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Research Article

Green tea catechins upregulate superoxide dismutase and catalase in fruit flies

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Chinese Longjing green tea is an excellent source of polyphenol antioxidants. HPLC analysis revealed that Longjing green tea catechin extract (GTC) contained 62% epigallocatechin gallate (EGCG), 19% epigallocatechin (EGC), 9% epicatechin gallate (ECG), and 7% epicatechin (EC). Investigating the effect of GTC on the lifespan of *Drosophila melanogaster*, we observed that a 10 mg GTC/mL diet could prolong its 50% survival time by 36% and mean lifespan by 16%. This was consistent with 17% reduction in total body lipid hydroperoxide (LPO) level in GTC-treated flies compared to the control group. Supplementation of 10 mg GTC/mL diet increased the survival time only in wild type Oregon-R-C (OR) but not in two mutant fly lines, $SOD^{n108}/TM3$ (gene for superoxide dismutase (SOD) was knocked out) and $Cat^{n1}/TM3$ (gene for catalase was knocked out), when the flies were challenged with paraquat or hydrogen peroxide. Accordingly, SOD and catalase activities in OR wild type increased by 40 and 19%, respectively. RT-PCR analysis indicated that the genes for copper–zinc containing SOD (CuZnSOD), manganese containing SOD (MnSOD), and catalase were upregulated. It was concluded that prolonging lifespan by GTC in *D. melanogaster* was influenced, among others, by upregulation of endogenous antioxidant enzymes.

Keywords: Catalase / Flies / Green tea catechins / Lipid peroxidation / Superoxide dismutase

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1 Introduction

The free radical theory of aging emphasizes that the free radical species cause deterioration of an organism [1]. On one hand, aerobic organisms develop a cellular metabolism that takes oxygen as an electron acceptor but they continuously generate reactive oxygen species (ROS), namely hydroxyl radical, superoxide anion, and hydrogen peroxide; on the other hand, they possess antioxidant defense systems that can effectively remove these ROS [2]. One of antioxidant defense systems includes a series of enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase, responsible for the removal of ROS [3]. It is also known that other free radicals exist as products of nonenzymatic reactions of oxygen and oxidants. Antioxidants are also present in foods, including ascorbic acid, vitamin A, vitamin C, α-tocopherol, and plant flavonoids, that are responsible for the removal of ROS and other free radicals in cells [4]. These dietary antioxidants and antioxidant enzymes work synergistically or independently to scavenge the free radicals [5]. These antioxidant systems build a defense base to terminate the propagation of the free radical reactions, limit the formation of new free radicals, and slow down the aging process [6].

Tea catechins have been a subject of extensive studies for their various biological activities. Tea catechins refer to a group of catechin derivatives including mainly (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epicatechin (EC), accounting for about 10% dry weight of tea leaf [7]. Drinking green tea modulates favorably blood lipid profile [8], inhibits the lipogenesis [9] and decreases risk for coronary heart diseases [10, 11]. Tea catechins have demonstrated an antiatherosclerosis activity in rabbits fed an atherogenic diet [12]. Various animal models have shown that tea catechins are tumor inhibitory [13]. Drinking tea is associated

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Abbreviations: CuZnSOD, copper–zinc containing superoxide dismutase; **GTC,** green tea catechin extract; **LPO,** lipid hydroperoxide; **MnSOD,** manganese containing superoxide dismutase; **ROS,** reactive oxygen species; **SOD,** superoxide dismutase

with an increased plasma antioxidant capacity in humans [14]. Tea catechins scavenge superoxide, hydroxyl radical, and copper-mediated LDL oxidation more effectively than alpha-tocopherol [15 – 18]. Tea catechins have been shown to work synergistically with α-tocopherol and ascorbic acid as well as other organic acids such as citric acid, malic acid, and tartaric acid against oxidation [19, 20].

Fruit fly, *Drosophila melanogaster*, is one of the most commonly used models to investigate the genetic determinants of aging [21]. Tea catechins are one of excellent dietary antioxidants. The present study hypothesized that supplementation of tea catechins in the diet of *D. melanogaster* could prolong its lifespan and capacity of scavenging the free radicals. We focused on interaction between dietary tea catechins and gene expression of endogenous antioxidant enzymes namely SOD and catalase in *D. melanogaster*.

