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Rosa damascena Decreased Mortality in Adult *Drosophila*

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ABSTRACT The effects of a rose-flower extract, *Rosa damascena*, on the mortality rate of *Drosophila melanogaster* was evaluated in this study. *R. damascena* is a potent antioxidant that has many therapeutic uses in addition to its perfuming effects. Supplementing *Drosophila* with this rose extract resulted in a statistically significant decrease in mortality rate in male and female flies. Moreover, the observed anti-aging effects were not associated with common confounds of anti-aging properties, such as a decrease in fecundity or metabolic rate.

KEY WORDS: • ***anti-aging effect*** • **Drosophila melanogaster** • ***mortality rate*** • **Rosa damascena** • ***rose-flower extract***

INTRODUCTION

AGING IS A SIGNIFICANT RISK FACTOR that contributes to the development of human diseases such as cardiovascular disorders, cancer, diabetes, cataracts, neurodegenerative disorders, and osteoporosis. Aging is the outcome of the interactions of a plethora of genetic and environmental factors and biochemical pathways. As we age, we accumulate damage at molecular, cellular, and organ system levels, accompanied by the increasing inefficiency of our bodies to repair these types of damage.

Comparative genetic and functional studies between *Drosophila* and human have revealed that 60% of a set of 289 human disease genes have homologues in fruit flies and that about 100 of these genes are involved in various human endocrine and metabolic diseases.[1]

Accumulation of oxidative damage to cells and tissues in conjunction with the inefficiency of our antioxidant defense system is one of the mechanisms that is commonly used to explain aging. The oxidation pathway is perhaps the most studied and manipulated aging pathway, and a number of genetic and pharmacological manipulations targeting this pathway have attempted to delay aging. *Drosophila* and human share a number of important antioxidative enzymes such as cytosolic Cu/Zn and mitochondrial superoxide dismutase, catalase, and glutathione reductase.[2] A number of antioxidants have been evaluated for their potential anti-aging properties. Some of these antioxidants have increased the life span of model systems employed in anti-aging research, such as *Drosophila melanogaster* and *Caenorhabditis elegans*.[3]

Rosa damascena is a small plant from the Rosaceae family with aromatic light pink flowers. This plant flowers in spring and is grown widely in France, Bulgaria, Iran, and Turkey.[4] The major products taken from *R. damascena* are essential oil and rose water, obtained by steam distillation of flowers. In the rose essential oil, several volatile C_{13}-norisoprenoids such as damascenone have been identified, which are important odor constituents.[5] Also, several valuable phenolic compounds (*e.g.*, kampferol and quercitin glycosides) are extracted from petals of *R. damascena* after industrial distillation.[6] Although this rose species is mainly known for its perfuming effects, it has many medicinal uses. The majority of scientific documents are on *in vitro* and animal studies, but there are a few human data on the medicinal effects of *R. damascena*. In recent years, antioxidant,[7] antispasmodic, cardiovascular preventive, antibacterial, and skin protective effects of extracts from this plant have been reported in the medical literature.[8,9]

In the present study, we evaluated the anti-aging properties of this flower and found that *Drosophila* flies supplemented with an extract of this plant exhibited reduced mortality. Although our study does not reveal the causal mechanism behind our observed anti-aging effects, it does suggest that this rose species is worthy of continued investigation. To our knowledge, this is the first study that reports anti-aging effects of *R. damascena.*

MATERIALS AND METHODS

Rosa damascena

R. damascena flowers (voucher number 6526TEH) were dried in the shade, and the petals were pulverized to a coarse

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powder. Aqueous extract was prepared by adding 2.5 g of the powder to 100 mL of boiling water for 10 minutes. After filtration, the extract was lyophilized with a freeze-dryer and stored at 4°C (yield, 35.5% wt/wt).

Experimental methods

The details of the experimental methods used in our mortality, fecundity, metabolic rate, and CNS assays are outlined by Jafari *et al.*[10]

All *D. melanogaster* stocks used in these experiments were ultimately derived from a sample (called "IV") of the Amherst, MA, Ives population studied extensively by Rose and colleagues (see, *e.g.*, Rose *et al.*[11]). *R. damascena* was supplied to adults only. The freeze-dried powder of the flower was mixed into the yeast paste; the fruit fly adults preferentially consume this paste. There were four males and four females in each vial, with a total of 80 vials per dose per sex or 640 flies per dose. The flies were transferred every other day during the aging phase, which lasts 4 weeks in the population that we used in this study. At each transfer, data on the number of dead flies and their sex were recorded. After a number of dose-finding preliminary studies were performed, the final concentration of the rose powder in the yeast was 1, 1.5, and 2 mg/mL. Each dose was compared to a control group that was only exposed to yeast (0 mg/mL group). Adults were transferred to fresh vials, and survivors were counted every 2 days. All assays were conducted on flies that had undergone two generations of controlled-density rearing. When flies from different treatments were compared, all preliminary rearing was carried out in parallel.

