Cellular and Molecular Biology

Hu *et al. Cell. Mol. Biol.*2016, 62 (4): 116-122
ISSN: 1165-158X
doi: 10.14715/cmb/2016.62.4.20

*Original Research*

# Edible bird's nest enhances antioxidant capacity and increases lifespan in Drosophila Melanogaster

Q. Hu[1,#], G. Li[1,#], H. Yao[1], S. He[1], H. Li[1], S. Liu[1], Y. Wu[1], X. Lai[2*]

[1] School of Chinese Herbal Medicine, Guangzhou University of Chinese Medicine, Guangzhou 510006, China
[2] Dongguan Mathematical Engineering Academy of Chinese Medicine, Guangzhou University of Chinese Medicine, Dongguan 523808, China

**Abstract:** In this study, we aims to investigate the effects of edible bird's nest (EBN) on anti-aging efficacy. In order to investigate lifespan and mortality rate of flies, we treated flies with various doses of EBN. Besides, fecundity, water content and food are determined and heat-stress test is conducted after flies treating with different medium. Effects of EBN on total antioxidant activity (T-AOC), super-oxide dismutase activity (SOD), catalase activity (CAT), and malondialdehyde (MDA) were examined in *drosophila melanogaster*. Results indicated that flies in EBN treated group illustrated significantly lower mortality rates and longer median and maximum lifespan compared to control group ($P<0.05$). The fecundity in EBN-treated group was increased compared to control group. SOD levels and CAT activity were significantly increased, and MDA levels decreased in EBN-treated group compared to control group ($P<0.01$). In conclusion, EBN can extend lifespan, decrease mortality rate and increase survival rate in heat-stress test, and which can also promote SOD and CAT activity and reduce MDA levels. EBN is able to delay *drosophila melanogaster* aging, attributing to the increasing antioxidant enzyme activities and decreasing content of lipid peroxidation products in *drosophila melanogaster*.

***Key words:*** Edible bird's nest, Drosophila Melanogaster, anti-aging, anti-oxidation.

## Introduction

Edible bird's nests (EBNs) are produced by the swiftlets of the genera *Aerodramus*, *Apus* and *Collocalia* (1,2). A 'nest cement' is secreted to construct their nests. The nest cement was firstly recorded in "*Essentials of Materia Medica*", and the edible bird's nest was used to nourish lung yin deficiency (which means the function of the lung is weak), which is also the best remedy to regulate consumptive diseases. EBN is perceived as both food and medicine for quite a long time. In Traditional Chinese Medicine (TCM), EBN has functions of strengthening the functions of spleen and kidney (1,2). The EBN could also be helpful to regulate consumptive diseases and nourish the skin. Sialic acid-rich glycoproteins is a major constituent in EBN (3), which can enhance memory and immunity. Hydroxyl in polysaccharides combining with $O^{2-}$ and -OH can guard against oxidation by free radicals. Therefore, the activity of SOD is popped up by polysaccharides as to clean free radical. Together with the hydroxyl, the metal ion complex is formed to repress the production of free radical according to the previous study (4).

Recently, a few studies show that EBN is capable of prohibiting brain tissue from aging and eliminating oxygen free radicals (5,6). Aging always increases the risks of other diseases and diminishes the defensive capacity against environmental challenges. Additionally, EBN and its extracts illustrate some of the interesting bioactive properties, however, there is little literature writing about anti-aging effect of EBN so far. As the *drosophila melanogaster* with short lifespan and abundant manifestations of cellular senescence, which can be observed on the adult flies. Therefore, the EBN is a perfect animal model for gerontological research. This study aims to illuminate the anti-aging mechanism of EBN and provide a scientific basis of the valuable health-care product since ancient times.

## Materials and Methods

### Materials

Edible bird's nest was collected from Nha Trang, and Viet Nam was produced by *Aerodramus fuciphagu*s of the genera *Aerodramus*. Edible bird's nest was pestled to a fine powder. Distilled water was mixed with EBN for 14 hours, and then heated with slow fire for 10 min to thicken, which was used to prepare basal medium for experiment. The content of protein presented 59% in edible bird's nest gathered from Nha Trang (The protein amounts in EBN was determined by using the ultraviolet spectrophotometry assay). While the main component of sialic acid N-acetyl neuraminic acid accounted for 5.4% (The amounts in EBN was purified and determined by using the high performance liquid chromatography assay, HPLC).

### Fly culturing

The *drosophila melanogaster* was purchased from School of Life Sciences, Sun Yat-Sen University. The *drosophila melanogaster* was cultured in Guangzhou University of Chinese Medicine TCM research labora-

**Received** January 22, 2016; **Accepted** April 20, 2016; **Published** April 30, 2016

*** Corresponding author:** Dr. Xiaoping Lai, MD, Dongguan Mathematical Engineering Academy of Chinese Medicine, Guangzhou University of Chinese Medicine, Dongguan 523808, China. Email: laixpinggz@sina.com

[#] These authors contributed equally to this work, and are the co-first authors.

tories. Experimental flies were kept at 25°C (50% humidity) on a 12 hour light/dark cycle. Emerging adult flies were gathered within 8 hours and then separated by gender.

