Published in final edited form as:

Biogerontology. 2012 April ; 13(2): 105–117. doi:10.1007/s10522-011-9357-0.

Extension of *Drosophila* lifespan by *Rosa damascena* associated with an increased sensitivity to heat

Samuel E. Schriner¹, Niki S. Katoozi¹, Kevin Q. Pham¹, Maral Gazarian¹, Asghar Zarban², and Mahtab Jafari^{1,*}

Samuel E. Schriner: schriner@uci.edu; Niki S. Katoozi: katoozi@usc.edu; Kevin Q. Pham: kqpham@uci.edu; Maral Gazarian: mgazaria@uci.edu; Asghar Zarban: azarban@yahoo.com

¹Department of Pharmaceutical Sciences, University of California, Irvine, Irvine, CA, 92697

²Birjand University of Medical Sciences, Birjand, Iran

Abstract

Rosa damascena, or Damask rose, is a rose hybrid commonly harvested for rose oil used in perfumery and for rose water used to flavor food. The petal extract of *R. damascena* was recently found to decrease *Drosophila melanogaster* mortality without impairing reproductive fitness or metabolic rate. Here, we report that *R. damascena* extended both mean and maximum lifespan of the fly. The extract also protected against oxidative stress in flies, predominantly in females. However, it did not alter mitochondrial respiration or content, superoxide production, or the major antioxidant defenses, superoxide dismutase and catalase. The extract increased survival in both sexes when exposed to reduced iron, though surprisingly, it sensitized both sexes to heat stress (survival at 37° C), and appeared to down-regulate the major heat shock protein HSP70 and the small mitochondrial heat shock protein HSP22, at 25° C and after heat shock (4 hours at 37° C). We hypothesize that *R. damascena* extends lifespan by protecting against iron, which concomitantly leads to decreased HSP expression and compromising heat tolerance.

Keywords

Rosa damascena; botanical extract; heat shock; lifespan; aging

Introduction

Over approximately the last two decades, there has been an enormous research effort into the genetics of aging, primarily in model organisms. This was largely initiated by the finding that a single gene mutation, *age-1*, in the worm was capable of doubling its lifespan (Friedman and Johnson 1988). Since then, there have been a number of reports on mutations or genetic modifications that extend the lifespan of yeast, worms, flies, and mice (Bartke 2011; Kenyon 2010; Giannakou and Partridge 2007). While these studies significantly help our understanding of the aging process, they are of little benefit in human medicine due to the current difficulties associated with gene therapy, particularly germ line modification. The natural extension of these studies, therefore, is to search for compounds that can modulate these same pathways, which can then be easily administered. This strategy is exemplified by the exploration of rapamycin, as an inhibitor of the target of rapamycin complex, to extend lifespan in flies and mice (Bjedov et al. 2010; Harrison et al. 2009; Miller et al. 2011)

* Address for correspondence and reprints: Mahtab Jafari, Department of Pharmaceutical Sciences, 147 Biological Sciences Administration, University of California, Irvine, Irvine, CA 92697, USA, Tel: (949) 824-0145, Fax: (949) 824-2118, mjafari@uci.edu.

We have pursued an alternate strategy. Instead of searching for compounds that can modulate a particular ageing pathway, we searched for compounds, and plant extracts, that improved survival without bias to any particular mechanism. Using the fruit fly, *Drosophila melanogaster*, we indentified the petal extract of the rose, *Rosa damascena* as able to decrease fly mortality, without impairing reproductive fitness or metabolic rate (Jafari et al. 2008). *Rosa damascena* is a hybrid rose species predominantly grown in Turkey and Bulgaria to produce rose oil and rose water to be used in perfume, cosmetic and food industries. The extract has been found to have medicinal properties as well. It has anti-microbial activity, and has been reported to protect neurons against amyloid β toxicity, a major pathological component of Alzheimer's disease, and to protect rats against seizures (Basim and Basim 2003; Shokouhinejad et al. 2010; Awale et al. 2009; Ramezani et al. 2008). The active components of *R. damascena* are not known. *Rosa damascena* oil is composed of a large number of volatile organic compounds including various terpenes such as citrenellol, heneicosane, and disiloxane (Loghmani-Khouzani, et al. 2007). The marc, material left after rose oil is extracted, has significant polyphenol content, including quercetin, myricetin, kaempferol and gallic acid (Kumar et al. 2008). Though, the predominant molecules have been suggested to be glycosides of quercetin and kaempferol (Kumar et al. 2008).

Given that *R. damascena* decreased mortality, without any negative impact on fecundity or metabolic rate (Jafari et al. 2008), we considered it to be a potential anti-aging therapy, and further explored its biological activities in the fly. Here, we report that *R. damascena* extended both mean and maximum fly lifespan, while enhancing protection against iron toxicity. The extract compromised survival in response to heat, and decreased the expression levels of two heat shock proteins. The interpretation of these findings and possible underlying molecular actions of *R. damascena* are discussed.

Materials and methods

Measurement of polyphenol content

HPLC analysis of *R. damascena* extract was performed by Alkemists Pharmaceuticals (Costa Mesa, CA). Two-hundred and fifty mg of *R. damascena* was suspended in 10 mL methanol and sonicated for 1 h at 45° C. The samples were run on a C18 column with a mobile phase mixture of water and 0.025% trifluoroacetic acid in acetonitrile. Compounds were detected at 280 nm, and concentrations were estimated by comparison to known concentrations of standard compounds.

Fly strain, feeding and lifespan assays

Two strains of flies were used in this study, standard *w¹¹¹⁸* flies and B flies. B flies were obtained from M. R. Rose, and were originally derived from the South Amherst, Massachusetts population established by P.T. Ives in 1975, from an initial collection of 200 males and 200 females (Rose and Charlesworth 1981; Rose et al. 2002). These flies have been cultured at moderate to large population sizes and controlled densities (50–80 eggs per vial) for more than 700 generations with discrete generations cultured every 2 weeks. All studies in this work use the B flies, except for the survival analysis of *w¹¹¹⁸* flies in Fig. 1, C and D.