2 Materials and methods

2.1 Fly diet

The fly diet was prepared according to the standard formulation described by Roberts and Standen [22]. In brief, 1000 mL of diet contained 105 g of cornmeal, 21 g yeast, 105 g dextrose, and 13 g agar. Ethyl-4-hydroxybenzoate (0.4%) was added to inhibit the growth of mold. Acetic acid (0.5%) was added into the fly diet to provide a low pH diet (pH 4 – 5) in which tea catechins were found stable [23]. The mixture was cooked. For rearing the stocks, 15 mL of the cooked mixture was poured into each vial. For the experimental flies, 5 mL of the cooked mixture was prepared *per* vial.

2.2 Green tea catechin extract (GTC)

Chinese Longjing green tea was used to prepare GTC as previously described [24]. Dry green tea (700 g) was soaked in 4 L of boiling water for 30 min, the infusion was filtered and condensed in a rotary evaporator. Equal volume of chloroform was used to remove the caffeine. After the chloroform was removed, the aqueous phase was extracted using an equal volume of ethyl acetate. GTC (30 g) was obtained after the ethyl acetate was evaporated employing a rotary evaporator. The content of tea catechins was analyzed and quantified using a Shimadzu LC-10AD HPLC (Tokyo, Japan) equipped with a ternary pump delivery system [23].

2.3 Fly lines

Fly stocks used in the present study included the wild type, Oregon-R-C (OR), and two mutant fly lines, $SOD^{n108}/TM3$ (SOD^{n108}) and $OE^{-}/SM5 \times Cat^{n1}/TM3$ (Cat^{n1}) (OR and Cat^{n1} were obtained from the Bloomington Drosophila Stock Center, FlyBase ID FBst0000005 and FBst0004014; SOD^{n108} was the gift from Professor John P. Phillips, Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada). SOD^{n108} is a mutant with one pair of single SOD gene on 3 L chromosome being knocked out. In contrast, Cat^{n1} is a mutant with catalase gene on chromosome 3 L being knocked out by point mutation [25]. They were reared on the standard diet inside vials with 40×69 mm^2 diameter and incubated at a controlled incubator at 25°C at 60 – 70% humidity.

For the following survival study, the stock fly population was expanded. In brief, adult flies (n = 1200) from the same generation were allowed to mate and lay eggs. To avoid overcrowding, four males and six females were placed in one vial for mating and laying eggs for a three-day-period. Male flies (two-day-old) were pooled from each vial under carbon dioxide anesthesia. To avoid the factors that may affect the lifespan [26], only male flies were chosen in the present study.

2.4 Dose effect of GTC on lifespan

Male flies were divided into four groups with 200 flies in each group rearing in ten vials (n = 20 flies *per* vial). The first group was maintained on the control diet, while the rest three groups were fed one of the three diets containing 1, 5, and 10 mg GTC/mL diet, respectively. The supplementation of 1, 5, and 10 mg/mL of GTC corresponds to the GTC concentration in regular tea infusion and beverage consumed by humans. In China, 100 mL of tea infusion is prepared by pouring hot water onto 5 – 10 g dry tea leaf, which contains about 10% green tea catechins. The dead flies were counted every 2 – 3 days and the remaining alive flies were then transferred to a new vial containing the same diet. The feeding lasted 85 days.

2.5 Measurement of lipid hydroperoxides (LPO)

A LPO assay kit (Cayman Chemical, Michigan, USA) was used to measure the LPO concentration. In brief, hydroperoxides in the sample reacted with ferrous ions and resulted in the formation of ferric ions, which was detected using thiocyanate ion as the chromogen. The flies were maintained on either the control diet or the experimental diet containing 10 mg GTC *per* mL for a period of 20 days (n = 300 each group). The adult fruit flies (n = 100) were weighted and homogenized in 2 mL of HPLC grade water. Debris was spun down at the speed of 1500 g for 5 min at 4°C. The supernatant (500 μL) was aliquoted into a tube in triplicates. The sample was deproteinized and extracted using methanol/chloroform (v/v; 1:2) saturated with nitrogen gas and followed by centrifugation at $1500 \times g$ for 5 min at 0°C. FTS reagent 1 (50 μL) containing 4.5 mM ferrous sulfate in 0.2 M hydrochloric acid and 50 μL of FTS reagent 2 containing 3% methanolic solution of ammonium thiocyanate were added. After incubated for 5 min at room

temperature, absorbance of each sample was measured in a spectrometer at 500 nm in 1 mL quartz cuvette.