**Culture medium**

Flies were raised in vials contained a standard fly food, which was made of 10 g yeast extract, 90 g sucrose, 7.5 g agar and 100 g corn meal in 850 ml distilled water. 1% final v/v concentration of propionic acid was added to the food to inhibit fungus from growing. The food was served as medium later. To examine the effects of EBN on the biology of aging, the medium was supplemented with EBN as final w/w concentrations of 1g $Kg^{-1}$, 3g $Kg^{-1}$ or 9g $Kg^{-1}$, and which was equal to the effective daily dosage as human.

**Analysis on Lifespan and mortality rate**

To measure EBN's effect on flies' lifespan, four groups were set, including control group, 1g $Kg^{-1}$ EBN group, 3g $Kg^{-1}$ EBN group and 9g $Kg^{-1}$ EBN group for both genders. EBN was dissolved in distilled water and added to a media with boiled cornmeal at 1 g, 3 g and 9 g $Kg^{-1}$ concentrations. 200 flies collected in 5 vials in each group were raised in food vials with or without EBN in the life span analysis, and 360 flies collected in 9 vials per group were used in mortality rate analysis. 25°C and 50% to 60% relative humidity was needed during all experimental procedures. Experimental flies were transferred to fresh medium every 5 days (7). Lifespan assays were conducted by recording their mortality until all flies died while mortality rate assay for 29 days.

**Fecundity assessment**

One female fly was mated with one virgin male for 24 hours in a vial, and there are 40 pairs in total for each group (8). Then each pair was transferred to fresh medium to stay for another 24 hours to lay eggs. Repeat this procedure until the tenth day and we would count the total eggs laid in each vial daily. The mean egg production in ten days per female was calculated as the fecundity index.

**Water content and food intake assessment**

100 flies were collected in 10 vials per group and pretreated with or without various doses of EBN for 4 days at 25°C with 50% to 60% relative humidity. The spent media was changed with fresh media every day. In the water content assay, the weight of the flies was measured. The flies were then heated to 55°C for 1 hour and the dry weight of the flies was recorded. Later, the fly water content was calculated by subtracting the dry weight value from initial weight value. In food intake assessment assay, the flies were feed for 4 hours after 8 hours starvation. The food intake was measured by weight change in the 4 hours.

**Heat-stress test**

After a treatment with or without EBN at 25°C for 10 days, heat sensitivity of flies in each group were assessed by transferring the flies to fresh vials at 40°C thermal condition for 53 min. 100 flies were used for each group and the heat sensitivity was evaluated with survival rate after heat stress.

**Protein density**

After a treatment with or without EBN at 25°C for 30 days, flies were measured and 20 mg refrigerant homogenized in 100 µl phosphate buffered saline (PBS) on ice. The homogenates were centrifuged at 2500 r/min for 10 min at 4°C. To know the enzyme activity, resultant supernatant was measured immediately. Protein concentrations in the whole-fly homogenates were measured with BCA Protein Assay Kit (Beyotime Institute of Biotechnology, 20120830, China).

**Ferric reducing antioxidant power**

Fly homogenates for each group were centrifuged at 12000 ×g for 5 min at 4°C, the supernatant was assayed using FRAP Assay Kit (Beyotime Institute of Biotechnology, 20120829, China). In FRAP assay, antioxidants were added to motivate a rapid reduction in ferric-tripyridyltriazine ($Fe^{III}$-TPTZ) into a blue-colored product ferrous-tripyridyltriazine ($Fe^{II}$-TPTZ) (9). Known concentrations of $Fe^{2+}$ solutions of FRAP reagent was used to create a standard curve, which allowed the $Fe^{2+}$ concentration of fly samples to be calculated thereby determining antioxidant capacity. The FRAP reagent was prepared according to the kit instructions, and the absorbance was measured at 595 nm.

**Antioxidant enzyme activity**

Antioxidant activity changes in groups were evaluated by measuring CAT and SOD activity with CAT assay kit (Nanjing Jiancheng Bioengineering Institute, 20120830, China) and SOD assay kit (Nanjing Jiancheng Bioengineering Institute, 20120828, China). Briefly, experimental female and male flies raised with or without EBN at 25°C for 30 days were gathered and homogenized with cold buffer. Enzyme activity assay was obtained from collected supernatants, which were assayed by CAT and SOD.