Flies were fed *R. damascena* extract based on the methods described in Jafari *et al.* (2008). Five mg/mL of *R. damascena* extract were mixed in a yeast solution (2 g yeast/50 mL 1% acetic acid), and 75 μ L of this mixture was overlaid on standard *Drosophila* banana-molasses food composed of 9% carbohydrate and 4% yeast. Flies were maintained at 25° C under constant illumination for all experiments. For lifespan studies, 200 flies for each

treatment group (100 per sex) were housed 10 to a vial (5 males and 5 females). This density was maintained as long as feasible. Flies were given fresh food every two days and deaths were recorded at these times. Survival analyses were calculated based on the number of deaths recorded and evaluated by the Mantel-Cox log-rank test. For mitochondrial respiration, superoxide ($O_2^{\cdot-}$), SOD and catalase assays, 800 flies for each treatment group (400 per sex) and experiment were fed for two weeks in a manner described above. This time frame was selected as it was sufficient to mediate a detectable difference in life span, but permits the study of relatively young animals, which would minimize normal age related changes.

Mitochondrial isolation and functional assays

Fifty flies per sample were homogenized in ice-cold isolation buffer (225 mM mannitol, 75 mM sucrose, 10 mM MOPS, 1 mM EGTA, 0.5% fatty acid free BSA, PH 7.2) using a glass-teflon dounce homogenizer, and then filtered through two layers of cotton gauze. A mitochondrial enriched pellet was obtained by centrifugation for 10 min at $6000 \times g$ and re-suspended in 150 μ L ice-cold isolation buffer. Fifty μ L of this suspension were used for each respiration or superoxide measurement.

Mitochondrial respiration rates were measured using a Clark-type oxygen electrode and monitoring system (Hansatech Instruments, Norfolk, England). An NADH-linked substrate (pyruvate in combination with malate, 5 μ mol each) was added to isolated mitochondria suspended in 1 mL of respiration buffer (225 mM mannitol, 75 mM sucrose, 10 mM KCl, 10 mM Tris-HCl, 5 mM KH_2PO_4 , pH 7.2) at 30° C with stirring. To this, 125 nmol ADP was added to generate state 3 respiration. State 3 respiration represents the maximal ability to generate ATP, and is generally 5–6-fold higher than state 4 respiration, which is determined after ADP is consumed. The ratio of state 3 to state 4 defines the respiratory control ratio (RCR), which is used to evaluate the functional quality of the mitochondrial preparations. Ratios of approximately 5 or greater are considered to be functionally acceptable. Uncoupled (maximal rate of O_2 consumption) respiration rates were measured after addition of 0.5 nmol of the uncoupler FCCP (Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazine).

Rates of mitochondrial superoxide production were measured with the fluorescent dye MitoSOX (Invitrogen, Carlsbad, CA) according to a protocol modified from Robinson *et al.* (2006). Freshly isolated mitochondria were incubated in the presence of 10 mM succinate as an electron donor, 0.8 μ M MitoSOX, and 2.5 mM ADP to induce state 3 respiration. Mitochondria were incubated for 5 min at 30° C, after which 15 μ M antimycin was added to block the electron transport chain and maximize superoxide production. Superoxide production was then taken as the rate of MitoSOX oxidation measured at an excitation of 510 nm and emission of 579 nm and normalized to the amount of mitochondrial protein used. Alternatively, *in vivo* $O_2^{\cdot-}$ levels were compared based on the relative levels of the TCA cycle enzyme aconitase (Hausladen and Fridovich 1996). Aconitase activities were measured by the production of *cis*-aconitate at 240 nm (Hausladen and Fridovich 1996).

Enzyme activities and protein measurements

Fifty flies per sample were homogenized in 2 mL of ice cold 50 mM potassium phosphate buffer, pH 7.0. Homogenates were centrifuged at $10,000 \times g$ for 10 min at 4° C, and the supernatants were used for enzymatic analyses. Superoxide dismutase (SOD) activities were determined by an indirect method using xanthine/xanthine oxidase as a superoxide generator and nitroblue tetrazolium (NBT) as the target (Winterbourn *et al.* 1975). The SOD activities of the total fly homogenates were determined from a standard curve generated with purified Cu, Zn-SOD protein. One unit of SOD is defined as the amount of protein required to inhibit

NBT reduction (at 560 nm) by 50%. MnSOD levels are considered to be the level of SOD activity in the presence of 5 mM KCN that inhibits Cu, Zn SOD proteins. Catalase activity was measured by direct decomposition of H₂O₂ (240 nm) in 50 mM potassium phosphate buffer, pH 7.0 (Beers and Sizer 1952). Citrate synthase was measured by a method based on Williams *et al.* (1998). The reduction of dithionitrobenzoic acid is measured at 412 nm in the presence of 0.3 mM acetyl-coenzyme A, and 0.5 mM oxaloacetic acid in 100 mM Tris-HCl, pH 8.0. Fumarase was measured by the conversion of L-malate to fumarate in 50 mM phosphate buffer, pH 7.6 measured at 240 nm. SOD and catalase enzyme activities were reported as normalized to citrate synthase activity.

Heat, starvation, and oxidative stress challenges

Two hundred flies for each treatment group (100 per sex) were fed *R. damascena* at 5 mg/mL for one week after which they were subjected to oxidative challenge of 12.5 mM paraquat, 5% H₂O₂, or 80 mM iron-nitrilotriacetate (Fe-NTA) in a 5% glucose solution on filter paper. Control flies fed 5% glucose only exhibited no deaths during the time frame of these experiments. Fe-NTA solution was mixed according to Awai *et al.* (Awai et al. 1979). Flies (5 males, 5 females per vial) were housed in 8 dram vials. Deaths were recorded every 4 h until the completion of the assay. For heat tolerance, 200 flies were housed at 37° C, and deaths recorded every h. Tolerance to starvation was measured as the ability to survive in the presence of agarose alone, to provide moisture. Deaths were recorded every 8 hours. Survival was measured by both the Mantel-Cox log-rank and Mann-Whitney analysis of average life spans.