2.6 Paraquat treatment

Paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride; Pq^{2+}) (Sigma, St. Louis, MO, USA) was used to examine the resistance of flies against oxidative stress. The chemical generates superoxide anions by NADH-dependent reduction. According to Michaelis and Hill [27], sluggishness of normal male flies would be observed after 12 h, and nearly 90% flies died after 48 h when 20 mM paraquat was added to the diet. Both OR flies (n = 400 in 20 vials) and SOD^{n108} mutant flies (n = 400 in 20 vials) were maintained on their corresponding control diet and experimental diet containing 10 mg GTC/mL, and incubated at 25°C. Every 2 days, flies were transferred into new vials containing corresponding fresh diet. At day 20, adult flies in two groups were first starved for 2 h, and then transferred into new vials containing a filter paper saturated with 1 mL of 20 mM paraquat in a 6% glucose solution. Every 4–6 h, dead flies were counted until all flies died.

2.7 Hydrogen peroxide (H_2O_2) treatment

H_2O_2 was also used to examine the resistance of flies against oxidative stress. Dietary H_2O_2 is not a free radical but it is a ROS, which is unstable and generates a hydroxyl radical in the presence of some metal ions. OR flies (n = 400) and cat^{n1} mutant flies (n = 400) were maintained on their corresponding control diet or experimental diet containing 10 mg GTC/mL and incubated at 25°C (400 each with 20 *per* vial). Similarly, adult flies were transferred into new vials containing the fresh diet every 2 days. At day 20, flies in all the groups were first starved for 2 h, and then were transferred into new vials containing a filter paper saturated with 1 mL of 30% H_2O_2 in a 6% glucose solution. Every 4–6 h, dead flies were counted until all of the flies died.

2.8 Enzyme activity assay

In each enzyme assay, 600 flies were used with 300 from the control group and 300 from the tested group. Flies (n = 20 *per* vial) were being reared on a 5 mL of the control diet or the experimental diet containing 10 mg/mL of GTC at 25°C. Every 2 days, flies were transferred into new vials containing their corresponding fresh diet. At day 20, flies were first starved for 2 h, and sacrificed by storing at –80°C for 10 min.

2.9 SOD activity

SOD was quantified using SOD assay kit (Cayman chemical), which detects the rate of formazan dye formed from tetrazolium salt by the action of superoxide radicals generated by xanthine oxidase and hypoxanthine. Flies (n = 100 in 10 vials) in each sample were homogenized in 1 mL of cold 20 mM HEPES buffer (pH 7.2, with 1 mM EGTA, 210 mM mannitol, and 70 mM sucrose). The homogenized mixture was centrifuged at the speed of 1500 × g for 5 min at 4°C. The supernatant was transferred into a new tube on ice and then subjected to centrifugation at 10 000 × g for 15 min at 4°C. The supernatant contained the cytosolic copper–zinc containing SOD (CuZnSOD), and the pellet contained mitochondrial manganese containing SOD (MnSOD). The supernatant was removed into a new tube and the mitochondrial pellet was suspended in 0.5 mL of cold HEPES buffer. The sample (10 μL) in triplicates was used for each test. The diluted radical detector containing tetrazolium salt (200 μL) was added onto 96-well plates together with 10 μL of sample. The reaction was initiated by adding 20 μL of diluted xanthine oxidase followed by shaking the plate for 20 min at room temperature. After incubation, the absorbance was recorded at 450 nm in a microplate reader.

2.10 Catalase activity

Catalase was measured using a catalase assay kit (Sigma). This method is based on the measurement of the hydrogen peroxide substrate remaining after the action of catalase present in the sample. The remaining hydrogen peroxide is measured in the presence of 3,5-dichloro-2-hydroxybenzenesulfonic acid, which couples oxidatively to 4-aminoantipyrine in the presence of hydrogen peroxide and horseradish peroxidase to give a red quinoneimine dye. Flies (n = 100) were homogenized in 1 mL enzyme dilution buffer followed by centrifugation at the speed of 1500 × g for 5 min at 4°C. The supernatant was transferred into a new tube on ice and diluted by mixing 3 μL of the supernatant with 42 μL 1 × assay buffer (5 mM potassium phosphate buffer, pH 7.0) in triplicates. The resultant sample (10 μ>L) was diluted again with 65 μL of 1 × assay buffer. Then, 25 μL of 200 mM hydrogen peroxide solution was added as the substrate solution to initiate the reaction. At exactly 1 min, 900 μL of stop solution (15 mM sodium azide) was added. The reaction mixture (10 μL) was mixed with 1 mL of color reagent containing 0.25 mM 4-aminoantipyrine, 2 mM 3,5-dichloro-2-hydroxybenzenesulfonic acid, and freshly added peroxidase (0.8–1.2 U/mg). After incubation for 15 min at room temperature, absorbance of each sample was measured in a spectrometer at 520 nm.

