Original Research Article

Anti-aging activity of *Xylaria striata* in *Drosophila melanogaster*

ABSTRACT

Aims: To evaluate the application potential of *Xylaria striata* in anti-aging field.

Methodology: Extracting the fruit body of *Xylaria striata* by ultrasonic-assisted extraction method. In this study, Drosophila melanogaster was used as an anti-aging organism material. After feeding with different concentrations of extract, the natural survival time, survival time under oxidative stress and survival time under UV irradiation of *Drosophila melanogaster* were all measured. In addtion, the *in vivo* activity of SOD、CAT and MAD, protein concentration and body weight were determined to evaluate the anti-aging effect of ethanol extract from *Xylaria striata*.

Results: The results showed that the ethanol extract of *Xylaria striata* could extend the lifespan of *Drosophila melanogaster* under both irradiation and oxidative stress condition. And the ethanol extract could enhance the activity of CAT in *Drosophila melanogaster*, especially at concentration of 50 μM, and the content of MAD in *Drosophila melanogaster* were decreased significantly.

Conclusion: This study clarified the anti-aging activity of *Xylaria striata* in *Drosophila melanogaster* and it would provide some theoretical basis for its further development and utilization in anti-aging drugs and health food.

Keywords: Xylaria striata; anti-aging; Drosophila melanogaster; lifespan.

1 INTRODUCTION

The higher fungi contain compounds with many novel structure, which have variety and remarkable biological activity[1]. Because of potential medicinal and economic value, they have drawn an increasing attention and become a highlight research field of science and industry in recent years.

The genus *Xylaria*, a big family of mycomycetes, has great value in application according to a large amount of literature reports. There were many kinds of compounds such as terpenoids, sterols, alkaloids, polyketones, polysaccharides, cyclic peptides and carboxylic acids[2-4] had been isolated from the *Xylaria* genus, which exhibited antioxidant, antimicrobial, antitumor, enzyme inhibitory abilities. *Xylaria striata Pat*. 1887, one kinds of summer-living mushroom belong to the family of *Xylaria*, mainly grows on decayed barks and lived roots of broad-leaved woodland. It was used as folk medicine in China[5]. After finding its diverse biological activities in the preliminary screening of large numbers of edible and medicinal mushrooms, our research group started a systematic study on *Xylaria striata* from cultivation method to chemical components since 2013[6-7]. These research results clarified its effect of anti-animal and plant pathogens[8], anti-tumor[9], and promoting sleep[10].

Furthermore, another mushroom from the same genus named Xylaria nigripes has been widely used to prevent and treat senile diseases in China. It can promote the effect of antidepressants[11] and alleviate depressive symptoms in patients with epilepsy[12].Hence, the main objectives of this study were to evalute the anti-aging activity of Xylaria striata using Drosophila melanogaster as the model organism by assessing the lifespan of flies, the activity of SOD and CAT, the content of MDA and protein and its stress resistant ability under H_2O2 and UV irradiation treatment.

2 MATERIALS

2.1 Biomaterial

Xylaria striata was obtained in 2013 from Qingyi town, Mianyang, Sichuan province of China, and identified by professor Xin-sheng He of microbiology, Southwest University of Science and Technology. The voucher specimen was preserved in the Microbiology Laboratory of the same university. *Drosophila melanogaster*, reared under the condition of temperature 25 ± 1℃, humidity 60%-80%.

2.2 Reagents

Protein content determination kit, superoxide dismutase (SOD) determination kit, catalase (CAT) determination kit, and malondialdehyde (MDA) content determination kit are all purchased from Nanjing Jiancheng bioengineering institute; Corn flour, agar and yeast extract was purchased from Bejing Aobaoing Bio-Tech Co., Ltd.; Ethyl ether, ethanol, 30% hydrogen peroxide and other reagents were purchased from ChengDu Chon Chemicals Co., Ltd.; All reagents are analytical pure grade and pure water is made in the laboratory.

2.3 Equipments

Biochemical incubator(SPX- 80B, Shanghai Kuntian instrument Co., Ltd.), Thermo Scientific Microplate Reader(Multiskan Spectrum, Thermo Fisher), Ultraviolet lamp(LP-GDZJ40W, LONGPRO CO.,Ltd.), Ultrasonic cleaner(KQ-200KDE, Kunshan Ultrasonic Instrument Co., Ltd.).

3 METHODS

3.1 Sample pretreatment

The cultivated fruiting body[13] of *Xylaria striata* was dried at 50 ℃ and ethanol-assisted ultrasonic extraction process was carried out at the ratio of material to liquid（1:30 g:mL) for three times, 10 min of each. Then, the extract was obtained by vacuum distilling of the filtered supernatant at 65 ℃ and preserved at 4℃ untill use.

3.2 Diet preparation and Fly husbandry

The wild-type *Drosophila melanogaster* Canton-S (CS) flies, selected as the experiment organism for later assay, were reared at temperature 25 ± 1 ℃, humidity 60%-80%. Before the experiment, *Drosophila melanogasters* were mass reared in 500 mL erlenmeyer flask containing 100 mL of standard cornmeal diet[14] (13% cornmeal w/v, 8% sugar w/v, 2.4% Agar w/v, 1.6% yeast extract w/v and 0.4% acetic acid v/v). To avoid overcrowding, 200 flies of each bottle was enough. When the progeny drosophila was going to hatch, all adult flies would be transferred to the new culture bottle. Afterwards, the flies eclosed within 24 h were collected and segregated according to their sex.