Gene expression assays

Approximately 400 Flies were fed 5 mg/mL *R. damascena* or an identical control diet for two weeks. These flies were either directly frozen in groups of 50 or subjected to 4 h treatment at 37° C prior to freezing. RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Samples were treated with DNase (New England Biolabs, Ipswich, MA) at 37° C for 10 min to remove contaminating DNA. DNase was heat inactivated by incubation at 75° C for 10 min in the presence of 5 mM EDTA. RNA was then purified by use of the RNeasy kit (Qiagen, Hilden, Germany). RNA quantity and quality was measured by spectrophotometry. One µg of RNA from each sample was converted to DNA by the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). Samples were diluted 100-fold. Quantitative PCR was performed on a MiniOpticon real-time PCR system with SYBR green dye (Bio-Rad, Hercules, CA). Relative amplification was calculated by the threshold cycle of each respective gene divided by the threshold cycle of the reference gene, RNA polymerase II. Primer sequences and product sizes are as follows. RNA pol II forward: AGGCGGCGAGGACATGGAT, and reverse: CGACGGCTGGTAGTGACCGC. HSP70 forward: ACCAAGGGGTGTGCCCCAGA, and reverse: CTTGGCCTTGCCCGTGCTCA. HSP22 forward: TTGGCGGATGGCCGAGGAGA, and reverse: AGCGCCACACTCCAAACGGG. Primers were designed by NCBI/Primer-BLAST.

Statistical analyses

Fly survival was evaluated by the Mantel-Cox log-rank test and mean lifespans were evaluated by the Mann-Whitney nonparametric test. The remaining experiments were evaluated by 2-way analysis of variance (ANOVA). *P*-values less than 0.05 were considered statistically significant, and were calculated using Prism (GraphPad Software, San Diego, CA).

Results

Polyphenol content in *R. damascena* extract

Polyphenols have been shown to exhibit antioxidant activity, and could potentially contribute to the activity of the *R. damascena* extract. We used HPLC to measure the content of 4 principal previously shown to be present in the extract. These were myricetin, quercetin, kaempferol, and gallic acid. This was done to assess their presence in this extract, and to standardize the extract used in this study relative to any other extract of *R. damascena*. The particular extract used here was composed of 0.330% myricetin, 0.040% gallic acid 0.051% quercetin, and 0.003% kaempferol.

Rosa damascena Extended *Drosophila* lifespan

Previously, we reported that supplementing flies with *R. damascena* extract in their diets could decrease mortality, defined as the proportion dead after 28 days (Jafari et al. 2008). Here we investigated the ability of the extract to increase mean lifespan and the mean age of death of the longest-lived 10%. When supplemented to our B flies, *R. damascena*, increased overall survival in both males and females (Fig. 1 and Table 1) and also increased both mean lifespan and the average age of death of the longest-lived 10% (Table 1) relative to control diet fed flies. We also examined the effect of *R. damascena* on a different strain of flies, *w¹¹¹⁸*. Here, *R. damascena* showed a sex-specific effect with an improved lifespan in *w¹¹¹⁸* males, but not females (Fig. 1 and Table 1), though the extract does appear to have a beneficial effect late in life in the *w¹¹¹⁸* females (Table 1).

Rosa damascena protected B flies against oxidative stress and iron

Oxidative stress has been implicated in aging for over 60 years (Harman 1956). Therefore, we examined whether *R. damascena* could enhance protection against three types of oxidative insults: paraquat, which generates superoxide, H_2O_2 , and iron-nitilotriacetate (Fe-NTA). The extract exhibited minimal or no protection in males against paraquat and H_2O_2 (Fig 2A and C). However, the extract did seem to enhance protection in females against these two agents (Fig 2B and D), though to a modest degree. In contrast, *R. damascena* afforded a positive protective effect in both sexes when challenged with Fe-NTA (Figure 2C).

Rosa damascena did not alter the major antioxidant defenses, mitochondrial content or function, ROS production, or total soluble protein

Animals have multiple antioxidant defenses. The major antioxidant enzymes in the fly include the superoxide dismutases (SODs), which convert superoxide to H_2O_2 , and catalase, which converts H_2O_2 to water and oxygen. We investigated whether changes in the activities of these enzymes could explain the protective effect of *R. damascena*. We found that *R. damascena* feeding had no effect on total SOD activities (which includes both the cytosolic and mitochondrial forms) or on the mitochondrial form alone (Figure 3A and B) in either sex. Nor did *R. damascena* modulate catalase activities (Figure 3C).

Because of the increasing implication of mitochondrial function in aging and disease (Fearon and Faux 2009; Ballinger 2005; Wang et al. 2007; Choi et al. 2005; Henchcliffe and Beal 2008; Baron et al. 2007; Martin 2006; Johannsen and Ravussin 2009), we investigated whether *R. damascena* could modulate mitochondrial function resulting in either increased consumption of oxygen, or decreased production of mitochondrial reactive oxygen species (ROS). A decreased endogenous production of ROS and/or elevated oxygen utilization could confer protective effects against exogenous oxidative stress. *Rosa damascena* had no effect on mitochondrial respiratory activity (Table 2). Using the mitochondrial-localized superoxide-sensitive dye, MitoSOX, we found no evidence that *R. damascena* could

decrease superoxide levels in isolated mitochondria (Fig 4A). We also investigated whether *R. damascena* might decrease superoxide levels *in vivo* by measuring the activity of aconitase, a TCA cycle enzyme that is especially sensitive to mitochondrial ROS levels. Consistent with our respiration and MitoSOX data, *R. damascena* feeding had no effect on mitochondrial aconitase activities (Fig 4B). Elevated mitochondrial content could also offer a protective effect, as the net increase in mitochondria would elevate whole animal oxygen consumption potentially resulting in a decreased ROS production. Some lifespan-extending treatments, such as dietary restriction (DR) and resveratrol, have been reported to increase mitochondrial content (Nisoli et al. 2005; Baur et al. 2006; Lagouge et al. 2006). *Rosa damascena* had no effect on mitochondrial content as measured by two parameters, total fly mitochondrial protein (Fig 5A) and citrate synthase activity (Fig 5B). Because of the potential concern that *R. damascena* may mediate its effects through a DR-associated mechanism, we also inquired whether its feeding altered total soluble protein levels. A decrease in soluble protein has been shown to occur in DR treated flies (Magwere et al. 2006). *Rosa damascena* had no effect on total soluble protein (Fig. 5C).