Because some compounds may increase life span simply by substantially depressing fecundity (total number of eggs laid by each female fruit fly per day),[12] a fecundity assay is an important check for artifactual life span enhancement. We evaluated age-specific fecundities and the number of eggs laid each day by each individual female for a period of 10 days.

TABLE 1. FRACTION OF *DROSOPHILA* THAT DIED DURING AGING PHASE WITH *R. DAMASCENA*

Replicate	*Sex*	*Dose (mg/mL)*	*Mortality (mean ± SD)*	P *value*
1	Male	0 (control)	0.45 ± 0.03	
		1	0.42 ± 0.03	.48
		1.5	0.45 ± 0.03	.92
		2	0.38 ± 0.03	.05
	Female	0 (control)	0.46 ± 0.03	
		1	0.41 ± 0.03	.29
		1.5	0.37 ± 0.03	.04
		2	0.37 ± 0.03	.04
2	Male	0 (control)	0.61 ± 0.03	
		2	0.47 ± 0.03	.0004
	Female	0 (control)	0.74 ± 0.03	
		2	0.56 ± 0.03	.000017

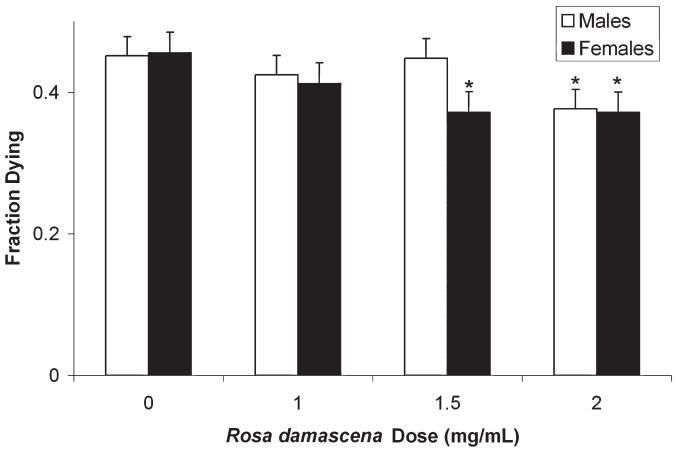

FIG. 1. Fraction dying with *R. damascena* at 1, 1.5, and 2 mg/mL. There is a decrease in the fraction dying with 1.5 and 2.0 mg/mL in male flies and a decrease in the fraction dying with 2 mg/mL in female flies (*$P < .05$).

As a further test for artifactual effects, compounds that had a beneficial effect on mortality, but did not significantly depress fecundity, were assayed for their effect on metabolic rate.[12,13] This assay was used to ascertain whether there had been an artifactual decrease in mortality due to hypometabolism. CO_2 production in drugged flies was compared to that of a control group handled in parallel and assayed simultaneously. We used flow-through respirometry to measure the rate of CO_2 release from groups of flies following the methods of Williams *et al.*[14]

Male virility was used as an assay for a variety of possible secondary drug effects on fly behavior, including reduced activity and other forms of central nervous system depression. For each assay of virility, two male flies—one that was supplemented with *R. damascena* and one that was not supplemented—were placed in a mating vial with a virgin female fly, who had not been exposed to the *R. damascena*. Among the mating vials, half had marked supplemented males; the other half had marked control males. In this experiment each male was scored according to his status (control or supplemented), marked status (marked or not marked), and mating status (mated or not mated). The data were inserted into a contingency table that was analyzed using a log likelihood model.

The experimental conditions such as the size of the vials and the amount of food in the vial were similar for fecundity, metabolic rate, and central nervous system depression assays.

RESULTS

R. damascena at 2 mg/mL resulted in a statistically significant decrease in mortality (Table 1 and Fig. 1). It resulted in 23% and 22% reduction in the fraction dying in the male and female flies, respectively. We were able to confirm the beneficial effects of this dose on mortality in a sec-

ond replicate (Table 1 and Fig. 2). In this replicate assay, 2 mg/mL *R. damascena* resulted in 27% and 22% reduction in the fraction dying in the male and female flies, respectively. Consequently, we proceeded with further testing of fecundity. There was no statistically significant difference in fecundity with any doses relative to the control (Fig. 3).

We proceeded on to a metabolic rate assay, to check for the other major source of pharmacological artifact. As shown in Table 2, *R. damascena* did not have a significant adverse effect on metabolic rate at the doses that we tested on either sex. Accordingly, we continued to an assay of male mating success. As shown in Table 3, there was no significant increase or decrease in mating success in this assay.

