Effects of bioactive extracts from four edible mushrooms on the lifespan of Drosophila melanogaster

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The effects of bioactive extracts from Ganoderma lucidum, Lentinula edodes, Agaricus blazei and Auricularia auricula-judae on the lifespan of Drosophila melanogaster were studied. The results showed that 5 mg/ml of L. edodes and A. blazei extracts extended the lifespan of male and female flies by 40.53 and 6.03%, and 32.13 and 2.69%, respectively. An extract from A. auricula extended the lifespan of male flies by 31.41% at 5 mg/ml and female flies by 16.85% at 20 mg/ml. While an extract from G. lucidum extended the lifespan of male flies by 42.32% at 80 mg/ml and female flies by 29.24% at 5 mg/ml. These results suggest that the extracts can prolong the lifespan of D. melanogaster in a dose- and sex-dependent manner. Extracts of G. lucidum, L. edodes, A. blazei, A. auricula may be potential anti-aging agents.

Keywords: Ganoderma lucidum; Lentinula edodes; Agaricus blazei; Auricularia auricula-judae; Drosophila melanogaster; lifespan

Introduction
Aging is a physiological phenomenon in the life process, ordinarily expressed as a decrease in fertility and an increase in mortality (Kirkwood and Austad 2000). It leads to various diseases, such as hypertension, coronary artery heart disease, diabetes and tumors. At present, the population age (over 60 years old) is increasing rapidly in developed and developing countries, costing billions in aging-related diseases every year. Aging has become a global issue; thus, the investigation of anti-aging methods and screening of anti-aging medicines are important.

Ganoderma lucidum, Lentinula edodes, Agaricus blazei and Auricularia auricula-judae are well-known as edible mushrooms and traditional medicines in China for anti-aging, regulating the immune system and inhibiting tumor cell growth. Studies on their anti-aging effects on organisms such as Caenorhabditis elegans (Chuang et al. 2009), piglets (Xue et al. 2009) and rats (Ma et al. 2005) have also been reported.

Drosophila melanogaster is a model organism studied exhaustively by scientists. The effects of diverse substances on its lifespan, such as alpha-1,2-mannosidase I (Liu et al. 2009), superoxide dismutase (Magwere et al. 2006), cerium (Huang et al. 2010) and resveratrol (Bass et al. 2007), have been investigated. However, to the best of our knowledge, it has not been used in the study of bioactive extracts from medicinal mushrooms. Here, we describe the effects of extracts from four mushroom species on the lifespan of D. melanogaster.

Materials and methods

Materials
The strains of G. lucidum, L. edodes, A. blazei and A. auricula were supplied by Yuewei Edible Fungi Technology Co. Ltd. D. melanogaster was wild-type and purebred, obtained from College of Life Science, South China Normal University.

Extraction of bioactive compounds
Two kg of the fruiting bodies were soaked successively in 14 L of pure water, absolute ethanol and petroleum ether for 24 h. Each extract was filtered twice through qualitative filter paper. The filtrate was concentrated in a rotary evaporator at 60°C and dried to constant weight in an oven at 60°C. Each extract used for experimental purposes was a mixture of the three extracts.

Preparation of media
Components of the basic medium (BM) were as follows: 100 g of ultrafine cornmeal, 135 g of brown sugar, 15 g of agar, 1.5 g of benzoic acid dissolved in 30 ml of 95%
ethanol, 10 g of dry yeast, and 740 ml of distilled water. BM was prepared according to the method of Zhong (2004).

Medium for test group (MTG) was prepared based on the BM. Mushroom extract was added to the preparation process of BM at 60°C, resulting in concentrations of 5, 20 and 80 mg/ml, which were designated MTG-5, MTG-20 and MTG-80, respectively.

A 1.5-ml aliquot of media were injected into a test-tube (75×15 mm) by syringe and solidified at an inclined plane. The tube was sealed by a cotton plug. Before the experiments, the tubes with fresh media were kept in an incubator at 25°C for 24 h to ensure there was no contamination.

**D. melanogaster survival test**

Ripe fruit flies were reared for mating and oviposition in a conical flask (50 ml) containing BM. When the larvae were in the imagochrysalis stage, the parental flies were removed. The neonatal flies were gathered 10 h after emergence of pupae. They were sorted according to sex and grouped according to somatotype approximation. The separated flies were reared in tubes with BM or the MTGs. Each tube contained 20 flies.