***Rosa damascena* sensitized B flies to heat and down-regulated HSP22 and HSP70**

Genetic or pharmacological treatments that extend lifespan are often associated with enhanced resistance to stress, including environmental stresses (Vermeulen and Loeschcke 2007; Kirkwood and Austad 2000). We therefore examined the ability of *R. damascena* to protect flies against heat and starvation. We found that *R. damascena* provided no protection against starvation (Fig 6A and B). However, the extract appeared to compromise survival at 37° C (Fig. 6 C and D).

Because *R. damascena* sensitized flies to heat, we investigated whether *R. damascena* could affect the expression levels of heat shock proteins. We investigated two HSPs that are thought to play a role in aging, HSP70 and HSP22 (Tower 2011; Yang and Tower 2009). HSP70 is the principal heat shock protein in animals. HSP22 is located within the mitochondrion and massively up-regulated (>60-fold) during the aging process (King and Tower 1999). We found that *R. damascena* feeding resulted in decreased expression levels of both HSP22 and HSP70 (Figure 7A and B), both at room temperature (~22° C) and after treatment with heat shock (4 h at 37° C). We also estimated whether *R. damascena* affected the ability of flies to up-regulate these proteins in response to heat. HSP22 was up-regulated 49 ± 6 fold in control males and 52 ± 28 fold in *R. damascena*-fed males ($P = 0.31$, Mann-Whitney test, $n = 6$). HSP70 was up-regulated 22 ± 4 fold in control males and 39 ± 9 fold in *R. damascena*-fed males ($P = 0.39$, Mann-Whitney test, $n = 6$). Therefore, it appears that *R. damascena* does not block the heat shock response of these proteins, but rather acts in some fashion to lower their baseline expression levels.

Discussion

Previously, we reported that the petal extract from the rose, *Rosa damascena*, was able to decrease *Drosophila* mortality without any negative effects on egg laying, mating behavior, or metabolic rate (Jafari et al. 2008). These findings suggested that further investigation of the action of *R. damascena* in the fly was warranted. Here, we examined the impact the extract has on mean and maximum lifespan, and determined whether it can improve tolerance to stress. Because of the potential involvement of reactive oxygen species (ROS) and mitochondrial function in aging and disease (Harman 1956; Beckman and Ames 1998; Wallace et al. 1998; Wallace 2005), we also determined the ability of *R. damascena* to modulate mitochondrial function, decrease ROS levels, and/or elevate antioxidant defenses. We found that *R. damascena* extended lifespan in both sexes of the B flies, by a magnitude of up to 32%. In *w¹¹¹⁸* flies, the extract extended male lifespan up to 14%, and had no effect in females, suggesting strain and sex-specific effects. In B flies, the extract also appeared to

preferentially protect females against oxidative insult (H_2O_2 and paraquat), though it afforded protection to both sexes against iron (Fig 2). *Rosa damascena* had no effect on mitochondrial function, mitochondrial content, ROS production, or antioxidant defenses (Table 2 and Figs. 3, 4 and 5). However, it appeared to compromise survival at 37° C and down-regulated two heat shock proteins (Figs 6 and 7), suggesting a potential trade-off between resistance to iron toxicity and tolerance to heat in B flies.

Since aging has been associated with a loss in stress resistance, and treatments that extend lifespan are often associated with increased stress resistance (Vermeulen and Loeschcke 2007; Kirkwood and Austad 2000), we first investigated whether *R. damascena* could confer elevated protection against various stresses, in addition to an increased lifespan. Therefore, it was expected that *R. damascena* would confer an improved stress tolerance in both sexes as it extended lifespan in both sexes. What was unexpected was the sex specificity in resistance to paraquat and H_2O_2 , where *R. damascena* seemed to afford a protective effect in females, but not in males (Fig 2A and B). Since *R. damascena* extended lifespan in both sexes, we might infer that its action is not strongly related to protection against oxidative stress. This is also consistent with our findings that the extract had no effect on antioxidant enzymes, superoxide levels, mitochondrial content, or mitochondrial respiratory parameters in either sex. These results are not entirely surprising; as some have argued that oxidative stress plays a minimal role in lifespan (Lapointe and Hekimi 2010; Salmon et al. 2010; Speakman and Selman 2011).

Rosa damascena however, appeared to protect both sexes against iron toxicity (Fig 2C). This is significant, as loss of iron homeostasis has been suggested to drive mitochondrial dysfunction and the aging process (Xu et al. 2010). Iron has been shown to accumulate in flies as they age, and to shorten fly lifespan in a dose dependent manner when supplemented in the diet (Massie et al. 1993). The same study also showed that a natural product, black tea (*Camellia sinensis*) blocked the age-dependent accumulation of iron, and extended lifespan. Because tea prevented the increase in iron accumulation, the authors concluded that it must somehow block the uptake of iron, as there is no mechanism of export in the fly. They also investigated the action of individual components of tea including caffeine, catechin, epicatechin, theobromine, and tannic acid. None of these compounds blocked the uptake of iron, nor did they increase fly lifespan when supplemented in the diet, and the authors remained uncertain as to how black tea blocked iron uptake. Nevertheless, a contributing factor in the action of *R. damascena* may be the prevention of iron uptake. Alternatively, flies contain multiple proteins such as transferrin, ferritin, and frataxin that bind iron and regulate iron metabolism (Dunkov and Georgieva 2006; Kondapalli et al. 2008). Up-regulation of these proteins could be involved in the beneficial effects of *R. damascena* on fly lifespan and iron tolerance.

While *R. damascena* protected flies against iron, it sensitized them against heat (Fig 6). We followed this observation by finding the down-regulation of two important heat shock proteins, HSP22 and HSP70. Heat shock proteins are group of functionally related proteins that are up-regulated in response to heat and variety of other stressors, such as heavy metals, starvation, desiccation, and hypoxia. We were particularly interested in HSP22 and HSP70, as they are up-regulated during aging in the fly, with the levels of HSP22 increasing enormously (King and Tower 1999). This age-dependent increase in expression suggests that these proteins may be especially important in protecting the flies against elevated stress occurring during aging. Therefore it was surprising to find that *R. damascena* treated flies had decreased levels of these proteins. However, the relationship of HSP22 and aging is not quite clear. Over-expression of HSP22 has been shown to increase fly lifespan, protect flies against paraquat, and improve their locomotor ability (Morrow et al. 2004) and HSP22 was found to be elevated in flies selected for increased longevity (Kurapati et al. 2000).