**Malondialdehyde (MDA) level assay**

The malondialdehyde (MDA) level was quantified using the thiobarbituric acid (TBA) method (Nanjing Jiancheng Bioengineering Institute, 20120823, China). Briefly, female and male flies homogenate were collected for MDA analysis, before the flies were treated with or without various doses of EBN for 30 days at 25°C. Flies tissue homogenate reacted with solution mixed with 15% trichloroacetic acid (TCA) and 0.375% TBA in 0.2 N HCl. The mixture was heated in a 95°C water bath for 40 min and cooled to room temperature. After a centrifugation at 3500 r/min for 10 min, each supernatant absorbance was determined at 532 nm, and 1, 1, 3, 3-tetramethoxypro-pane was used as a standard for quantification.

**Statistical analysis**

Three independent replicates were conducted for all experiments, and statistical analysis was calculated using the SPSS20.0 statistical package (IBM Corporation). A student's *t*-test was performed for experimental comparisons with control groups. Data were showed as the mean±SD, and significant difference was remarked with a *P* value of less than 0.05.

**Table 1.** The influence of EBN on mortality in drosophila (mean ± SD, n=360).

| Group | Mortality rate (%) | |
|---|---|---|
| | Female (♀) | Male (♂) |
| Control | 18.2±6.2 | 24.3±6.8 |
| 1g $Kg^{-1}$ EBN | 15.4±5.7 | 19.2±5.5 |
| 3g $Kg^{-1}$ EBN | 11.6±5.4* | 16.5±5.2* |
| 9g $Kg^{-1}$ EBN | 8.9±5.9** | 11.7±5.8** |

## Results

### Edible bird's nest reduces the mortality rate of *Drosophila Melanogaster*

Accompanying reproductive decline, aging is associated with high mortality. Mortality rate was evaluated in this study. Flies treated with or without EBN for 29 days were calculated. The data are shown in Table 1. For female, mortality rate dropped from 18 percent in control group to 11.6 and 8.9 in 3 g and 9 g Kg-1 EBN group respectively, and male achieved similar results ($P$<0.05 or $P$<0.01). The rates showed that EBN increased the survival rate.

### EBN increase life span on drosophila

To examine whether EBN can extend flies' lifespan, we supplemented flies with 1 g, 3 g and 9 g $Kg^{-1}$ EBN after adult eclosion at 25°C during the whole experiment. As it is shown in Table 2, a remarkable increase was found in maximum lifespan of female and male, comparing with all EBN-supplemented groups with EBN-free control group ($P$<0.01). The medial life time jumped from 60 days to 69 days in female and jumped from 47 days to 56 days in male when the flies were treated with 9 g $Kg^{-1}$ EBN. The results suggested that lifespan was extended with extra EBN added to the diets. Moreover, the lifespan extension caused by EBN was concentration-dependent. The survival curve was shown in Figure 1 (A, B).

### EBN-treated diets increase the fecundity of flies

Reproduction is related to lifespan and repression of reproduction and is also an efficient path to extend lifespan. Fecundity was detected when administrating of EBN. From the study, we found that fecundity was not affected in the EBN-treated groups. To further study the fecundity of the flies treated with EBN, control group was set. It turned out that flies fed with EBN had greater egg-laying capability than control group ($P$<0.01 or $P$<0.05). However, there was no significant difference among EBN-treated groups in egg-laying capability ($P$>0.05).

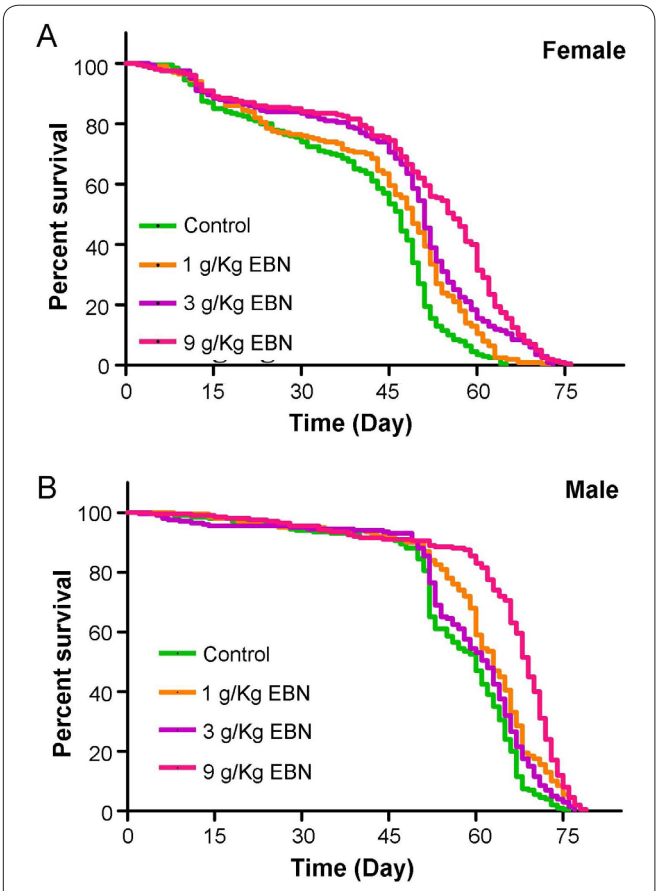


**Figure 1.** The survival curves of *Drosophila Melanogaster*. The dose curves response of EBN in the survival: (A) survival rate for female; (B) survival rate for female; flies were treated with series doses of EBN. Data were expressed as mean ± SD (n= 200).