Each bioactive extract was tested at three concentration levels (5, 20 and 80 mg/ml) in the media for the relative test groups, i.e. MTG-5, MTG-20 and MTG-80. BM was used as the control group. In each group, males and females were prepared in four parallel tubes. After living in BM for 12 days, flies in the test groups were transferred to the MTGs and cultivated until the last one had died. Flies in the control group were cultivated in BM until the last one had died.

The experiment was conducted in an incubator with 50–70% relative humidity and 25±1°C. Media were replaced every 4 days and mortality was recorded daily.

**Statistical analysis**

Average lifespan, half death time and maximum lifespan were calculated. Significant differences among groups in each index were tested at 5% level of confidence, according to Duncan’s multiple range test (Duncan 1955).

**Results**

**Effect of bioactive extract from L. edodes on the lifespan of D. melanogaster**

As shown in Table 1, 5 mg/ml of the extract markedly prolonged the average lifespan (in days) and half death time (i.e. time in days at which 50% of the flies had died) of both sexes. In the MTG-5 group, the average lifespan was lengthened by 40.53% for male and 6.03% for female; half death time was also improved by 64.04% for male and 11.30% for female.

The 20 mg/ml extract exhibited a similar effect in male flies. However, the maximum lifespan decreased with increasing concentration. In addition, the effect on average lifespan and half death time was weakened at higher doses. This implies that the lower dose of bioactive extract from *L. edodes* is most effective in extending the lifespan of *D. melanogaster*.

**Effect of bioactive extract from A. blazei on the lifespan of D. melanogaster**

As shown in Table 2, both 5 and 20 mg/ml of extract dramatically prolonged lifespan of male flies. The difference between the two was small, except for maximum lifespan, where the 5 mg/ml extract had a superior effect. In the MTG-5 group, lifespan average, half death time and maximum lifespan were improved by 32.13, 38.59 and 9.12%, respectively. This suggests that 5 mg/ml of *A. blazei* extract can extend the lifespan of male *D. melanogaster* effectively.

In comparison, the lifespan of female flies was barely influenced by the extract, except for maximum lifespan, which showed an increase of 13.64% at 5 mg/ml, but it dropped significantly at 20 and 80 mg/ml. A dose above 5 mg/ml needs to be tested to ascertain whether *A. blazei* extract can extend the lifetime of female *D. melanogaster*.

**Effect of bioactive extract from A. auricula on the lifespan of D. melanogaster**

As shown in Table 3, the average lifespan and half death time in male flies were clearly improved. For the MTG-5 and MTG-20 groups, the average lifespan was lengthened by 31.41 and 28.90%, respectively, while the half death time was increased by 33.70 and 34.24%, respectively. Nevertheless, maximum lifespan was influenced only slightly or even decreased, which suggests that the ideal dose of *A. auricula* extract may be in the range 5–20 mg/ml for lengthening the lifespan of male *D. melanogaster*.

Regarding female flies, 20 mg/ml of the extract showed a marked increase compared to control, with average lifespan, half death time and maximum lifespan improved by 16.85, 24.61 and 10.54%, respectively. Thus, 20 mg/ml is an ideal dose of *A. auricula* extract in prolonging the lifespan of female *D. melanogaster*.

**Effect of bioactive extract from G. lucidum on the lifespan of D. melanogaster**

Table 4 shows that, for male flies, the average lifespan of the MTG-20 and MTG-80 groups increased compared to the control group. Nevertheless, only the MTG-80 group showed an improvement in maximum lifespan. In no group was the half death time prolonged in male flies. In the
Table 1. Effect of bioactive extract from Lentinula edodes on the lifespan of Drosophila melanogaster.

| Medium | Sex | Average lifespan (days) | Half death time (days) | Maximum lifespan (days) |
|--------|-----|-------------------------|------------------------|-------------------------|
| BM     | ♂️  | 35.21 ± 0.30b*          | 29.67 ± 3.79c          | 62.40 ± 4.35a           |
| MTG-5  | ♂️  | 49.48 ± 2.06a           | 48.67 ± 1.53a          | 58.20 ± 1.68b           |
| MTG-20 | ♂️  | 46.41 ± 3.27a           | 47.00 ± 4.58a          | 53.30 ± 2.40c           |
| MTG-80 | ♂️  | 36.43 ± 0.41b           | 37.33 ± 1.15b          | 45.30 ± 2.16d           |
| BM     | ♀️  | 43.09 ± 0.98b           | 41.33 ± 2.31bc         | 63.40 ± 4.88a           |
| MTG-5  | ♀️  | 45.69 ± 1.24a           | 46.00 ± 2.00a          | 57.00 ± 3.46b           |
| MTG-20 | ♀️  | 44.16 ± 0.78ab          | 43.33 ± 3.51ab         | 51.20 ± 1.47c           |
| MTG-80 | ♀️  | 38.50 ± 0.62c           | 37.67 ± 1.53c          | 42.10 ± 0.99d           |