Therefore, we would predict that a decrease in HSP22 expression to be detrimental. However, another group reported the opposite effect, that HSP22 over-expression shortened lifespan and sensitized flies to oxidative stress (Bhole et al. 2004). The latter group argued that these differences might be attributed to the promoters being used in the respective studies and their resulting expression patterns. Nevertheless, the relationship between HSPs and aging may be complex, and it is not implausible that a decrease in HSP22, and/or HSP70 could correlate to an enhanced lifespan. Our results also suggest that *R. damascena* does not impair the heat shock response. At least one HSP, HSP27 has been implicated specifically in iron metabolism. Over-expression of HSP27 resulted in decreased iron levels in cultured cells and mice (Chen et al. 2006; Zhang et al. 2010). We favor the possibility that *R. damascena* directly protects against cellular stress, iron in particular, which in turn, results in lowered baseline levels of HSP22 and HSP70.

The extract of *R. damascena* contains many different compounds, which can be crudely divided into two groups, those present in rose oil, and those present in the marc, material left after extraction of the rose oil. Rose oil is typically obtained by steam distillation of the petals, and is composed of numerous volatile compounds. Citrenellol, heneicosane, and disiloxane are the three most prominent (Loghmani-Khouzani, et al., 2007). The marc, which is comparable to the material used in this study, is particularly enriched in polyphenols, with the predominant compounds being gallic acid, quercetin, myricetin, and kaempferol (Kumar et al. 2008). We initially considered that the polyphenols present in our extract might explain its effects. Polyphenols have been suggested to have multiple health benefits, with quercetin, in particular, having been shown to increase mitochondrial biogenesis in the brain and skeletal muscle of mice (Davis et al. 2009), activate the mammalian Sir2 homolog, SIRT1, and extend lifespan in the worm (Kampkotter et al. 2008). The discordance in oxidative stress resistance and lifespan extension in males suggests that the polyphenol component may be insufficient to explain the beneficial effects of *R. damascena*. This is not surprisingly as the total polyphenol content of the extract used in this study may be less than 1%. The predominant polyphenols detected were myricetin (0.33%), gallic acid (0.04%), and quercetin (0.05%), totaling some 0.4% of the extract. Therefore, it is likely that some other compounds are responsible for the activity of *R. damascena*. While we would expect the volatile oils to be predominantly removed from our extract through boiling during its preparation, or evaporated during feeding, there may remain a significant amount to mediate the observed protective effects. Future work will examine the action of individual polyphenolic and terpene compounds on fly lifespan, resistance to iron and heat, and HSP gene expression.

In summary, we have found that the petal extract of the rose, *R. damascena*, is able to extend the lifespan of the fruit fly. However, it has a sex specific protective effect against oxidative stress, and no effect on mitochondrial content, function, or antioxidant defenses. The extract did protect both sexes against iron, while compromising survival at 37° C. We hypothesize that compounds present in *R. damascena* extract directly afford enhanced protection to the fly, resulting in enhanced survival, whilst causing endogenous defenses to be down-regulated, correlating with an impaired response to heat.

Acknowledgments

We would like to thank Dr. Douglas C. Wallace for the use of his equipment and facilities. This work was funded by an R21 grant awarded to MJ awarded by the National Center for Complementary and Alternative Medicine.

