

# Effects of Rosemary Extract on the Lifespan and Antioxidant System of *Drosophila*

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**Abstract.** The main components of Rosemary extract are Caffeic acid, Rosmarinic acid and Carnosic acid. This article studies the effects of Rosemary extract on delaying aging. Feeding *drosophila* with different dose of Rosemary extract, the lifespan of *drosophila* is different either. Every test group can prolong the lifespan of *drosophila* in some degrees. With the increase of Rosemary extract, aging is delayed in a dose-response relationship. This article also studies the content of maleic diadehyde (MDA), the activity of catalase (CAT) and superoxide dismutase (SOD) on the 30<sup>th</sup> day of *drosophila* being raised. The result shows that 3mg/mL test group could significantly decrease MDA content and increase the activities of CAT and SOD. Rosemary extract can improve the antioxidant enzyme activity, inhibit the lipid peroxidation, and have the function of delaying aging, which might be one of the reasons for prolonging the lifespan of *drosophila*.

**Keywords:** Rosemary extract, *Drosophila*, Lifespan, Antioxidant.

## 1. Introduction

*Drosophila* is a multicellular eucaryon with the advantages of short life, strong fertility, high pure breed and simple feeding, whose metabolism and physiologic condition are essentially similar with mammal [1]. English scientists find the *drosophila* has aging gene which is similar with human's, and therefore, it is used for aging experiment researches.

Rosemary, belongs to lamiaceae and is a perennial, evergreen and shrub-like plant [2]. The extract of Rosemary is extracted from the plant of Rosemary and is the series of compounds with antioxidant activity and is natural antioxidant with high efficiency, main components of which are phenolic diterpenoids, flavonoid and little triterpenoid compound [3]. The researches indicate extract of Rosemary has much activity with immunoregulation, anti-inflammation and anti-cancer and is widely used in food industry [4]. But the reports that the extract of Rosemary can delay aging are few, and therefore, this research intends that the extract of Rosemary with different dosages is used to feed *drosophila* to study the effect from it to *drosophila* lifetime and antioxidant enzyme system and to provide reference to anti-aging activity researches of extract of Rosemary.

## 2. Materials and Methods

### 2.1. Drugs

Extract of Rosemary provided by Tianjin Jianfeng Co., Ltd., superoxide dismutase(SOD), catalase(CAT), malondialdehyde (MDA) and commercial kit for coomassie brilliant blue purchased from Nanjing Jiancheng Bioengineering Research Facility, other reagents are national analytical reagents or biochemical reagents.

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## 2.2. Method of HPLC

Chromatographic column: Hypersil ODS-2(250×4.6mm, 5μm), mobile phase: A: 0.1% phosphoric acid solution, B: acetonitrile, flow speed: 1mL/min, wavelength: 280nm, injection volume: 20μL.

## 2.3. Experimental Animal Culture

Collect wild-type Oregon drosophila with black abdomen which is eclosion but unmated within 8 h, put it in constant-temperature incubator with the temperature of 25°C and relevant humidity of 60%~70% to feed. The experiment selects males as objects of study.

## 2.4. Preparation for Culture Medium of Drosophila

- Basic culture medium

Corn meal 72g, glucose 72g, agar 6g, dried yeast powder 10g, 75% ethanol 40mL (including 0.1% ethyl p-hydroxybenzoate), water 750mL. Pour agar and glucose into pot, add water, heat and boil them together to make them dissolve fully, use left water to turn corn meal and dried yeast powder into a paste, pour well mixing corn meal paste into pot to boil it until it is slightly sticky, add yeast powder paste to fully mix, add 75% ethanol of 45mL after fire is ceased, and then, mix them well.

- Sample culture medium

Weigh a certain amount of extract of Rosemary and add them into heated basic culture medium; and then, make sample culture medium of 1 mg/mL, 3 mg/mL and 5 mg/mL respectively.

## 2.5. Survival Experiment for Drosophila

Get male fruit flies with aging of 2d to make into four groups randomly; each group with the number of 200; and put into 10 test tubes. The control group is cultured on the basic culture medium, the other three groups are cultured on culture medium of extract of Rosemary of 1 mg/mL, 3 mg/mL and 5mg/mL respectively. Change new culture medium every two days and record the number of death until all the fruit flies died. Finally, count up the death time of half the number, average lifetime and high lifetime (the average of survival days of 20 fruit flies which died finally).