Notes: *In a column with same sex, data followed by different alphabetical letters (a, b, c, d) were significantly different at the p < 0.05 level, according to Duncan’s multiple range test. For example, data followed by “a” was significantly different to data followed by b, c, or d, but not significantly different to other data followed by “a” or “ab”.

Table 2. Effect of bioactive extract from Agaricus blazei on the lifespan of Drosophila melanogaster.

| Medium | Sex | Average lifespan (days) | Half death time (days) | Maximum lifespan (days) |
|--------|-----|-------------------------|------------------------|-------------------------|
| BM     | ♂️  | 44.57 ± 2.08b           | 46.00 ± 1.15b          | 73.50 ± 3.44c           |
| MTG-5  | ♂️  | 58.89 ± 9.29a           | 63.75 ± 15.84a         | 80.20 ± 1.54a           |
| MTG-20 | ♂️  | 58.17 ± 1.80a           | 64.00 ± 3.46a          | 76.90 ± 1.44b           |
| MTG-80 | ♂️  | 34.10 ± 10.7b           | 34.25 ± 10.50b         | 56.00 ± 3.77d           |
| BM     | ♀️  | 49.80 ± 1.27ab          | 47.75 ± 0.50a          | 64.50 ± 6.02b           |
| MTG-5  | ♀️  | 51.14 ± 4.86a           | 51.50 ± 6.55a          | 73.30 ± 5.49a           |
| MTG-20 | ♀️  | 48.86 ± 4.72ab          | 51.75 ± 8.18a          | 65.50 ± 4.11b           |
| MTG-80 | ♀️  | 44.55 ± 2.50b           | 45.00 ± 2.94a          | 54.20 ± 3.19c           |

Table 3. Effect of bioactive extract from Auricularia auricula-judae auricula on the lifespan of Drosophila melanogaster.

| Medium | Sex | Average lifespan (days) | Half death time (days) | Maximum lifespan (days) |
|--------|-----|-------------------------|------------------------|-------------------------|
| BM     | ♂️  | 44.57 ± 2.08b           | 46.00 ± 1.15c          | 73.50 ± 3.44a           |
| MTG-5  | ♂️  | 58.57 ± 1.92a           | 61.50 ± 1.73a          | 72.20 ± 1.54a           |
| MTG-20 | ♂️  | 57.45 ± 4.36a           | 61.75 ± 5.31a          | 73.80 ± 3.11a           |
| MTG-80 | ♂️  | 53.85 ± 4.91a           | 56.00 ± 4.24b          | 66.60 ± 3.09b           |
| BM     | ♀️  | 49.80 ± 1.27c           | 47.75 ± 0.500c         | 64.50 ± 6.021b          |
| MTG-5  | ♀️  | 52.33 ± 3.44bc          | 53.00 ± 3.91b          | 69.90 ± 3.31a           |
| MTG-20 | ♀️  | 58.19 ± 2.56a           | 59.50 ± 2.64a          | 71.30 ± 0.67a           |
| MTG-80 | ♀️  | 56.32 ± 2.68ab          | 58.00 ± 4.16a          | 64.70 ± 0.48b           |

Table 4. Effect of bioactive extract from Ganoderma lucidum on the lifespan of Drosophila melanogaster.

| Medium | Sex | Average lifespan (days) | Half death time (days) | Maximum lifespan (days) |
|--------|-----|-------------------------|------------------------|-------------------------|
| BM     | ♂️  | 35.21 ± 0.30b           | 29.67 ± 3.79a          | 62.40 ± 4.35b           |
| MTG-5  | ♂️  | 34.85 ± 3.33b           | 39.67 ± 13.87a         | 59.80 ± 0.42b           |
| MTG-20 | ♂️  | 45.91 ± 1.73a           | 49.67 ± 10.69a         | 62.40 ± 3.37b           |
| MTG-80 | ♂️  | 50.11 ± 4.55a           | 51.67 ± 15.57a         | 65.20 ± 1.93a           |
| BM     | ♀️  | 43.09 ± 0.98b           | 41.33 ± 2.31b          | 63.40 ± 4.88b           |
| MTG-5  | ♀️  | 55.69 ± 3.76a           | 57.33 ± 2.31a          | 70.20 ± 2.93a           |
| MTG-20 | ♀️  | 49.21 ± 3.78b           | 45.33 ± 9.07b          | 61.20 ± 2.39b           |
| MTG-80 | ♀️  | 55.62 ± 3.67a           | 57.43 ± 4.04a          | 61.30 ± 0.67b           |