References

- Awai M, Narasaki M, Yamanoi Y, Seno S. Induction of diabetes in animals by parenteral administration of ferric nitrilotriacetate. A model of experimental hemochromatosis. *Am J Pathol.* 1979; 95 (3):663–673. [PubMed: 377994]
- Awale S, Tohda C, Tezuka Y, Miyazaki M, Kadota S. Protective Effects of *Rosa damascena* and its Active Constituent on A β (25–35)-induced Neuritic Atrophy. *Evid Based Complement Alternat Med.* 2009 nep149 [pii]. 10.1093/ecam/nep149
- Ballinger SW. Mitochondrial dysfunction in cardiovascular disease. *Free Radic Biol Med.* 2005; 38 (10):1278–1295. S0891-5849(05)000869 [pii]. 10.1016/j.freeradbiomed.2005.02.014 [PubMed: 15855047]
- Baron M, Kudin AP, Kunz WS. Mitochondrial dysfunction in neurodegenerative disorders. *Biochem Soc Trans.* 2007; 35 (Pt 5):1228–1231. BST0351228 [pii]. 10.1042/BST0351228 [PubMed: 17956319]
- Bartke A. Single-gene mutations and healthy ageing in mammals. *Philos Trans R Soc Lond B Biol Sci.* 2011; 366 (1561):28–34. 366/1561/28 [pii]. 10.1098/rstb.2010.0281 [PubMed: 21115527]
- Basim E, Basim H. Antibacterial activity of *Rosa damascena* essential oil. *Fitoterapia.* 2003; 74(4): 394–396. S0367326X03000443 [pii]. [PubMed: 12781814]
- Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK, de Cabo R, Sinclair DA. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature.* 2006; 444 (7117):337–342. nature05354 [pii]. 10.1038/nature05354 [PubMed: 17086191]
- Beckman KB, Ames BN. The free radical theory of aging matures. *Physiol Rev.* 1998; 78 (2):547–581. [PubMed: 9562038]
- Beers RF Jr, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem.* 1952; 195 (1):133–140. [PubMed: 14938361]
- Bhole D, Allikian MJ, Tower J. Doxycycline-regulated over-expression of hsp22 has negative effects on stress resistance and life span in adult *Drosophila melanogaster*. *Mech Ageing Dev.* 2004; 125 (9):651–663. S0047-6374(04)00154-X [pii]. 10.1016/j.mad.2004.08.010 [PubMed: 15491684]
- Bjedov I, Toivonen JM, Kerr F, Slack C, Jacobson J, Foley A, Partridge L. Mechanisms of life span extension by rapamycin in the fruit fly *Drosophila melanogaster*. *Cell Metab.* 2010; 11 (1):35–46. S1550-4131(09)00374-X [pii]. 10.1016/j.cmet.2009.11.010 [PubMed: 20074526]
- Chen H, Zheng C, Zhang Y, Chang YZ, Qian ZM, Shen X. Heat shock protein 27 downregulates the transferrin receptor 1-mediated iron uptake. *Int J Biochem Cell Biol.* 2006; 38 (8):1402–1416. S1357-2725(06)00049-5 [pii]. 10.1016/j.biocel.2006.02.006 [PubMed: 16546437]
- Choi J, Rees HD, Weintraub ST, Levey AI, Chin LS, Li L. Oxidative modifications and aggregation of Cu,Zn-superoxide dismutase associated with Alzheimer and Parkinson diseases. *J Biol Chem.* 2005; 280 (12):11648–11655. M414327200 [pii]. 10.1074/jbc.M414327200 [PubMed: 15659387]
- Davis JM, Murphy EA, Carmichael MD, Davis B. Quercetin increases brain and muscle mitochondrial biogenesis and exercise tolerance. *Am J Physiol Regul Integr Comp Physiol.* 2009; 296 (4):R1071–1077. 90925.2008 [pii]. 10.1152/ajpregu.90925.2008 [PubMed: 19211721]
- Dunkov B, Georgieva T. Insect iron binding proteins: insights from the genomes. *Insect Biochem Mol Biol.* 2006; 36 (4):300–309. S0965-1748(06)00010-5 [pii]. 10.1016/j.ibmb.2006.01.007 [PubMed: 16551544]
- Fearon IM, Faux SP. Oxidative stress and cardiovascular disease: novel tools give (free) radical insight. *J Mol Cell Cardiol.* 2009; 47 (3):372–381. S0022-2828(09)00225-9 [pii]. 10.1016/j.yjmcc.2009.05.013 [PubMed: 19481547]
- Friedman DB, Johnson TE. A mutation in the *age-1* gene in *Caenorhabditis elegans* lengthens life and reduces hermaphrodite fertility. *Genetics.* 1988; 118 (1):75–86. [PubMed: 8608934]
- Giannakou ME, Partridge L. Role of insulin-like signalling in *Drosophila* lifespan. *Trends Biochem Sci.* 2007; 32 (4):180–188. S0968-0004(07)00064-3 [pii]. 10.1016/j.tibs.2007.02.007 [PubMed: 17412594]

- Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol.* 1956; 11 (3): 298–300. [PubMed: 13332224]
- Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, Nadon NL, Wilkinson JE, Frenkel K, Carter CS, Pahor M, Javors MA, Fernandez E, Miller RA. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature.* 2009; 460 (7253):392–395. nature08221 [pii]. 10.1038/nature08221 [PubMed: 19587680]
- Hausladen A, Fridovich I. Measuring nitric oxide and superoxide: rate constants for aconitase reactivity. *Methods Enzymol.* 1996; 269:37–41. [PubMed: 8791635]
- Henchcliffe C, Beal MF. Mitochondrial biology and oxidative stress in Parkinson disease pathogenesis. *Nat Clin Pract Neurol.* 2008; 4 (11):600–609. ncpneuro0924 [pii]. 10.1038/ncpneuro0924 [PubMed: 18978800]
- Jafari M, Zarban A, Pham S, Wang T. *Rosa damascena* decreased mortality in adult *Drosophila*. *J Med Food.* 2008; 11 (1):9–13.10.1089/jmf.2007.546 [PubMed: 18361732]
- Johannsen DL, Ravussin E. The role of mitochondria in health and disease. *Curr Opin Pharmacol.* 2009; 9 (6):780–786. S1471-4892(09)00135-0 [pii]. 10.1016/j.coph.2009.09.002 [PubMed: 19796990]
- Kampkotter A, Timpel C, Zurawski RF, Ruhl S, Chovolou Y, Proksch P, Watjen W. Increase of stress resistance and lifespan of *Caenorhabditis elegans* by quercetin. *Comp Biochem Physiol B Biochem Mol Biol.* 2008; 149 (2):314–323. S1096-4959(07)00386-7 [pii]. 10.1016/j.cbpb.2007.10.004 [PubMed: 18024103]
- Kenyon CJ. The genetics of ageing. *Nature.* 2010; 464 (7288):504–512. nature08980 [pii]. 10.1038/nature08980 [PubMed: 20336132]
- King V, Tower J. Aging-specific expression of *Drosophila hsp22*. *Dev Biol.* 1999; 207 (1):107–118. S0012-1606(98)99147-3 [pii]. 10.1006/dbio.1998.9147 [PubMed: 10049568]
- Kirkwood TB, Austad SN. Why do we age? *Nature.* 2000; 408 (6809):233–238.10.1038/35041682 [PubMed: 11089980]
- Kondapalli KC, Kok NM, Dancis A, Stemmler TL. *Drosophila* frataxin: an iron chaperone during cellular Fe-S cluster bioassembly. *Biochemistry.* 2008; 47 (26):6917–6927.10.1021/bi800366d [PubMed: 18540637]
- Kumar N, Bhandari P, Singh B, Gupta AP, Kaul VK. Reversed phase-HPLC for rapid determination of polyphenols in flowers of rose species. *J Sep Sci.* 2008; 31 (2):262–267.10.1002/jssc.200700372 [PubMed: 18172921]
- Kurapati R, Passananti HB, Rose MR, Tower J. Increased *hsp22* RNA levels in *Drosophila* lines genetically selected for increased longevity. *J Gerontol A Biol Sci Med Sci.* 2000; 55 (11):B552–559. [PubMed: 11078089]
- Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P, Auwerx J. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1 α . *Cell.* 2006; 127 (6):1109–1122. S0092-8674(06)01428-0 [pii]. 10.1016/j.cell.2006.11.013 [PubMed: 17112576]
- Lapointe J, Hekimi S. When a theory of aging ages badly. *Cell Mol Life Sci.* 2010; 67 (1):1–8.10.1007/s00018-009-0138-8 [PubMed: 19730800]
- Loghmani-Khouzani H, Sabzi Fini O, Safari J. Essential oil composition of *Rosa damascena* mill cultivated in central Iran. *Scientia Iranica.* 2007; 14 (4):316–319.
- Magwere T, Goodall S, Skepper J, Mair W, Brand MD, Partridge L. The effect of dietary restriction on mitochondrial protein density and flight muscle mitochondrial morphology in *Drosophila*. *J Gerontol A Biol Sci Med Sci.* 2006; 61(1):36–47. 61/1/36 [pii]. [PubMed: 16456193]
- Martin LJ. Mitochondriopathy in Parkinson disease and amyotrophic lateral sclerosis. *J Neuropathol Exp Neurol.* 2006; 65(12):1103–1110. 00005072-200612000-00001 [pii]. 10.1097/01.jnen.0000248541.05552.c4 [PubMed: 17146283]
- Massie HR, Aiello VR, Williams TR. Inhibition of iron absorption prolongs the life span of *Drosophila*. *Mech Ageing Dev.* 1993; 67 (3):227–237. [PubMed: 8326745]
- Miller RA, Harrison DE, Astle CM, Baur JA, Boyd AR, de Cabo R, Fernandez E, Flurkey K, Javors MA, Nelson JF, Orihuela CJ, Pletcher S, Sharp ZD, Sinclair D, Starnes JW, Wilkinson JE, Nadon