3.3 Longevity assay

Refers to Proshkina *et al*[15], 10 newly eclosed male flies, reared on a standard cornmeal medium with ethanol extract from *Xylaria striata*. The extract was dissolved in water and mixed with the standard diet at final concentrations of 50, 100 and 200 μM. Negative control diets contained only water while positive control contained ascorbic acid at final concentrations of 100 μM. During the rearing process, diet was replaced with fresh medium every five days and the numbers of alive flies were recorded every two days. The test was stopped until all flies were dead. All the treatments were carried out with 3 replicate. Median lifespan was calculated using the method reported by Kaplan–Meier[16] previously.

3.4 Oxidative stress resistance

The situation of group dividing is the same as Longevity assay. Newly eclosed male flies were fed on standard diet with the extract from *Xylaria striata* for 25 days, followed by an oxidative challenge of 9% H2O2 in 6% glucose solution on filter paper strips[17]. The number of death was recorded every 4 h until all flies were dead. Median lifespan was calculated as the same as 3.3.

3.5 Irradiation resistance

The flies were treated at a constant distance of 10 cm from the ultraviolet lamp and irradiation time was 20 min[18]. We strictly controlled the surrounding temperature at 25□ to decrease heat stroke from the lamp during irradiation.Then, the flies were transferred to vials. Dead flies were counted every day until all flies died. Life span was studied in the same way.

3.6 SOD、CAT、MDA，Protein content and weight assays

After 25 days of feeding, flies were transferred into empty tube for 2 hours. After anesthesia, the flies were homogenized in an ice bath at the ratio of weight to normal saline at 1:19 (g:mL). The supernatant was obtained after the homogenate was centrifuged in 2500 r $\cdot min^{-1}$ for 20 min at 4 □. The activity of CAT(catalase) and SOD(superoxide dismutase), the content of MDA(malondialdehyde) and protein were determined by the Thermo Scientific Microplate Reader according to the instruction described by the kit. Flies were anesthetized and weighed every ten flies. For each concentration, 3 replicate were set up.

3.7 Statistical Analysis

All the data collected were repeated 3 times, and the data were displayed in the form of means±SD. The statistical analysis was performed with SPSS 20.0 software, and the difference analysis between groups was performed by One-way ANOVA analysis. Significant differences were expressed by: * P < 0.05; ** P < 0.01; *** P < 0.001.

4 RESULTS AND DISCUSSION

4.1 Longevity assay

Drosophila melanogaster has been widely used in anti-aging experiments as a model organism because of its short lifespan and easy reproduction[19]. The anti-aging ability of the drugs or its toxicity can be reflected at a certain level by comparing the lifespan of *Drosophila melanogaster* before and after feeding the drugs. As shown in Table 1, the ethanol extract of *Xylaria striata* can significantly shorten the mean lifespan of *Drosophila melanogaster*, and with the increasing of the dosage, the more significant the shorten effect is. In addition, the maximum lifespan and half survival time of *Drosophila melanogaster* were also reduced. Therefore, there may be some biologically toxic substances in the ethanol extracts of *Xylaria striata*. It has been reported by Yuan et al.[8] that *Xylaria striata* has an inhibitory effect on plant and animal pathogens, which can also prove that it may contain some toxic substances.

Table 1 The effect of *Xylaria striata* on lifespan

Treatment	Concentration (μM)	Max±SD (day)	H±SD (day)	M±SD (day)
Control	-	45.3±1.2	35.7±1.6	30.6±2.1
Xylaria striata	50	32±0.9*	20±2**	28.2±0.15

	100	41.3±6.2	31.3±4.7	34.1±3.7
	200	38±4.3	15.3±1.9***	24.2±2.1**
Ascorbic acid	100	48.9±3.2	39.0±3.4**	37.7±0.7*

Note: Max, maximum lifespan, days; M, mean lifespan, days; H, half survival lifespan; *p < 0.05, **p < 0.01, ***p<0.001

4.2 Oxidative stress resistance

In vivo, hydrogen peroxide reacts with oxygen to produce hydroxyl radicals, which can induce acute oxidative stress damage and shorten the survival time of *Drosophila melanogaster*[20]. As shown in Table 2, the ethanol extract of *Xylaria striata* could prolong the maximum lifespan, half survival time and mean lifespan of *Drosophila melanogaster* under oxidative stress. When the concentration of extract was 100 μM, the maximum lifespan and the mean lifespan were obviously extended, and the prolongation of the mean lifespan was extremely significant at the concentration of 200 μ M.

