Prolongevity Effects of an Oregano and Cranberry Extract are Diet Dependent in the Mexican Fruit Fly (Anastrepha ludens)

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Botanicals have numerous health benefits. Here, we used the Mexican fruit fly to screen 14 compounds and botanicals for their prolongevity effects and found an oregano and cranberry mixture (OC) improved survival. We then evaluated prolongevity effects of OC within the context of diet composition. Individual flies were fed 0%, 1%, or 2% OC in one of the three diets containing sugar and yeast extract (SY) at a ratio of 3:1, 9:1, or 24:1. We found that prolongevity effects of OC depended upon dose, gender, and diet composition. The greatest increase in longevity was observed in females fed the SY24:1 diet with 2% OC compared to the non-supplemented diet. OC did not reduce egg laying and, hence, did not compromise fecundity under any dietary condition tested here. This study reveals the prolongevity effects of OC and supports the emerging view that benefits of botanicals on aging depend on diet composition and gender.

Key Words: Life span—Botanical extract—Aging intervention—Reproduction—Egg laying.

The health benefits of diets high in fruit and vegetable content have been well established. Extracts from various plants, fruits, and herbs have diverse biological activities, including antimicrobial infection, anti-inflammation, anticancer, and antcardiovascular diseases as well as prevention of several other types of diseases, such as diabetes and neurodegenerative disorders (1). Many of these properties are attributable to the presence of a variety of phytochemicals, including polyphenolics and flavonoids (2). These phytochemicals often exert their influence through multiple mechanisms, acting as antioxidants, ligands for receptors and activators or inhibitors of enzymes, and transcription factors linked to disease pathways (3–5). Although botanical extract can affect a broad range of biological signatures of aging, few have been directly demonstrated to provide prolongevity effects.

Oregano (Origanum vulgare) and cranberry (Vaccinium macrocarpon) are prominent examples of botanicals offering multiple health benefits (6,7). Oregano is widely used as a food and drink additive and contains high levels of phytochemicals, especially carvacrol and thymol (6,8). Extracts from oregano exhibit antimicrobial activity against a variety of pathogens (6). Oregano oil, for example, has been found to be more effective than benomyl, a commercial fungicide, at inhibiting mycelial growth of a number of phytopathogenic fungi (9). Anticancer effects of oregano have been demonstrated in several cell-based and animal models as well as in clinical investigations and appear to be at least partly attributable to antioxidant activity (10,11). Oregano extracts also exhibit selective proapoptotic and cytotoxic effects on colon cancer cells, suggesting that the wide use of oregano as a spice in the Mediterranean region contributes to the relatively low regional risk for colon cancer (10). In a 3-month supplement study of patients with mild hyperlipidemia, oregano has been shown to improve lipid profiles, antioxidant status, and endothelial function in patients (12). Biochemical studies indicate that oregano extracts potentially inhibit both diabetes-related alpha-glucosidase and hypertension-related angiotensin-converting enzyme-1 (ACE-1) (13). These findings suggest that oregano supplementation is potentially effective for management of diabetes and hypertension, two common age-related conditions (14). Together with evidence that oregano can reduce mortality and improve reproductive success in a commercial farm animal setting (15), the range of potential applications of this dietary intervention appears quite broad.

Cranberry provides a rich source of phytochemicals (16,17). Consumption of cranberry juice is popularly regarded as effective in the treatment and prevention of urinary tract infection (UTI) in humans (18). Indeed, recent clinical studies have demonstrated that cranberry consumption for a 12-month period significantly decreases the incidence of symptomatic UTIs, especially among women with recurrent infections (19,20). Randomized control trials in older women with recurrent UTI further indicate that cranberry is equally effective as the commonly prescribed...
antibiotic, Trimethoprim, but has less adverse effects, especially reduced risk of developing antimicrobial resistance (21). Cranberry constituents, especially proanthocyanidins with A-type linkages, are thought to exert antimicrobial activity by inhibiting adhesion of P-fimbriated uropathogenic *Escherichia coli* to uroepithelial cells, thereby suppressing bacterial growth and improving urinary tract health (22). Alongside this antimicrobial action, other studies indicate that cranberry has the potential to exert multiple biological effects through its antioxidant properties and by engaging many signaling pathways, such as Jun kinase (JNK), mitogen-activated protein kinase, and NFκB signaling pathways (7, 18, 23).

Consistent with the proposal that cranberry may offer widespread health benefits, dietary supplementation of cranberry reduces the levels of total and low density lipid cholesterol as well as the total: high density lipid cholesterol ratio without affecting the glycemic control in type 2 diabetic patients on glucose-lowering agents, suggesting that persons with impaired glucose tolerance can benefit from cranberry consumption (24). In addition, a number of in vitro and in vivo experiments have revealed a chemopreventive action of cranberry. For instance, cranberry extracts can inhibit the proliferation and invasion of several types of tumor cells in vitro and tumor formation in vivo, including Rev-2-T-6 murine lymphoma cells and tumors, SGC-7901 cancer cells, and human tumor xenografts in mice (25).

Cranberry also stimulates the generation of anti-lymphoma antibodies, suggesting the potential for protective effects against malignant lymphoma in immune competent hosts (26). Cranberry appears to exert these effects by inducing apoptosis in tumor cells and by reducing the expression of tumor metastasis–related matrix metalloproteinases and promoting anti-inflammatory activity (23). Finally, current evidence also suggests that cranberry reduces the risk of cardiovascular disease and blunts the toxicity of Alzheimer’s disease–related A-beta peptide (17, 27).