### EBN exerts no effect on food intake and water content in flies

Diet influences life span both in humans and animal models. To evaluate compensatory feeding, the quantity of food consumed by the flies were measured. In our study, the result is displayed in Table 4, on which EBN-treated groups showed no significant difference in both food intake and fly water content assays ($P$>0.05) compared with control group. Our data showed that the intakes of flies treated with EBN was greater than control group and the water content was higher. We found that more food was consumed by flies on EBN-treated diets.

**Table 3.** Fecundity change in EBN-treated flies (mean ± SD, n=40).

| Group | Egg-yielding amount |
|---|---|
| Control | 309±16.1 |
| 1g $Kg^{-1}$ EBN | 318±18.0* |
| 3g $Kg^{-1}$ EBN | 322±22.4** |
| 9g $Kg^{-1}$ EBN | 321±21.0** |

*P<0.05, **P<0.01compered to control.

**Table 2.** Life span results in EBN-treated flies (mean ± SD, n=200).

| Group | Life span | | maximum life span | | Medial death time | | Life extension rate | |
|---|---|---|---|---|---|---|---|---|
| | Female (♀) | Male (♂) | Female (♀) | Male (♂) | Female (♀) | Male (♂) | Female (♀) | Male (♂) |
| Control | 56.82±12.49 | 40.23±15.59 | 71.20±2.59 | 59.95±2.96 | 60 | 47 | -- | -- |
| 1g $Kg^{-1}$ EBN | 60.65±12.79* | 43.21±16.71 | 75.55±0.95** | 63.85±3.28** | 63 | 49 | 0.067 | 0.074 |
| 3g $Kg^{-1}$ EBN | 60.46±13.80* | 46.85±16.94** | 73.90±2.10** | 69.80±2.22** | 62 | 51 | 0.028 | 0.165 |
| 9g $Kg^{-1}$ EBN | 65.04±13.21** | 50.13±15.96** | 76.50±1.15** | 70.75±2.31** | 69 | 56 | 0.145 | 0.246 |

*P<0.05, **P<0.01compered to control.

**Table 4.** Water content and food intake changes in EBN-treated flies (mean ± SD, n=10).

| Group | Food intake (g) | | Water content (g) | |
|---|---|---|---|---|
| | Female (♀) | Male (♂) | Female (♀) | Male (♂) |
| Control | 0.0130±0.0006 | 0.0135±0.0006 | 0.092±0.021 | 0.104±0.011 |
| 1g $Kg^{-1}$ EBN | 0.0127±0.0007 | 0.0129±0.0008 | 0.095±0.029 | 0.099±0.027 |
| 3g $Kg^{-1}$ EBN | 0.0125±0.0005 | 0.0130±0.0005 | 0.104±0.014 | 0.124±0.038 |
| 9g $Kg^{-1}$ EBN | 0.0126±0.0006 | 0.0129±0.0006 | 0.105±0.014 | 0.124±0.040 |

**EBN enhances resistance to heat-stress on flies**

Heat sensitivity of each deficiency strain was assessed by comparing to the survival after heat stress. On the tenth day, the result of a stress test is revealed in Table 5. From the table above, we found that EBN-treated (3 and 9 g $Kg^{-1}$) group had higher survival rate ($P$<0.01), and the survival increase caused by EBN was also concentration-dependent. The results showed that the EBN may be associated with the resistance to high temperature.

**Protein density shows no difference in flies**

The role of protein in regulation of drosophila lifespan has been studied and summarized extensively by some researchers. We conclude that there was no correlation between lifespan and protein density because no statistically significant difference present in fly protein density between EBN-treated and control flies on day 30 (Table 6, $P$>0.05).

**EBN promotes total antioxidant activity**

Ferric reducing antioxidant power can reflect total antioxidant activity. The formation of FeII-TPTZ in the sample was detected in this method. Measurements were performed till 30th Day. The $Fe_{III}$-TPTZ compound was returned to the ferrous ($Fe_{II}$) by 1 g, 3 g and 9 g $Kg^{-1}$ EBN-treated fly homogenates 0.1052, 0.1178, 0.1497 mmol $Fe^{2+}$ equivalent per gram, respectively. Control group fly homogenates had a lower antioxidant activity (0.0740 mmol $Fe^{2+}$ equivalent per gram) compared with experimental groups in female ($P$<0.01). We found that the female fly homogenates had higher antioxidant activity than male. Compared with male control group (0.0710 mmol $Fe^{2+}$ equivalent per gram), EBN-treated male fly homogenates showed significant different antioxidant activity (0.0947, 0.1097, 0.1209 mmol $Fe^{2+}$ equivalent per gram for 1 g, 3 g and 9 g $Kg^{-1}$ EBN-treated fly homogenates, respectively) ($P$<0.01). The antioxidant activity caused by EBN was concentration-dependent.