Table 2 The effect of oxidative stress on lifespan

Treatment	Concentration (μM)	Max±SD (h)	H±SD (h)	M±SD (h)
Control	0	23.3±3.1	16.7±1.1	17.2±0.6
	50	22±2	17±1.3	17±0.6
Xylaria striata	100	29.3±3.3*	18±4	18.3±0.3*
	200	21.3±2.3	15.3±1.1	18.7±0.3**
Ascorbic acid	100	26±2	18.7±2.3	18.5±0.5**

Note: *p < 0.05, **p < 0.01, ***p<0.001

4.3 UV irradiation resistance

Ultraviolet radiation can cause changes in some physiological functions and damages the body. Herein, we determined if the *Xylaria striata* could repair UV irradiation damage by observing the lifespan changes of *Drosophila melanogaster* under UV irradiation stress. According to a previous report, the sensitivity of *Drosophila melanogast*er to ultraviolet light was closely related to the melanin content[21]. So, this method could also show the effect of the tested agent on melanin production in *Drosophila melanogaster*. As can be shown in Table 3, a low dose of extract could significantly prolong the lifespan of *Drosophila melanogaster* after UV irradiation. Medium, high dose groups could also prolong lifespan, but the effect was inferior to the low dose group. This may be due to the variety of substances in the extract. As the increasing of dose, the increasing biotoxicity resulted in a decrease in lifespan. In addition, we found ascorbic acid can't prolong the lifespan, which indicated that it unable repaired the radiation damage.

Table 3 Effect of UV irradiation on lifespan

Treatment	Concentration (μM)	Max±SD (day)	H±SD (day)	M±SD (day)
Control	0	12.5±0.7	6±1.3	5.2±0.4
	50	17±1.4*	11.5±0.7*	10.4±2.3*
Xylaria striata	100	15±1.4	10±2.8	8.5±1.5
	200	14.5±1.5	9.3±2.9	9±2.5
Ascorbic acid	100	12.5±0.7	9.5±0.7	9.4±0.4

Note: *p < 0.05, **p < 0.01, ***p<0.001

4.4 SOD、CAT 、MAD, protein content and weight assay

After feeding on the extract, the overall living condition can be indirectly reflected by determining the weight and protein content of *Drosophila melanogaster*. In Table 4, there were no significant changes in the weight and protein content of flies.

Superoxide dismutase (SOD), one kind of free radical enzyme, can scavenge the superoxide anion in body, and prevent the body from being oxidized. Hydrogen peroxide reacts with oxygen can produce hydroxyl radicals, which are harmful to the body. However, hydrogen peroxide decomposition *in vivo* can be catalyzed by catalase (CAT) to reduce the generation of hydroxyl free radicals[22]. Hence, SOD and CAT are two kinds of representative enzymes in antioxidant system. The activities of these two enzymes can indirectly evaluate the free radical scavenging ability of the organism[23].

Malondialdehyde (MDA) is a kind of lipid peroxidation product produced by the reaction of free radicals and unsaturated fatty acids. MDA residues in body can be further cross-linked with proteins and peptides to accelerate the aging of the body [24].

As can be seen in Table 4, The ethanol extract of *Xylaria striata* has no positive effect on SOD. But the extract at low dose can promote the activity of CAT to reduce the free radicals. Furthermore, the extract can also significantly decrease the content of MDA which is consistent with the content of free radicals. It is implied that the MDA content can also indirectly reflect the content of free radicals and the degree of lipid peroxidation. So, it is conducted that the ethanol extract of *Xylaria striata* keeps body much away from peroxidation and prevent it from aging too quickly.

Table 4 Effect of *Xylaria striata* on SOD/CAT/MDA

Treatment	Concentration (μM)	Protein content (g/L)	CAT (U/mL)	MDA (nmol/mL)	SOD (U/mg)	Weight (mg/10 flies)
Control	0	1.08±0.16	61.91±5.92	2.65±0.40	43.21±8.5	6.7±0.76
	50	1.32±0.23	87.47±11.34*	0.9±0.27***	34.59±6.71	6.9±0.32
Xylaria striata	100	1.37±0.22	69.46±6.94	1.75±0.11**	36.51±6.91	6.6±0.83
	200	1.3±0.23	58.49±10.34	1.03±0.26***	29.45±2.91	6.2±0.26
Ascorbic acid	100	1.37±0.23	92.27±13.23*	2.65±0.27	34.11±6.2	5.9±0.07

Note: *$p < 0.05$, **$p < 0.01$, ***$p<0.001$

5 CONCLUSIONS

From the previous report that Xylaria striata can promote pentobarbital-induced sleep by not only increasing the number of falling asleep and prolonging sleeping time but also reducing sleep latency[25], this mushroom had potential to be as a functional food used in the field of geriatrics. Above all, the anti-aging activity of *Xylaria striata* was evaluated by measuring the survival time under various conditions, the activity of SOD/CAT and the content of MAD/protein of *Drosophila melanogaster*. The results showed that, although the ethanol extract of *Xylaria striata* could shorten the lifespan of *Drosophila melanogaster* under natural conditions which indicated its biologically toxic, it could extend the lifespan of flies in the longevity test of two stress models by repair both ultraviolet radiation damage and hydrogen peroxide oxidative stress damage. The results of enzyme activity and MAD content showed that the ethanol extract of *Xylaria striata* could not only block the source of free radicals but also eliminate the reaction products of free radicals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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