MTG-80 group, the average lifespan was increased by 42.32% and maximum lifespan was increased by 4.49%, implying that 80 mg/ml of G. lucidum extract can effectively extend the lifetime of male D. melanogaster.

Regarding female flies, extracts at 5 and 80 mg/ml significantly increased the average lifespan and half death time. The extract at 5 mg/ml also increased maximum lifespan, reaching 10.73%, implying that a 5 mg/ml extract of G. lucidum can lengthen the lifespan of female D.
Bioactive extracts from four species of edible/medicinal mushrooms were capable of extending the lifespan of *D. melanogaster*. Extracts from *L. edodes* and *A. blazei* prolonged the lifespan of male and female flies by 40.53 and 6.03% and 32.13 and 2.69%, respectively, at 5 mg/ml. Extracts of *A. auricula* prolonged the lifespan of male flies by 31.41% at 5 mg/ml and female flies by 16.85% at 20 mg/ml. Extracts from *G. lucidum* prolonged the lifespan of male flies by 42.32% at 80 mg/ml and female flies by 29.24% at 5 mg/ml. Therefore, it can be concluded that these extracts can extend the lifespan of *D. melanogaster* in a dose- and sex-dependent manner.

The efficacy of extracts may also depend on the health of the fruit flies as Liu et al. (2006) found that half death time tended to reflect a prolonging of lifespan in weak fruit flies and maximum lifespan in strong fruit flies and a tolerance to overdose. Thus, *L. edodes* extract increased the lifetime of weak versus strong flies of both sexes. *A. blazei* extract could extend the lifetime of strong flies and weak male flies. *A. auricula* extract could extend the lifetime of weak flies and strong female flies. While, *G. lucidum* extract could extend the lifetime of strong flies and weak female flies.

At present, anti-aging implies an improvement in the physical quality of life and extending longevity within the allowable range of genetic characteristics. Several methods have been tested, including gene therapy, nutritional modulation, hormonal supplementation and intervention via antioxidants and other compounds (Rattan 2004).

The effect of mushroom extracts in extending the longevity of *D. melanogaster* may be partially due to their antioxidant properties. It has been reported that *G. lucidum* extract increased the activity of superoxide dismutase, catalase and the levels of reduced glutathione in aged mice, and decreased the levels of malondialdehyde (Cherian et al. 2009). An ethanol extract of *G. lucidum* was also reported to activate dehydrogenases and complexes of the electron transport chain in the heart/brain mitochondria of aged rats, with improved mitochondrial function possibly involved in the antioxidant properties of *G. lucidum* (Ajith et al. 2009; Sudheesh et al. 2009). Similarly, polysaccharides from *L. edodes*, *A. blazei* and *A. auricula* display a free-radical scavenging function (Ma et al. 2005; Ker et al. 2005; Xue et al. 2009). *G. lucidum* polysaccharides showed an anti-skin-aging function via affecting gene expression and promoting damaged DNA repair (Xie et al. 2008). The possibility that mushroom extracts may extend the lifespan of *D. melanogaster* at the gene level needs further investigation.

As shown in Tables 1–4, female flies in the BM group lived significantly longer than males, which could, according to Viña and Borrás (2010), be due to differences in free radical production between the sexes. Viña and Borrás (2010) indicated that estrogens promoted expression of anti-aging-related genes, leading to less free radical production in females. Phytoestrogens or similar compounds may, on the other hand, lengthen the lifespan of animal species in which females live longer than males, which may explain the gender difference in the lifespan-extending effect of the extracts. In our opinion, the previous results for male flies may be attributed to the action of extracts on their hormone.

Studies on the anti-aging effects of bioactive components from mushrooms are still in their infancy. *D. melanogaster* has been investigated in-depth as an animal model and its experimental use will advance research on the anti-aging function of mushrooms. Further studies are underway to isolate and purify the anti-aging components from mushrooms and explore their anti-aging mechanism on *D. melanogaster*, especially at the hormone level.

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