DISCUSSION

The present study demonstrates that *R. damascena* decreases the mortality rate of adult *Drosophila* without significantly affecting any secondary physiological mechanisms that could have caused an artifactual extension of life span. The observed anti-aging property of *R. damascena* was not associated with statistically significant reductions in fecundity, metabolic rate, or male mating success.

Caloric restriction has been shown to increase longevity in invertebrates and mammals. There is the potential that the presence of *R. damascena* in the food resulted in lower levels of food consumption and an effect similar to dietary restriction. It is well established that major depression in fecundity is associated with dietary restriction sufficient to significantly increase *Drosophila* longevity.[15] As shown in our fecundity results (Fig. 3), *R. damascena* supplemented at various doses to *Drosophila* cohorts produced no significant fecundity differences in treatment groups relative to the control. In these measurements, there was no statistically significant decrement in reproductive output due to *R. damascena* feeding. Since egg production is proportional to food

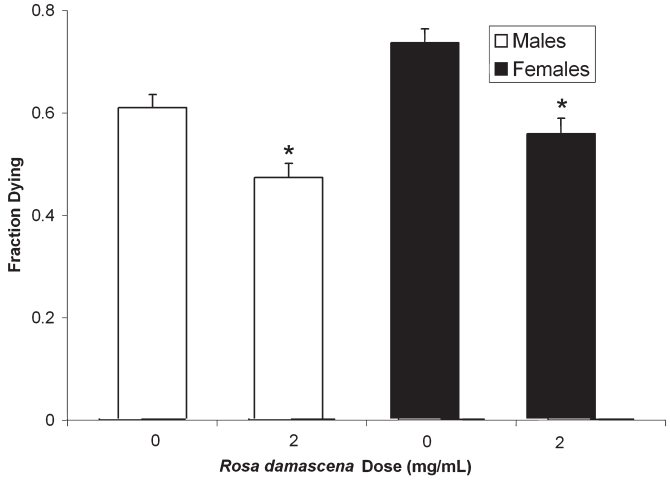

FIG. 2. Fraction dying with *R. damascena* at 2 mg/mL. There is a decrease in the fraction dying with 2.0 mg/mL in male and female flies (*$P < .05$).

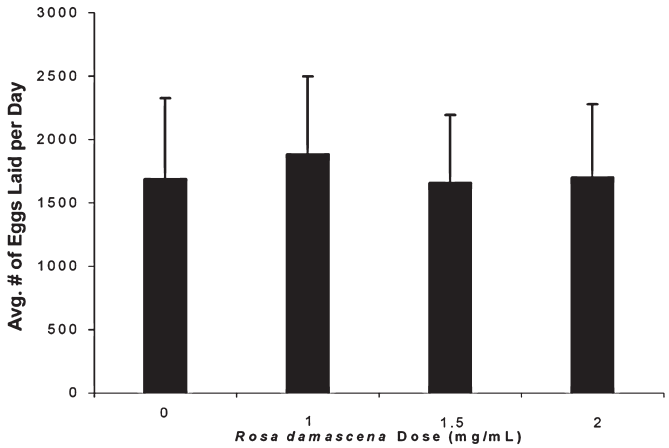

FIG. 3. Fecundity with *R. damascena* at 1, 1.5, and 2 mg/mL. No decrease in fecundity is seen with 1, 1.5, and 2 mg/mL.

level, the absence of a statistically significant decline in fecundity also indicates that the *Drosophila* females were not lowering their levels of *R. damascena*-supplemented food consumption.[16]

We evaluated the impact of *R. damascena* on the metabolic rate and observed that this botanical did not change the metabolic rate. It is well established that poikilotherms experience life span extension as their metabolic rate decreases.[17,18] As humans are homeotherms with fairly stable metabolic rates, compounds that act via gross lowering of metabolic rates, producing hypometabolic syndromes, are not appropriate candidates for anti-aging interventions.

The impairment of nervous system function is a common adverse effect associated with a number of botanical and pharmaceutical agents. In *Drosophila*, studying changes in locomotion is not enough to establish good neurological function. In *Drosophila*, two tests of nervous system function are fairly obvious. A reduction in the ability of supplemented flies to learn is a reasonable indicator of nervous system impairment. Another possible test is male mating function. Male *Drosophila* flies have to perform a fairly elaborate series of behaviors before females will mate with them; failure to accomplish these behaviors individually, or in the correct sequence, normally results in a failure to mate. An excellent test of general nervous system depression in fruit flies thus would be the mating success of supplemented males compared with nonsupplemented males, within the same cohort, in competition as pairs. The lack of *R. damascena*-induced changes in mating behavior showed that this plant did not impair the nervous system.