2.11 RNA isolation

Total RNA was isolated from 12 flies in triplicates by *TRI*-zol Reagent (Invitrogen Corporation, California, USA). *TRI*zol Reagent (800 μL) was added and the flies were homogenized in a tube. It was then centrifuged at

12 000 × *g* at 4°C for 10 min. The homogenized samples were incubated at room temperature for 5 min followed by addition of 160 μL of chloroform. After 3 min, the samples were subjected to centrifugation at 12 000 × *g* at 4°C for 15 min. The upper layer was transferred into a new tube with 400 μL of isopropanol and then stored at −20°C overnight. After overnight incubation, samples were subjected to centrifugation at 12 000 × *g* at 4°C for 10 min. The supernatant was discarded and the RNA pellets were mixed with 1 mL of 75% ethanol for washing. The pellets were re-pelleted by centrifugation and subjected to be air-dried. Finally, 25 μL of DEPC-treated water was used to resuspend the RNA pellet. For DNase digestion, 3 μL 10 × DNase buffer, 0.5 μL of DEPC-treated water, and 1.5 μL of DNase (Promega Corporation, Madison, WI, USA) were added and incubated at room temperature for 15 min followed by addition of 3 μL of EDTA and then incubation at 65°C for 10 min. Sodium acetate (4 μL, 3 M) and 3 μL of DEPC-treated water were mixed. Pure ethanol (80 μL) was introduced and then the samples were stored at −80°C overnight. After incubation, the samples were centrifuged at 12 000 × *g* at 4°C for 15 min and 1 mL of 70% ethanol was added afterwards for washing. Followed by being re-pelleted and air-dried, the RNA pellet was dissolved in 30 μL of DEPC-treated water and stored at −80°C. The quantity and purity of RNA were determined by absorbance reading at 260 and 280 nm. The quantity of RNA (mg/mL) was calculated by the formula $OD_{260} \times 40$ mg/mL and the ratio of OD_{260}/OD_{280} was greater than 1.8, showing a high purity of RNA.

2.12 Primer sequences

Primer sequences of CuZnSOD, MnSOD, and catalase were followed as published previously [28 – 30]. The forward and reverse primer sequences for CuZnSOD were 5′-taaattgattaattcattcg-3′ and 5′-acatcggaatagattatcgc-3′; for MnSOD, they were 5′-gcagatatgttcgtggcccgta-3′ and 5′-agttgcagtttgcccgacttct-3′; for catalase, they were 5′-ttcctggatgagatgtcgcact-3′ and 5′-ttctgggtgtgaatgaagctgg-3′, respectively.

2.13 Reverse Transcriptase PCR

A reverse transcription-PCR (RT-PCR) assay was carried out to quantify the mRNA level. RNA (5 μg) was responsible for cDNA synthesis together with the addition of Oligo dT, 5 × 1st strand buffer (with DTT), 10 mM dNTP, RNAse inhibitor, and M-MLV reverse transcriptase (Promega Corporation). The final volume was diluted to 20 μL. cDNA was amplified by PCR reaction. In each reaction, 0.4 μL 10 mM dNTP, 1 μL cDNA template, 5 mM of each primer, 10 × PCR buffer, 1 U Taq polymerase (Promega Corporation), and ddH_2O were added to obtain a final volume of 20 μL. The PCR products were separated on a 1.5% agarose gel, stained with 0.01 μg/mL ethidium bromide, and photographed.

2.14 Statistics

Data were expressed as mean ± SD. Student's *t*-test was used for statistical evaluation of differences between groups (SigmaStat version 2.01, SigmaStat Advisory Statistical Software, MO, USA), and two-way analysis of variance (ANOVA) was used for statistical evaluation of differences between groups at different time intervals (SPSS version 11.0, Statistical Package for the Social Sciences software, SPSS, Chicago, USA). $p < 0.05$ was considered statistically significant. To simplify the statistical analysis, $p < 0.05$ was indicated if *p*-value was smaller than 0.05 and bigger than 0.01, while $p < 0.01$ was indicated if *p*-value was smaller than 0.01.

3 Results

Chinese Longjing green tea is an excellent source of polyphenol antioxidants. The HPLC analysis revealed that GTC contained 62% EGCG, 19% EGC, 7% EC, and 9% ECG (Fig. 1).