**Table 5.** Heat-stress Test (mean ± SD, n=10).

| Group | Heat-stress resistance (%) | |
|---|---|---|
| | Female (♀) | Male (♂) |
| Control | 69±10.1 | 70±7.6 |
| 1g $Kg^{-1}$ EBN | 71±7.9 | 71±9.2 |
| 3g $Kg^{-1}$ EBN | 76±7.6** | 76±8.4** |
| 9g $Kg^{-1}$ EBN | 84±10.5** | 83±9.7** |

*P<0.05, **P<0.01compered to control.

**Table 6.** Protein concentrations in the whole-fly homogenates (mean ± SD, n=9).

| Group | Female (mg/ml) | Male (mg/ml) |
|---|---|---|
| Control | 0.881±0.015 | 0.946±0.064 |
| 1g $Kg^{-1}$EBN | 0.910±0.065 | 0.919±0.064 |
| 3g $Kg^{-1}$EBN | 0.922±0.075 | 0.927±0.016 |
| 9g $Kg^{-1}$EBN | 0.881±0.046 | 0.922±0.04 |

**EBN enhance antioxidant enzymes activity**

Enhancing the enzymatic detoxification system can be a way to slow down aging and prolong lifespan. To learn the mechanism where EBN protected against oxidative stress, the changes in free radical scavenging enzymes were estimated in flies treated with or without EBN, such as catalase and SOD. The superoxide anion $O_2^-$, one of the prominent radical species could be converted to $H_2O_2$. $H_2O_2$ turned to $H_2O$ and $O_2$ in turn by CAT, by the action of SOD. As Figure 2 (C, D) was shown, both CAT and SOD activity enhanced in the EBN-fed groups compared with controls ($P$<0.01). Data showed that the female fly homogenates had much higher catalase and SOD activity than male. After flies were treated with EBN for 30 days, the catalase activity grew from 0.997 U/mg (control group) to 1.539 U/mg (9 g $Kg^{-1}$ EBN) in female flies, and 0.998 U/mg (control group) to 1.231U/mg (9 g $Kg^{-1}$ EBN) in male flies. In SOD assay, we observed a dose-dependent 9.58 U/mg, 10.09 U/mg and 13.65 U/mg change in the 1 g, 3 g and 9 g $Kg^{-1}$ EBN-treated flies versus the control (8.431 U/mg) respectively in female ($P$<0.01) and a dose-dependent 8.682 U/mg, 9.207 and 10.31 U/mg change in the 1g, 3g and 9 ng $Kg^{-1}$ EBN-treated flies versus the control (8.007 U/mg), respectively in male ($P$ < 0.01), as seen in Figure 2E, F.

**EBN helps reducing malondialdehyde (MDA) level**

Malondialdehyde is the secondary product that accelerates aging. EBN-treated flies had lower MDA levels compared with controls of the same gender ($P$<0.01). Significant difference was found in MDA level between female and male ($P$<0.01). As shown in Figure 2G, H, MDA levels were 0.145 nmol/mg, 0.133 nmol/mg and 0.131 nmol/mg in the 1 g, 3 g and 9 g $Kg^{-1}$ EBN-treated groups correspondingly in female. MDA levels descended from 0.311 nmol/mg to 0.108 nmol/mg after male flies being treated with EBN. We conclude that EBN is able to decrease MDA level in flies.

## Discussion

EBN plays a vital role in molecular mechanisms that involving in aging, low mortality rates, average lifespan and maximum lifespan, as well as levels of oxidative damage to proteins and lipids. Mechanisms are linked mutually and their operation by EBN decelerate aging. The animal model *drosophila melanogaster*