## 2.6. Measuring Methods of Overall SOD Activity, CAT Activity and MDA Content of Drosophila

Get male fruit flies with aging of 2d to make into four groups randomly, each group with the number of 100, and put them into 5 test tubes. The culture method is same with the survival experiment. At first, make the fruit flies hungry for 2h in the 30<sup>th</sup> d, weigh them after they are suffocated by industrial nitrogen, freeze and store them in -80°C, and then, measure the content of antioxidase activity and MDA content in fruit flies.

Each tube is used as a sample. Add a certain amount of normal saline into tubes to make up 2% of tissue homogenate, make centrifugal turning at the speed of 2500r/min for 10min under 4°C, and then get liquid supernatant to measure overall SOD activity, CAT activity and MDA content and protein content of drosophila. The concrete method must be operated strictly in accordance with kit specification.

## 2.7. Statistical Analysis

Utilize SPSS statistical software to measure, the value is expressed by means±standard deviation (S.D.), and mean difference among different experimental groups is measured by Student t test.

## 3. Results

### 3.1. Main Components of Rosemary Extract

Known from Figure 1, the main components of Rosemary extract are Caffeic acid (a), Rosmarinic acid (b) and Carnosic acid (c). The retention time of them is 9.106min, 16.098min and 53.183min respectively.

### 3.2. Effect From Extract of Rosemary to Drosophila With Black Abdomen Lifetime

Known from Table 1, the death time of half the number, average lifetime and high lifetime of each dosage group are higher than that of control group. With the concentration increase of extract of Rosemary, the lifetime of drosophila is rising, the lifetime of drosophila in 3mg/mL group is highest; the extract content of Rosemary is continued to be added, while the lifetime of drosophila is shortened, therein, the average

lifetime in 1mg/mL and 3mg/mL has significant difference compared with control group ( $P<0.05$ ,  $P<0.01$ ). Compared with control group, the extending scale of the death time of half the number, average lifetime and high lifetime in 3mg/mL is 22.9%, 17.49% and 12.0% respectively.

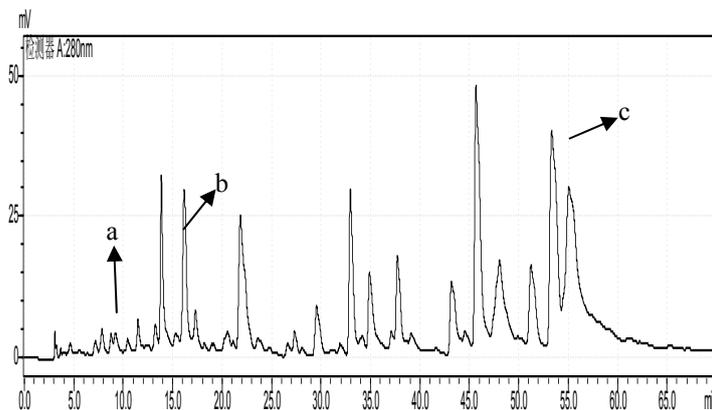


Fig.1 HPLC of effects of rosemary

Table 1 Effects of Rosemary extract on life-span in *Drosophila*

group	50%survival(d)	average life span(d)	maximum life span(d)
control group	48	49.04±7.07	72.75
1mg/mL	54	54.74±4.73*	77.40
3mg/mL	59	57.62±5.63**	81.45
<b>5mg/mL</b>	52	53.78±5.26	76.35

Compared with control group \* ( $P<0.05$ ), \*\* ( $P<0.01$ )

Known from Figure 2 of survival curve of *Drosophila*, the survival rate in each dosage group is higher than that of control group after the male *Drosophila* grows for 30d, especially, the survival curve of 3mg/mL group has significant difference compared with control group.

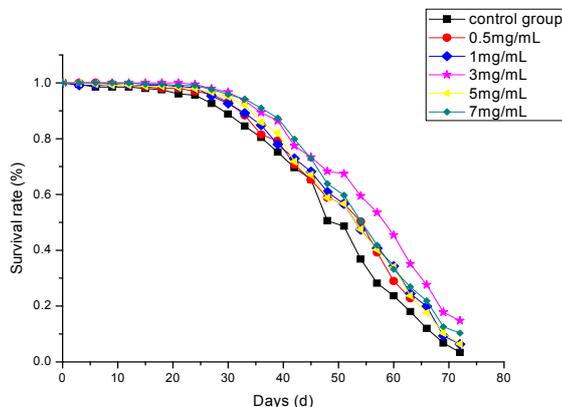


Fig.2 Lifespan curve of wild type flies

### 3.3. Effect From Extract of Rosemary to SOD, CAT and MDA

- Effect from extract of Rosemary to MDA content

Known from Figure 3, the MDA contents in each dosage group are lower than that of control group and have significant difference ( $p<0.01$ ). Within a certain amount of concentration, the MDA content decrease progressively similar with dose-response relationship with the concentration increase of extract of Rosemary, therein, the MDA content in 3mg/mL group is the lowest.