The present study demonstrated that the antiaging effect of GTC was dose-dependent. As shown in Fig. 2, maximum lifespan of GTC supplemented groups was longer than the control flies. When the lifespan was calculated by the survival time of the last dead fly, all the GTC supplemented groups reached a maximum lifespan of 79 days, but the control group only reached 75 days. The increase was nearly 5% of the total lifespan. In addition, 50% survival time increased as the doses of GTC increased. It was also found that 10 mg/mL of GTC was the most effective dose with 50% survival time reaching 57 days compared with the control value (42 days), increasing by 36%. As shown in Fig. 2, the GTC groups showed a dose-dependent increase in mean lifespan. The control group had a mean lifespan of 51 days, whereas the three GTC groups had 54, 55, and

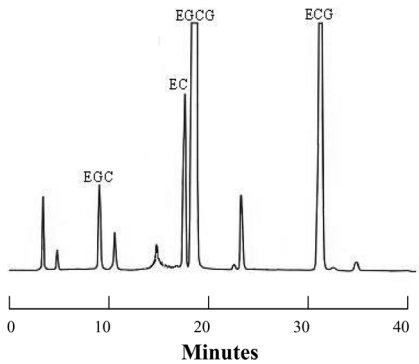
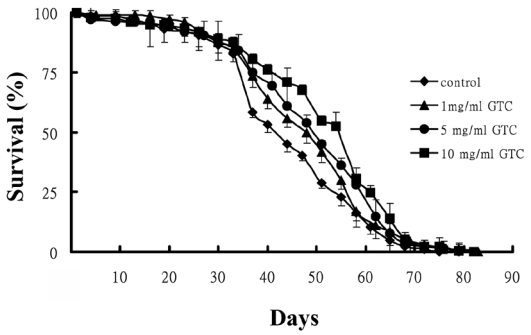


Figure 1. HPLC chromatogram of GTC isolated from Chinese Longjing green tea.



	Maximum Lifespan of Last Fly (Days)	50 % Survival (Days)	Mean Lifespan (Days)
Control	75	42	51 ± 2.0^{c}
1 mg/ml	79	48	54 ± 1.9^{bc}
5 mg/ml	79	51	55 ± 2.0^{b}
10 mg/ml	79	57	59 ± 2.8^{a}

Figure 2. Lifespan curve of wild type flies (OR) fed diets containing 0 mg/mL (control), 1, 5, and 10 mg GTC/mL. Data were expressed as the maximum lifespan of the last fly, 50% survival time, and mean lifespan ($n = 200$ flies) for each group. Data are expressed as mean ± SD. Means at the same column with different superscripts differ significantly at $p < 0.05$.

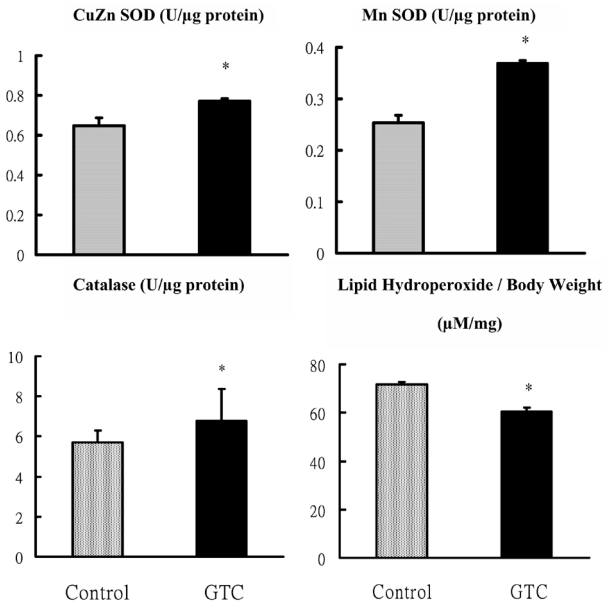
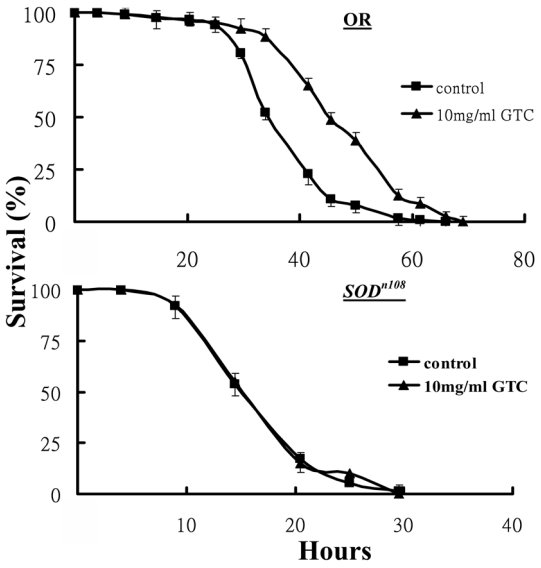


Figure 3. Effect of GTC supplementation (10 mg/mL diet) for 20 days on the whole body LPO level, and enzymatic activity of CuZnSOD, MnSOD, and catalase, compared with the control diet (0 mg GTC/mL diet). The wild type (OR) flies ($n = 300$/group, $n = 20$/vial) were incubated at 25°C for 20 days. Data are expressed as mean ± SD. * differs significantly from that of the control value at $p < 0.05$.