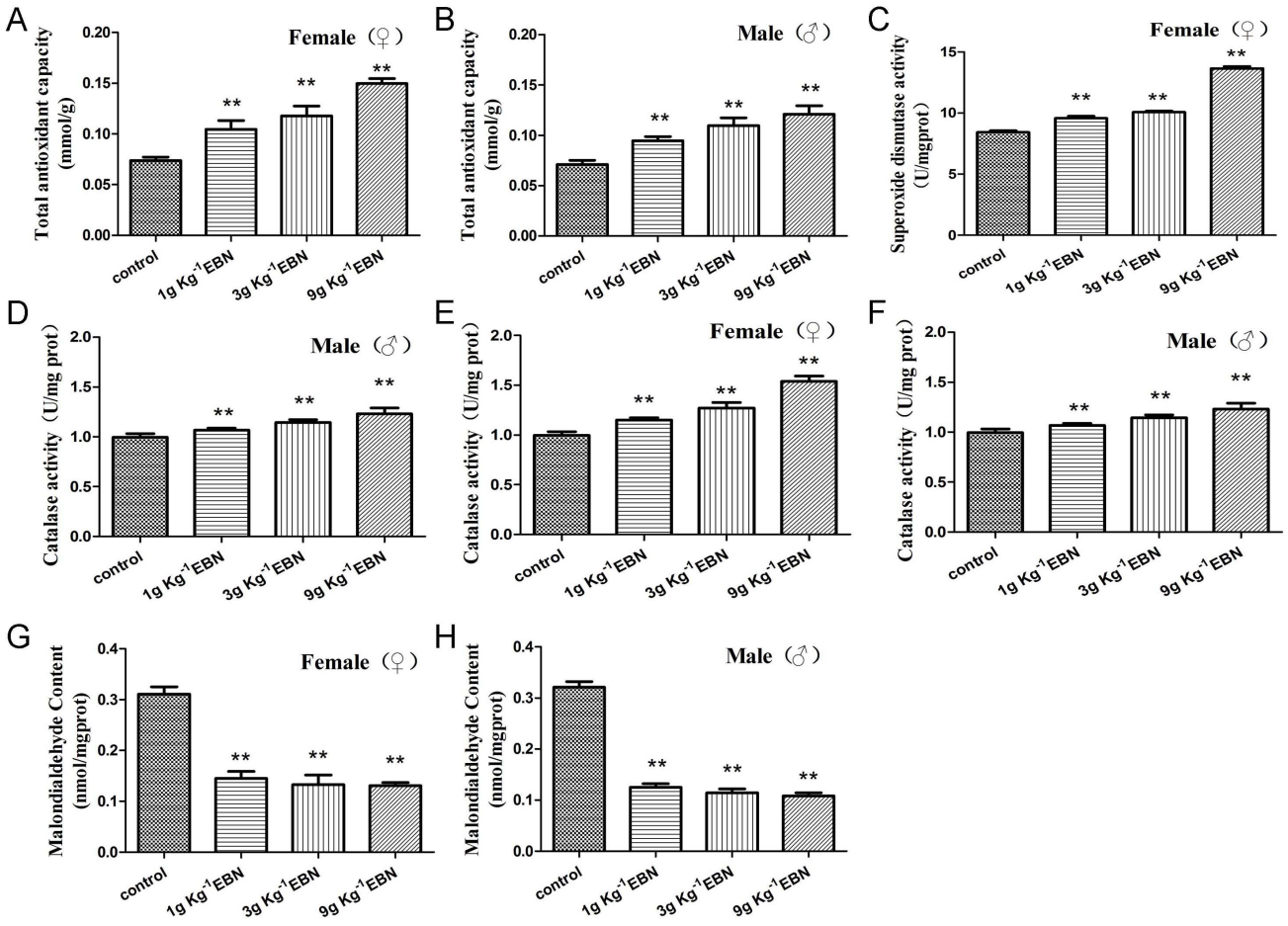


**Figure 2.** Antioxidant enzyme activity and MDA level. Flies were treated with series doses of EBN for 29 days and flies homogenate were collected under suitable conditions. The antioxidant enzyme (SOD and CAT) activity and MDA level were measured using relative kits. The parameters were investigated as the followings: (A) total antioxidant capacity in female; (B) total antioxidant capacity in male; (C) SOD activity in female; (D) SOD activity in male; (E) CAT activity in female; (F) CAT activity in male; (G) MDA level in female; (H) MDA level in male. Data were expressed as mean ± SD (n=9).

applied in our study has been considered as a perfect research model for aging study since 1915 because of its high fecundity, small genome size, and short generation time (10,11). Besides, the lipid metabolism and transport system of *drosophila melanogaster* are similar to mammals, and *drosophila* survival experiment has been used as an anti-aging test method (12). Different fly strains have different lifespan, even if the same line will be different in different experimental conditions. Factors, such as temperature, humidity, component of the medium, photoperiod (13,14) can contribute to the phenomenon.