- Effect from extract of Rosemary to SOD and CAT

Known from Figure 4, the CAT activity in each dosage group is higher than that of control group compared with control group, but only 3mg/mL has significant difference( $p<0.01$ ). Known from Figure 5, SOD activity in each dosage group is higher than that of control group. Compared with control group, 1mg/mL group and 3mg/mL group have more significant difference( $p<0.01$ ), 5mg/mL group has significant

difference( $p < 0.05$ ). It shows a certain amount of extract of Rosemary can increase endogenous antioxidase activity(SOD, CAT) in drosophila.

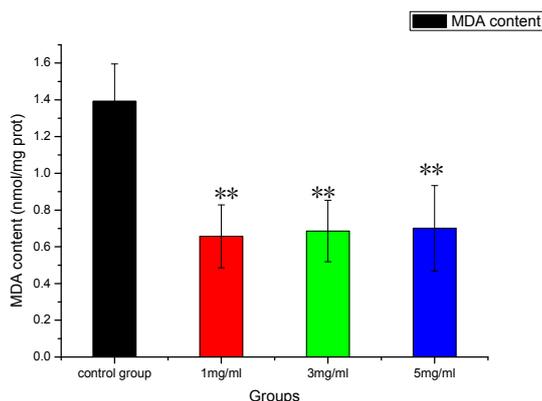


Fig.3 Effects of Rosemary extract on MDA content in Drosophila. Compared with control group \*\*( $P < 0.01$ ).

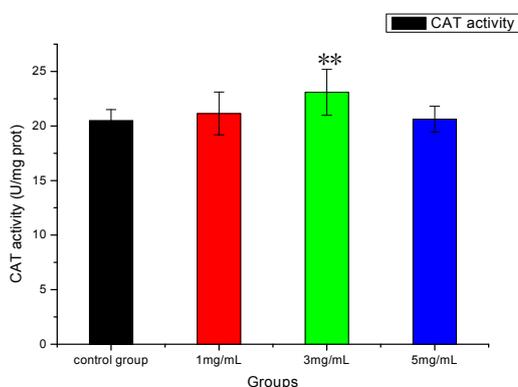


Fig.4 Effects of Rosemary extract on CAT activity in Drosophila. Compared with control group \*\*( $P < 0.01$ ).

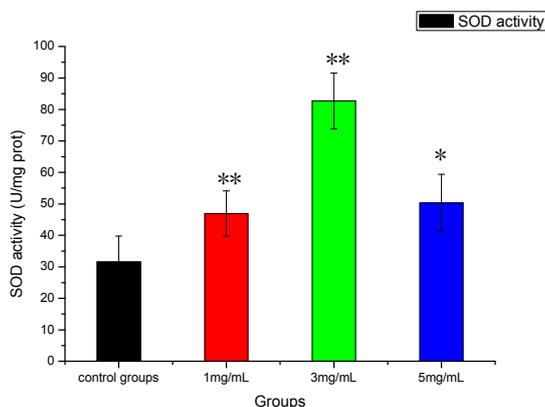


Fig.5 Effects of Rosemary extract on SOD activity in Drosophila. Compared with control group \*( $P < 0.05$ ), \*\*( $P < 0.01$ ).

#### 4. Discussion

From the survival experiment of drosophila, each dosage group of extract of Rosemary can lengthen lifetime of drosophila. With the concentration increase of extract of Rosemary, it shows dose-effect relationship within a certain concentration. The death time of half the number, average lifetime and high lifetime in 5mg/mL group are lower than that of the other two groups and has no significant difference compared with control group, which may be caused by heave smell of extract of Rosemary that the acceptable daily intake of drosophila is effected or that extract of Rosemary with high concentration may produce a certain amount of toxin.

Researches at home and abroad show extract of Rosemary is high-effect natural antioxidant, the main components of which are carnosol, carnosic acid, rosmanol and rosmarinic acid [5]. The researches of Wang Hong find rosmarinic acid can increase anti-oxidase activity, eliminate free radicals, reduce oxide lipid production and play a role of anti-aging [6,7]. The experiment results show 1mg/mL group and 3mg/mL group can increase anti-oxidase activity in drosophila significantly, and meanwhile, restrain lipid peroxide MDA production and effectively delay aging after the drosophila is feed with extract of Rosemary for 30 d.

## 5. Acknowledgements

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