59 days of mean lifespan, respectively. Supplementation of 5 and 10 mg GTC/mL diet increased significantly the mean lifespan by 8 and 16% compared with that of the control.

The present study investigated the effect of 10 mg of GTC on total body LPO level in *D. melanogaster*, demonstrating that supplementation of 10 mg GTC/mL diet could



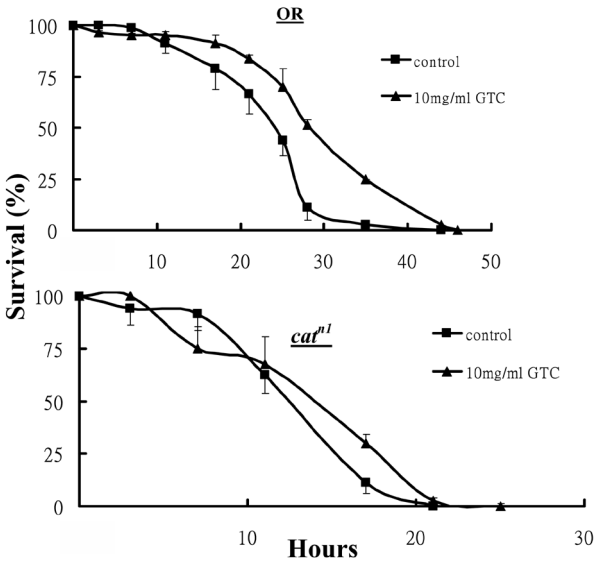
	Maximum Survival Hour of Last Fly (Hours)	50% Survival (Hours)	Mean Survival Time (Hours)
Group (OR)			
Control	61	35	35±3.9
10 mg/ml	66	45	43±4.5*
Group (SOD^{n108})			
Control	29	16	15±2.4
10 mg/ml	29	16	15±2.2

Figure 4. Effect of paraquat treatment on the survival time of wild type flies (OR) and the mutant flies (SOD^{n108}) fed the diets containing 0 mg/mL (control) or 10 mg GTC/mL. Data were expressed as the maximum survival hour of last fly, 50% survival time and mean survival time ($n = 400$ flies) for each group. Data are expressed as mean ± SD. * differs significantly from that of the control value at $p < 0.01$.

lower the LPO formation by 16.7% ($p < 0.05$). As shown in Fig. 3, the LPO level for the control was 72 ± 2 μM/mg of flies, whereas it for the GTC group was 60 ± 2 μM/mg of flies.

The major finding in the paraquat treatment was that supplementation of 10 mg GTC *per* mL diet only prolonged the survival time of OR wild type flies ($p < 0.01$) and it did not affect SOD^{n108} (Fig. 4). About 50% flies in the control group died at 35 h, but the survival percentage of the GTC group remained 89%, its 50% survival time was prolonged to 45 h. The increase in 50% survival time of the GTC group was nearly 29% compared with the control. For the mutant flies (SOD^{n108}), no significant difference in the resistance was observed between the GTC group and the control group (Fig. 4).

A similar trend was observed in the hydrogen peroxide treatment experiment. As shown in Fig. 5, GTC supplementation in diet (10 mg/mL) significantly increased the resistance in OR flies ($p < 0.01$) but not in Cat^{n1} mutant flies. In the control OR flies, 50% flies died at 25 h, when 75% GTC supplemented flies were still alive. The increase in 50% sur-



	Maximum Survival Hours of Last Fly (Hours)	50% Survival (Hours)	Mean Survival Time (Hours)
Group (OR)			
Control	35	25	22±1.5
10 mg/ml	44	28	26±3.4*
Group (*Cat*^{n1})			
Control	17	13	12±1.5
10 mg/ml	21	14	13±2.7

Figure 5. Effect of hydrogen peroxide treatment on the survival time of wild type flies (OR) and the mutant flies (*Cat*^{n1}) fed the diets containing 0 mg/mL (control) or 10 mg GTC/mL. Data were expressed as the maximum survival hour of last fly, 50% survival time, and mean survival time ($n = 400$ flies) for each group. Data are expressed as mean ± SD. * differs significantly from that of the control value at $p < 0.01$.

vival of GTC group was nearly 12% compared with the control. In contrast, GTC supplementation had no effect on 50% survival time in *Cat*^{n1} mutant flies (Fig. 5).