Ye *et al.* (15) suggests that wild male flies or virgin flies with strong vitality should be used for gerontology experiments and lifespan or maximum lifespan should be replaced by median life time to save time. From this study, we know that EBN-treated flies significantly extend maximum life span and the high-dose EBN-treated flies significantly extend life span in both genders, especially 9 g $Kg^{-1}$ EBN-treated flies which extend 5 days more than positive controls. The high-dose EBN also declines the mortality rate efficiently. Harman (16) is the first researcher to correlate radiation damage, aging and cancer with reactive oxygen free radicals. Harman performed some experiments, which showed that active oxygen free radical scavenger had the ability to extend lifespan under the appropriate concentration (17). 1g $Kg^{-1}$ to 9g $Kg^{-1}$ EBN concentrations are the appropriate concentration to extend flies' lifespan in this study. Flatt (18) remarks that lifespan is related with food intake and fecundity. However, no significant difference is found in both food intake and water content in flies between controls and EBN-treated flies in our study, and the fecundity ability for EBN-treated flies strikingly comparing with controls. Therefore, EBN can enhance fertility but exerts no effect on fly metabolism.

High ambient temperature has a negative effect on animal, human's health and their performance. In the heat-stress assay, the EBN-treated flies have higher survival rate compared to the control group, and the survival rate caused by EBN is concentration-dependent. High temperature better revs up the metabolism, accelerates blood circulation than normal temperature and shortens the life cycle. Similar conclusion is drawn as Yang *et al.* (19). In the heat-stress assay, our result shows that EBN is associated with resistance to various heat stresses. This result indicates that EBN serves to against aging by antioxidant activity. Enzymes such as alanine amino-transferase (ALT) and aspartate amino-transferase (AST) taking part in many important physiological and metabolic processes in the body, are inactive in high temperature.

The cells destruction or cell membrane permeability change is reflected by the change of enzyme activity (20). Some studies reported that there is a close relationship between Hsp70 expression and an increase in

thermo-tolerance after exposing to high temperature. Heat shock proteins (Hsps) expression helps to maintain homeostasis in body if it is induced by high temperature and to protect cell or organism from damaging stress factors (21,22). In addition, the thermal storage caused by high temperature accelerates the formation of free radicals and lipid peroxidation. In the meantime, SOD activity descents and the ability to clean superoxide anion radical is slashed. Therefore, they damage organism and hasten death (23).

Aging is a highly complex process and not all the aging process can be explained by free radical theory. Nevertheless, free radicals' role to spur the aging process is precisely examined by experimental basis (24,25). Not only do environmental stimuli outside accelerates the aging process, but also active oxygen free radicals in the organism influence the aging process. As free-radical theory proposed, the aging process and age-related diseases is largely determined by the damage caused by the reactive oxygen species generation in cells. Free radicals peroxidize membrane lipids fatty acids, form lots of cytotoxic aldehydes such as HNE and MDA (26). The secondary product MDA brings about aging by forming deposition polymerized with protein, peptide and lipid. The antioxidant enzymes can clean free radical specifically, such as SOD, which is an important free radical scavenger to curb lipid peroxidation chain reaction and the formation of lipid peroxidate.

The SOD activity and MDA level are regarded as mature and reliable indicators in the study of aging and anti-aging (27). As Ministry of Health published, SOD activity and MDA level for aged rats are tested as standard index to evaluate the anti-aging effect of healthy food. The SOD activity of *drosophila melanogaster* culminated in median age, and the MDA level grows with aging which is similar to the report on rats (28). In this study, after the administration of EBN for 30 days, a significant increase in SOD activity and a decrease in MDA is seen, and which enhances the CAT activity in EBN-treated flies. The main composition in nest cement is sialic acid-rich glycoproteins and polysaccharides. Polysaccharides plays a critical role in the antioxidant activity by reducing the free radical. Combining with $O^{2-}$ and -OH, polysaccharides guards against oxidation. The activity of SOD increased by polysaccharides is to clean free radical.

In conclusion, edible bird's nest is a natural treasure nutrition food of great value. EBN can prolong life span and maximum life span and to reduce the mortality rate under appropriate concentration. EBN plays a role in fecundity and has no effect on metabolism. After the flies were treated with EBN, their activity of SOD and CAT went up and the level of mitochondrial MDA went down. We attribute the life-extending effect of EBN to the increase of antioxidant enzyme activities and reduction of content of lipid peroxidation products in *drosophila melanogaster*. Additionally, there are significant differences between male and female in the activities of SOD and CAT and the level of MDA. Further study will be taken to elucidate the mechanisms.

**Acknowledgments**

This work was supported by the National Nature Science Foundation of China (81173498), Hong Kong, Macao and Taiwan Special Science and Technology Cooperation Project (2014DFH30010) and Guangdong Province Universities and Colleges Pearl River Scholar Funded Scheme (2011).

