to 4:1 in 4 min, and then was held for another 5 min. The peaks were identified according to the retention times of standards. The blueberry extracts in the present study mainly contained cyanidin-3-O-glu (49.2%) and petunidin-3-O-glu (20.1%) (Fig. 1).

2.4. Effect of blueberry extracts on longevity of OR flies fed the basal diet

Two independent trials were conducted. For each trial, newly eclosed male flies were divided into 3 groups (n = 200 each), and housed in 10 vials (20 flies per vial). The first group was maintained on the basal diet, while the two experimental groups were fed one of the Bbe and BBE diets. Dead flies were counted every 2–3 days and the remaining alive flies were transferred to a new vial containing the same diet. The feeding lasted 76 days (Fig. 2 A1 and A2). The maximum life spans in this study were calculated as the average life span of the 5% longest surviving flies. The same experiments described above were similarly repeated and the fruit flies were sacrificed at selected time points (days 0, 15, 25, 35, 45, 55) in order to quantify the expression of SOD, CAT, MTH, and Rpn11.

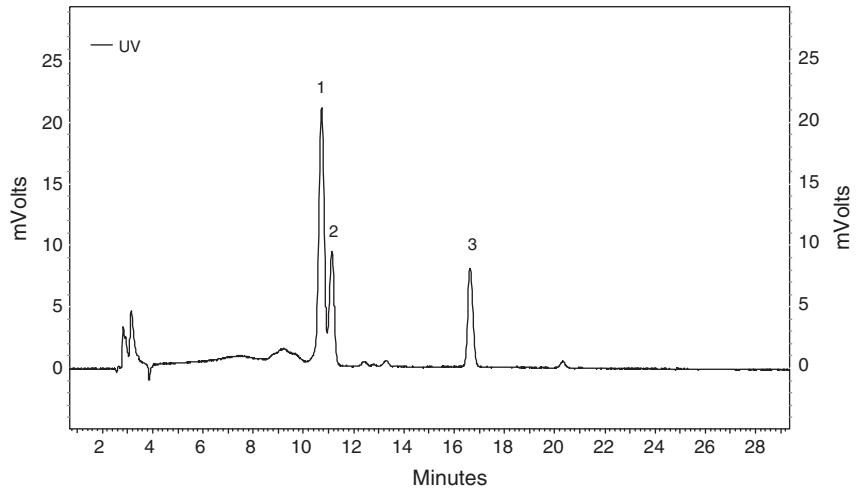

Fig. 1. HPLC chromatogram of blueberry extracts. Peaks: 1, cyanidin-3-O-glu; 2, petunidin-3-O-glu; 3, unknown.