The results of the present study revealed that GTC supplementation increased SOD and catalase activities in the OR flies. One unit (U) of activity of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. As shown in Fig. 3, CuZnSOD activity for the control was 0.25 ± 0.03 U/μg protein, but that in the GTC group was 0.33 ± 0.04 U/μg protein ($p < 0.05$). For MnSOD activity, the control value was 0.25 ± 0.01 U/μg protein, that in the GTC group was 0.37 ± 0.01 U/μg protein (Fig. 3). One unit (U) of catalase activity is defined as the amount of catalase is needed to decompose 1μm of hydrogen peroxide to oxygen and water *per* minute at pH 7.0 at 25°C at a substrate concentration of 50 mM hydrogen peroxide. As shown in Fig. 3, catalase activity for the control was 5.70 ± 0.12 U/μg protein; in contrast, that for the GTC group was 6.79 ± 0.42 U/μg protein ($p < 0.05$). In general, GTC group had a significant increase in CuZnSOD, MnSOD, and catalase activities by 32, 48, and 19%, respectively ($p < 0.05$).

GTC supplementation upregulated gene expression of CuZnSOD, MnSOD, and catalase. The expression level of each target gene was measured against β-actin. As shown in Fig. 6, the ratio of CuZnSOD gene expression to β-actin for the control was 0.65 ± 0.04, while that in the GTC group was 0.77 ± 0.01 with a significant increase by 18% ($p < 0.01$). The ratio of MnSOD gene expression to β-actin was 0.61 ± 0.01 in the control group. In contrast, the ratio for GTC group was 0.75 ± 0.01, increasing by 23% ($p < 0.01$). Similarly, the ratio of catalase gene expression to β-actin in the control group was 0.57 ± 0.02; whereas, the ratio in the GTC group 0.75 ± 0.02, increasing by 32% ($p < 0.01$).

4 Discussion

The results of our study revealed that GTC supplementation prolonged the mean lifespan and 50% survival time of fruit flies. These findings are consistent with that of Cui *et al.* [31], who investigated a Chinese medicine capsule containing 50% green tea extract, 30% spine date, and 20% Chinese wolfberry, finding *Drosophila's* lifespan increased by 33% when 7 mg/mL of GTC was added into the diet. The dose in the study of Cui *et al.* [31] was lower than that used

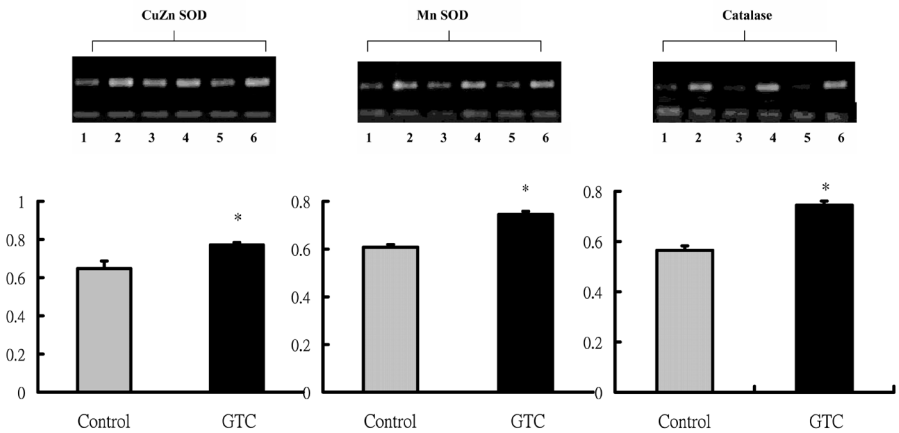


Figure 6. Effect of GTC supplementation (10 mg/mL diet) for 20 days on gene expression of CuZnSOD, MnSOD, and catalase, compared with the control diet (0 mg GTC/mL diet). The wild type (OR) flies ($n = 300$/group, $n = 20$/vial) were incubated at 25°C for 20 days. The values were normalized to the corresponding amount of β-actin (bottom bands). *Lanes 1, 3, 5 = control; Lanes 2, 4, 6 = GTC group. Data are expressed as mean ± SD, $n = 3$. * differs significantly from that of the control value at $p < 0.05$.

in the present study but it had a stronger antiaging activity, probably because the purity and composition of GTC used as well as the addition of the spine date and Chinese wolfberry were accountable. In male mice given a water solution containing 80 mg/L of GTC at the age of 13 months until death, it was found that mice had an increase in an average lifespan by 6.4% ($p < 0.01$) [32]. In a human cohort study, it was reported that daily consumption of green tea in sufficient amounts could prolong life by avoiding premature death, particularly death caused by cancer [33].