Although the anti-aging mechanism of *R. damascena* was not evaluated in our study, its antioxidation properties may explain our results. Among the many mechanisms of aging, the theory of reactive oxygen species-induced aging is widely studied and accepted. Many studies have evaluated the impact of antioxidants on life span extension in *Drosophila.* In a study by Bauer *et al.*,[19] two popular an-

tioxidants, resveratrol and lipoic acid, increased the life span. Resveratrol at 200 μM resulted in an average 16% and 10% increase in life span in female and male flies, respectively. Lipoic acid increased the life-span of female *Drosophila* only. Driver *et al*.[20] reported longevity benefit with vitamin E at a narrow therapeutic window. Vitamin E at 20 μg/mL increased the life span, and it was considered ineffective at lower doses and toxic at higher doses.[19–22]

R. damascena is the most commonly used source of rose extracts and oil, but a number of other *Rosa* species (*e.g*., *Rosa centifolia*, *Rosa gallica*, *Rosa alba*, and *Rosa rugosa*) with similar chemical composition have been identified and used for therapeutic purposes.[23] Often, *Rosa* species have more than 100 components, but the major components in all of them are polyphenols and rose alcohols such as geraniol, citronellol, and nerol.

In a study by Ng *et al*.,[24] *R. rugosa*, a commonly used Chinese herbal medicine, at 80 mg/kg/day increased the activities of antioxidant enzymes such as catalase and gluthathione peroxidase in senescence-accelerated mice. The rose extract also resulted in up-regulation of catalase and glutathione peroxidase gene expression and resulted in less lipid peroxidation in the liver, kidneys, and brain of senescence-accelerated mice. In addition to these positive impacts, the average life span of senescence-accelerated mice treated with *R. rugosa* was statistically longer than the control group.[24]

In a study by Jafari *et al*.,[10] a number of anti-diabetic compounds—metformin, glipizide, rosiglitazone, and pioglitazone—were screened for anti-aging effects. These compounds impact insulin and insulin growth factor-like signaling pathways, which are well-known longevity pathways. Only pioglitazone had a small but positive effect on longevity. Female longevity was increased by 1.1 days and male longevity by 0.9 days.[10]

In another study by Jafari *et al*.,[25] four Chinese herbals—Lu Duo Wei, Bu Zhong Yi Qi Tang, San Zhi Pian (Three Imperial Mushrooms), and Hong Jing Tian (*Rhodiola*)—were evaluated for their anti-aging properties. Although all of these herbal mixtures have antioxidant properties, only *Rhodiola*-fed flies exhibited decelerated aging and a decrease in the mortality rate without statistically significant physiological trade-offs that could generate an artifactual longevity benefit. *Rhodiola* increased survival by 3.5 days in males and 3.2 days in females. It decreased mortality rate by 27% in males and 30% in females. Based on a diet assay, it was concluded that *Rhodiola* did not impact food consumption, and thus the longevity-enhancing effects of *Rhodiola* were not produced by a caloric restriction effect but might be due to antioxidative protection against a possible accumulation of free radicals that occurs with aging.[25]

Table 2. Metabolic Rates of *Drosophila* at Different Dosages of *R. damascena*

Sex	*Dose (mg/mL)*	*CO_2 (μL/hour/fly) (average ± SD)*	P *value*
Male	0 (control)	1.42 ± 0.28	
	1	1.25 ± 0.24	.27
	1.5	1.37 ± 0.23	.43
	2	1.35 ± 0.28	.90
Female	0 (control)	1.72 ± 0.33	
	1	1.67 ± 0.27	.74
	1.5	1.74 ± 0.25	.59
	2	1.58 ± 0.25	.17

Table 3. Central Nervous System Competency of *Drosophila* as Measured by Mating Success with *R. damascena*

Dose (mg/mL)	*Frequency of mating success*	*Significance (*P*) of drug-mating interaction*
0 (control)	0.49	
1	0.51	.99
0 (control)	0.43	
1.5	0.57	.97
0 (control)	0.44	
2	0.56	.33

The above studies show that life span can be extended by manipulation of biological pathways involved in aging using pharmaceuticals and botanicals. However, the pharmacology of aging is likely to involve several pathways, given the multifold pathways that affect fruit fly aging.[26–28]

Although we did not evaluate the mechanism of *R. damascena*-induced deceleration of aging, we can postulate that the plant's antioxidant properties could have contributed to this effect.

In conclusion, we have presented evidence that *R. damascena* can extend *Drosophila* life span without impacting physiological mechanisms that can result in an artifactual longevity benefit.

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