## References

1. Yida Z, Imam MU, Ismail M, Hou Z, Abdullah MA, Ideris A, et al. Edible Bird's Nest attenuates high fat diet-induced oxidative stress and inflammation via regulation of hepatic antioxidant and infalmmatory genes. BMC Complement Altern Med 2015; 15: 310.
2. Hou Z, Imam MU, Ismail M, Ooi DJ, Ideris A, Aahmud R. Nutrigenomic effects of edible bird's nest on insulin signaling in ovariectomized rats. Drug Des Devel Ther 2015; 9: 4115-25.
3. Kathan RH, Weeks SI. Structure studies of collocalia mucoid: I. Carbohydrate and amino acid composition. Arch Biochem Biophys 1969; 134: 572-6.
4. Zhao LJ, Song XP, Li FY. Research Progress on Anti-oxidation of Polysaccharide and Its Derivatives. J shanghai Univ Eng Sci 2008; 22: 44-7.
5. Yew MY, Koh RY, Chye SM, Othman I, Ng KY. Edible bird's nest ameliorates oxidative stress-induced apoptosis in SH-SY5Y human neuroblastoma cells. BMC. Complement. Altern Med 2014; 14: 391.
6. Kim KC, Kang KA, Lim CM, Park JH, Jung KS, Hyun JW. Extract of Edible Bird's Nest Attenuated the Oxidative Stress-induced Matrix Metalloproteinase-1 by Regulating the Mitogen-activated Protein Kinase and Activator Protein-1 Pathway in Human Keratinocytes. J Korean Soc Appl Biol Chem 2012; 54: 347-54.
7. Huangfu J, Liu J, Sun Z, Wang M, Jiang Y, Chen ZY, et al. Anti-aging effects of astaxanthin-rich alga Haematococcus pluvialis on fruit flies under oxidative stress. J Agric Food Chem 2013; 61: 7800-4.
8. Hada B, Yoo MR, Seong KM, Jin YW, Myeong HK, Min KJ. D-chiro-Inositol and pinitol extend the life span of drosophila melanogaster. J Gerontol A Biol Sci Med Sci 2013; 68: 226-34.
9. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996; 239: 70-6.
10. Helfand SL, Rogina B. Molecular Genetics of Aging in the Fly: Is This the End of the Beginning? BioEssays 2003; 25: 134-41.
11. Piper MD, Skorupa D, Partridge L. Diet, Metabolism and Lifespan in Drosophila Exp Gerontol 2005; 40: 857-62.
12. Ferkovich SM, Shapiro JP. Increased egg-laying in Orius insidiosus (Hemiptera: Anchocoridae) fed artificial diet supplemented with embryonic cell line. Biol Control 2004; 31: 11-5.
13. Lv WS, Liu BS, Zeng EK. Experimental animal study of gerontology. Chin J Gerontol 1984; 2: 36-41.
14. Wang HD. A study on breeding management method of drosophila melanogaster used for gerontology experiment. Chin J Gerontol 1983; 1: 4-7.
15. Ye EC, Ai YH. Longevity index selection for gerontology experimental drosophila. Chin J Gerontol 1988; 8: 101-3.
16. Harman D. Aging: a theory based on free radical and radiation chemistry. J Gerontol 1956; 11: 298-300.
17. Harman D. Prolongation of the normal life span by radiation protection chemicals. J Gerontol 1957; 12: 257-63.
18. Flatt T. Plasticity of lifespan: a reaction norm perspective. Proc Nutr Soc 2014; 73: 532-42.
19. Yang HY, Liu HY, Yang B, Wu HM, Zhang XW. The effects of temperature and relative humidity on life span of drosophila melanogaster. J Tongji Univ (Med Sci) 2002; 01: 20-2.
20. Li QY, Yuan CL, Ge JR, Xie ZX, Zhu GB. The observation of enzymes activity in body at high temperature. Chin J Ind Hyg Occup Dis 1995; 13: 278-9.

21. Krebs RA. A comparison of Hsp70 expression and thermotolerance in adults and larvae of three Drosophila species. Cell Stress Chap 1999; 4: 243-9.
22. Dellavalle RP, Petersen R, Lindquist S. Preferential deadenylation of Hsp70 mRNA plays a key role in regulating Hsp70 expression in Drosophila melanogaster. Mol Cell Biol 1994; 14: 3646-59.
23. Li JH, Zhu GB, Cong H, He XB. The effect of high temperature on LDH and SOD. Chin J Ind Hyg Occup Dis 1993; 11: 358-9.
24. Ames BN, Shigenaga MK. Oxidants are a major contributor to aging. Ann N Y Acad Sci 1992; 21: 85-96.
25. Harman D. The aging process. Proceed Nat Acad Sci USA 1981; 78: 7124-8.
26. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal malonaldehydeand related aldehydes. Free Radic Biol Med 1991; 11: 81-128.
27. Szweda PA, Camouse M, Lundbem KC, Oberley TD, Szweda LI. Aging, lipofuscin formation, and free radical mediated inhibition of cellular proteolyticsystems. Ageing Res Rev 2003; 2: 383-405.
28. Yang C, Du YK, Wang J, Luan P, Yang QL, Huang WH, et al. Transplanted asipose-derived stem cells ameliorate testicular dysfunction in AD-galactose-induced aging rat model. J Cell Physiol 2015; 230: 2403-14.