A plausible explanation for GTC to extending the lifespan of OR flies is its free radical scavenging activity [34]. One of indications for aging is accumulation of LPO products. In fact, the present study clearly demonstrated that GTC group had a decrease in total body LPO level compared with the control group, suggesting that dietary GTC had antioxidant activity *in vivo* in *D. melanogaster*. Arking *et al.* [35] showed that genetic manipulated long-lived strain of *D. melanogaster* had a lower LPO level with greater SOD and catalase activities in every time points throughout the whole life compared with the short-lived strain. In rats, GTC feeding decreased plasma LPO level [3]. Another *in vitro* study also showed that GTC inhibited generation of superoxide, hydroxyl radicals, and LPO [36]. In addition, Zhang *et al.* [24] confirmed that GTC would prevent hemolysis of red blood cell membrane induced by 2,2′-azo-bis(2-amidinopropane)dihydrochloride (AAPH), a lethal free radical initiator. GTC had been proved to inhibit both spontaneous and photo-enhanced lipid peroxidation in mouse epidermal microsome [37]. In an *in vivo* study, lipid peroxidation in the plasma of rats fed a diet containing high perilla oil was significantly decreased when GTC was supplemented in diet [3]. GTC also demonstrated to inhibit Cu^{2+}-mediated oxidation of human low-density lipoprotein (LDL) and its activity was even stronger than that of ascorbic acid [16, 18]. It is speculated that antifree radical scavenging activity of GTC is attributable to its unique phenolic structure, which can donate an electron or proton to a free radical and exhibit the electron resonance of delocalization [15].

The most interesting finding in the present study was that GTC supplementation was associated with an increase in antioxidant enzymatic activity of CuZnSOD, MnSOD, and catalase. The observed increase in these antioxidant enzymes was consistent with upregulation of expression of both SOD and catalase in flies maintained on a diet containing 10 mg/mL of GTC. The present result was in agreement with the report of Sohal *et al.* [38], who found that flies had increased SOD activity by 26% and catalase activity by 73% in response to 34% increase in lifespan. The present result showed that GTC supplementation increased the mean lifespan by 16% in response to 32% activity enhancement in CuZnSOD, 40% in MnSOD, and 19% in catalase. However, Orr and Sohal [39] showed that overexpression of CuZnSOD alone did not increase the lifespan of the flies and the increase in the oxidative resistance induced by the paraquat challenge was insignificant. Similarly, overexpression of catalase gene alone did not increase the longevity of the flies [29]. It appears that longer lifespan of the flies is only associated with upregulation of expression of both SOD and catalase.

The present study clearly demonstrated that lifespan-prolonging effect of GTC in the fruit flies was associated with upregulation of genes for SOD and catalase. This was reflected from the observation that in paraquat and H_2O_2 challenge treatment, GTC prolonged the survival time only in OR wild type flies but it did not affect that of SOD^{n108} or Cat^{n1} mutants, in which gene of either SOD or catalase was knocked out. The present result was in agreement with that of Chan *et al.* [40], who found both activity and mRNA of SOD were markedly upregulated when the cultured rat brain astrocytes were incubated with tea catechins. Similar increase in activity and gene expression of SOD was observed when pheochromocytoma cells were incubated with tea catechins [41]. In mice, dietary catechin supplementation could protect epidermal cells against UV irradiation-induced damage by modulating SOD and catalase activity [42]. In other systems, GTC could also activate expression of these antioxidant enzymes [43–46]. The present study mainly focused on interaction of GTC with endogenous SOD and catalase but not on GPx as contribution of glutathione pathway in *D. melanogaster* toward antioxidant defense is very minimum [47].

In summary, the present study investigated the effect of GTC on the mean lifespan, activity, and gene expression of the antioxidant enzymes in *D. melanogaster*. The results showed that GTC supplementation could increase the mean lifespan and survival time of *D. melanogaster* under various oxidative stresses by upregulating the activity of SOD and catalase with reduction of the LPO level. The protective effect of GTC was associated at least in part with upregulation of the expression of endogenous SOD and catalase.

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