- NL, Strong R. Rapamycin, but not resveratrol or simvastatin, extends life span of genetically heterogeneous mice. *J Gerontol A Biol Sci Med Sci*. 2011; 66 (2):191–201. glq178 [pii]. 10.1093/gerona/glq178 [PubMed: 20974732]
- Morrow G, Samson M, Michaud S, Tanguay RM. Overexpression of the small mitochondrial Hsp22 extends *Drosophila* life span and increases resistance to oxidative stress. *FASEB J*. 2004; 18(3): 598–599. 03-0860fje [pii]. 10.1096/fj.03-0860fje [PubMed: 14734639]
- Nisoli E, Tonello C, Cardile A, Cozzi V, Bracale R, Tedesco L, Falcone S, Valerio A, Cantoni O, Clementi E, Moncada S, Carruba MO. Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. *Science*. 2005; 310 (5746):314–317. 310/5746/314 [pii]. 10.1126/science.1117728 [PubMed: 16224023]
- Ramezani R, Moghimi A, Rakhshandeh H, Ejtehad H, Kheirabadi M. The effect of *Rosa damascena* essential oil on the amygdala electrical kindling seizures in rat. *Pak J Biol Sci*. 2008; 11 (5):746–751. [PubMed: 18819571]
- Rose MR, Charlesworth B. Genetics of life history in *Drosophila melanogaster*. I. Sib analysis of adult females. *Genetics*. 1981; 97 (1):173–186. [PubMed: 6790340]
- Rose MR, Drapeau MD, Yazdi PG, Shah KH, Moise DB, Thakar RR, Rauser CL, Mueller LD. Evolution of late-life mortality in *Drosophila melanogaster*. *Evolution*. 2002; 56 (10):1982–1991. [PubMed: 12449485]
- Salmon AB, Richardson A, Perez VI. Update on the oxidative stress theory of aging: does oxidative stress play a role in aging or healthy aging? *Free Radic Biol Med*. 2010; 48 (5):642–655. S0891-5849(09)00786-2 [pii]. 10.1016/j.freeradbiomed.2009.12.015 [PubMed: 20036736]
- Shokouhinejad N, Emaneini M, Aligholi M, Jabalameli F. Antimicrobial effect of *Rosa damascena* extract on selected endodontic pathogens. *J Calif Dent Assoc*. 2010; 38 (2):123–126. [PubMed: 20232691]
- Speakman JR, Selman C. The free-radical damage theory: Accumulating evidence against a simple link of oxidative stress to ageing and lifespan. *Bioessays*. 2011; 33 (4):255–259. 10.1002/bies.201000132 [PubMed: 21290398]
- Tower J. Heat shock proteins and *Drosophila* aging. *Exp Gerontol*. 2011; 46 (5):355–362. S0531-5565(10)00291-3 [pii]. 10.1016/j.exger.2010.09.002 [PubMed: 20840862]
- Vermeulen CJ, Loeschcke V. Longevity and the stress response in *Drosophila*. *Exp Gerontol*. 2007; 42 (3):153–159. S0531-5565(06)00294-4 [pii]. 10.1016/j.exger.2006.09.014 [PubMed: 17110070]
- Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet*. 2005; 39:359–407. 10.1146/annurev.genet.39.110304.095751 [PubMed: 16285865]
- Wallace DC, Brown MD, Melov S, Graham B, Lott M. Mitochondrial biology, degenerative diseases and aging. *Biofactors*. 1998; 7 (3):187–190. [PubMed: 9568243]
- Wang X, Su B, Perry G, Smith MA, Zhu X. Insights into amyloid-beta-induced mitochondrial dysfunction in Alzheimer disease. *Free Radic Biol Med*. 2007; 43 (12):1569–1573. S0891-5849(07)00623-5 [pii]. 10.1016/j.freeradbiomed.2007.09.007 [PubMed: 18037122]
- Winterbourn CC, Hawkins RE, Brian M, Carrell RW. The estimation of red cell superoxide dismutase activity. *J Lab Clin Med*. 1975; 85(2):337–341. 0022-2143(75)90439-4 [pii]. [PubMed: 803541]
- Xu J, Marzetti E, Seo AY, Kim JS, Prolla TA, Leeuwenburgh C. The emerging role of iron dyshomeostasis in the mitochondrial decay of aging. *Mech Ageing Dev*. 2010; 131 (7–8):487–493. S0047-6374(10)00089-8 [pii]. 10.1016/j.mad.2010.04.007 [PubMed: 20434480]
- Yang J, Tower J. Expression of *hsp22* and *hsp70* transgenes is partially predictive of *Drosophila* survival under normal and stress conditions. *J Gerontol A Biol Sci Med Sci*. 2009; 64 (8):828–838. glp054 [pii]. 10.1093/gerona/glp054 [PubMed: 19420297]
- Zhang X, Min X, Li C, Benjamin IJ, Qian B, Ding Z, Gao X, Yao Y, Ma Y, Cheng Y, Liu L. Involvement of reductive stress in the cardiomyopathy in transgenic mice with cardiac-specific overexpression of heat shock protein 27. *Hypertension*. 2010; 55 (6):1412–1417. HYPERTENSIONAHA.109.147066 [pii]. 10.1161/HYPERTENSIONAHA.109.147066 [PubMed: 20439823]

Fig. 1.

The effect of *R. damascena* supplementation on survival. Five mg/mL *R. damascena* increased survival in both (A) male, and (B) female, B flies relative to flies fed a control diet. However, *R. damascena* supplementation increased overall survival in (C) male, but not (D) female, w^{1118} flies. *P*-values were calculated by Mantel-Cox log rank test. The sample sizes are as follows and are listed as control and *R. damascena*-fed respectively: B males, $n = 211$ and 186 ; B females, $n = 181$ and 155 ; w^{1118} males, $n = 240$ and 226 ; w^{1118} females, $n = 239$ and 239 .

Fig. 2.

Protective effects of *R. damascena* supplementation against oxidative insults. There was no statistically significant effect in *R. damascena* fed male flies against paraquat or H₂O₂ challenge (A and C), whereas females exhibited a modest enhanced survival against paraquat and H₂O₂ (B and D). *Rosa damascena* protected both sexes against Fe-NTA. *P*-values were calculated by Mantel-Cox log rank test, *n* = 100 for all groups.

Fig. 3.

Rosa damascena and antioxidant defenses. A two-week feeding of *R. damascena* had no effect on the major antioxidant defenses, total SOD (A), MnSOD (B), and catalase (C) enzyme activities. Data are presented as means $\pm$ SEM. $P > 0.05$ for diet, 2-way ANOVA, $n = 6$ (50 flies each) for all groups.

Fig. 4.

Rosa damascena supplementation and mitochondrial superoxide levels. Superoxide levels, measured by MitoSOX oxidation (A), and mitochondrial aconitase activities (B) were unaffected after a two-week feeding of *R. damascena* or a control diet. Data are presented as means $\pm$ SEM. $P > 0.05$ for diet, 2-way ANOVA for both, $n = 6$ (50 flies each).

Fig. 5.

Mitochondrial content and total soluble protein in *R. damascena*-fed and control flies. The levels of mitochondrial content via measurements of mitochondrial protein (A), and citrate synthase activities (B) were unaffected by *R. damascena* feeding. *Rosa damascena* feeding also had no effect on total soluble protein (C). Data are presented as means $\pm$ SEM. $P > 0.05$ for diet, 2-way ANOVA, $n = 6$ (50 flies each) for all groups.

Fig. 6.

Tolerance to starvation and heat in *R. damascena*-fed and control flies. A two-week feeding of *R. damascena* had no effect on the ability of flies to endure starvation (A and B). However, the identical treatment sensitized flies to survival at 37° C (C and D). *P*-values were calculated by the Mantel-Cox log rank test. The sample sizes are as follows and are listed as control and *R. damascena*-fed respectively: starvation-treated males, $n = 315$ and 309 , starvation-treated females, $n = 314$ and 306 , heat-treated males, $n = 320$ and 320 , heat-treated females, $n = 311$ and 311 .

Fig. 7.

Expression levels of heat shock proteins HSP22 (A) and HSP70 (B) in *R. damascena*-fed and control male flies. No heat shock indicated flies maintained at 24° C, whereas heat shock indicates flies subjected to a 4-hour exposure to 37° C prior to the collection of RNA. Data are presented as means $\pm$ SEM, and are shown as expression levels relative to flies fed a control diet, and are normalized to RNA Polymerase II. * $P < 0.01$, ** $P < 0.001$, 2-way ANOVA for diet, $n = 6$ for all groups.

Table 1
Mean life span and 90% mortality values for *R. damascena*-fed and control diet flies

Strain	sex	diet	mean	P	90%	P
B	♂	control	23.7 ± 0.8		41.8 ± 0.7	
B	♂	Rosa	29.0 ± 0.8	< 0.001	47.1 ± 1.2	< 0.001
B	♀	control	26.2 ± 0.9		47.8 ± 1.0	
B	♀	Rosa	34.5 ± 1.0	< 0.001	54.0 ± 1.2	< 0.001
w ¹¹¹⁸	♂	control	51.2 ± 1.3		79.3 ± 0.5	
w ¹¹¹⁸	♂	Rosa	58.6 ± 1.2	< 0.001	84.4 ± 0.4	< 0.001
w ¹¹¹⁸	♀	control	48.6 ± 1.2		76.8 ± 1.2	
w ¹¹¹⁸	♀	Rosa	50.8 ± 1.3	> 0.05	82.5 ± 0.6	< 0.001

Values are means ± SEM. Units are days. Ninety % mortality is the mean lifespan of the longest lived 10%. P-values were calculated by Bonferroni posttests, 2-way ANOVA for diet (control vs. Rosa). The interaction between diet and sex was P > 0.05, except for the mean lifespan in w¹¹¹⁸ flies, where P < 0.05, 2-way ANOVA.

Table 2
Mitochondrial respiratory parameters in *Rosa damascena* and control diet fed flies.

Sex	diet	state 3	state 4	RCR	uncoupled
♂	Control	353 ± 30	50 ± 6	7.9 ± 1.0	423 ± 48
♂	<i>Rosa</i>	363 ± 38	59 ± 6	6.3 ± 0.2	442 ± 62
♀	Control	416 ± 11	51 ± 5	8.5 ± 0.6	504 ± 49
♀	<i>Rosa</i>	398 ± 13	58 ± 7	7.9 ± 0.4	479 ± 30
<i>P</i> -values	diet	0.89	0.21	0.06	0.95
2-way ANOVA	sex	0.11	0.95	0.08	0.23

Rates are mean ± SEM, n = 15–17. Units are nmol O₂/min/mg protein except for RCR, which is a ratio of state 3/state 4.