Different botanical extracts have unique phytochemical profiles comprising various polyphenolics and flavonoids. A number of experiments have demonstrated synergistic or additive effects between different phytochemicals in exerting their biological functions (2, 28). For instance, synergistic interactions among anthocyanins, proanthocyanidins, and flavonol glycosides are reportedly coupled to the inhibitory effects of whole cranberry extract on the proliferation of cancer cells (29). The synergy is also evident in investigations combining extracts of different botanical sources. Combining water-soluble cranberry extract with extracts of oregano, rosemary, or *Rhodiola rosea*, for example, enhances inhibition of markers linked to diabetes and hypertension, including alpha-glucosidase and pancreatic alpha-amylase and ACE-I, respectively (28). Cranberry and oregano together, compared with either alone, also augment the inhibition of *Helicobacter pylori*, a bacterium linked to ulcers (30), and *Listeria monocytogenes*, a virulent food-borne pathogen causing Listeriosis, presumably by suppressing microbial urease and proline dehydrogenase activity (28). These studies suggest that developing maximally beneficial botanical extracts may require bringing together phytochemicals from different sources to enhance functional activities.

Despite numerous reports on the health benefits of botanical extracts and natural compounds contained within these extracts, little is known about their effects on longevity. Although available evidence indicates that blueberry extracts increase median life span in the nematode, *Caenorhabditis elegans* (31), for example, it remains to be determined whether this prolongevity effect can be obtained in other species. Resveratrol, a polyphenolic found in high concentrations in red grapes and a number of other plants, has been demonstrated to promote longevity in evolutionarily divergent species, including yeast, worms, flies, and fishes as well as mice fed a high fat diet (32–35). These findings have proved controversial, however, because others have failed to observe resveratrol-induced life-span extension (36, 37). Our recent findings are noteworthy in this context, demonstrating that the prolongevity effect of resveratrol is highly dependent upon diet composition and that treatment only slightly extends life span in calorie-restricted female mexflies (38). These findings, together with the wide variation in carbohydrate, protein, and fat content seen in human diets (39), suggest that diet composition may be a critical factor dictating the effects of botanical supplements.

In the current study using the Mexican fruit fly (mexfly) *Anastrepha ludens* (Loew) as a model organism, we first screened for potential prolongevity effects from a number of candidate compounds and botanical extracts selected on the basis of a variety of hypothesized actions in aging. Mexfly provides several advantages for use as a model system for screening compounds. First, extensive demographic studies of aging have been conducted in this species (40). In particular, the effects of dietary composition on life span and reproduction are well documented (41). Second, millions of mexflies are readily available on a daily basis from the site of the present study (Moscafrut facility, Chiapas, Mexico) (41), which enables usage of a large number of flies reared in identical conditions for screening. Third, unlike the commonly used laboratory fly, *Drosophila melanogaster*, mexfly females lay their eggs away from the food, allowing a direct assessment of egg laying or fecundity independent of the influence of food availability and composition on egg-laying behavior (38, 41). Capitalizing on this model, we screened 14 compounds and botanical extracts and identified a botanical product with a mixture of oregano and cranberry extract (OC) that exhibited robust prolongevity effects. With this lead, we explored this benefit on individually housed flies in the context of diet composition. The results demonstrate that the prolongevity effect of OC in mexfly is both diet and gender specific.
MATERIALS AND METHODS

Reagents

Compounds were purchased from Sigma-Aldrich Inc (St Louis, MO). Botanical extracts were obtained from Decas Cranberry Product Inc (Carver, MA) and Barrington Chemicals Inc (Harrison, NY).

Life-Span Assay Using Population Cages

To screen compounds or botanicals for longevity effects, approximately 2,000 adult mexflies of both sexes were housed in a large population cage (size: 0.5′ × 1′ × 2′ [W × L × H]) as described previously (42). Briefly, to get these adult flies, pupae were obtained from the mass rearing facility and placed in Petri dishes inside the population cages 1 day before the maximum emergence. After 24 hours, the Petri dishes with the empty pupal cases and non-emerged pupae were removed. Therefore, the age of all individual flies inside each cage was the same (within 24 hours). The amount of pupae placed in each cage was estimated by weight and the possible percentage of emergence so that there were approximately 2,000 flies per cage. The flies were then fed a standard solid diet with sugar: yeast: (9:1, 1.5% agar, and a specified concentration of a dietary supplement in each cage. Vivaria were maintained at 24°C ± 2°C, 65% ± 9% relative humidity, and 12:12 hours light–dark cycle. Each treatment was repeated with two cages of flies. Approximately 20 ml of fresh food was provided in a Petri dish to each cage once a week. Dead flies were collected, sorted by sex, and recorded every day for life-span measurements. The screening experiments were conducted in three batches, which were terminated after flies reached 107, 71, or 54 days, respectively. The control was set up and replicated in four separate population cages for each batch of experiments. The number of surviving flies was counted after the termination date. For each treatment, median life-span and survival curves were derived by combining data from four replicates.

Life-Span Assay Using Condominium Cages

Mass-reared virgin male and female flies were individually housed in clear Plexiglass cages (4 × 4 × 10 cm per unit), termed as condominium cages as described previously (41). Environmental conditions were the same as described previously for the population assay. Flies were individually provided 20 µl of one of the following nine diets and a 20-µl droplet of water was provided daily to each fly on a glass slide through an opening in each cage. The nine diets were designed by combination of one of the three different sugars: yeast extracts at a ratio of 3:1, 9:1, and 24:1 with one of the three concentrations of OC: 0%, 1%, and 2% (weight/volume). For each diet, sugar and yeast extract (SY) were first dissolved in double the amount of water by weight to make the sugar: yeast mother stock solution. The OC was then added to each mother stock solution to obtain the final concentration of 0%, 1%, and 2% OC in weight/volume. Eighty-two males and 82 females were randomly assigned into the 164-unit cages with the stipulation that females and males occupied alternate units to avoid mixing eggs from two females. Each unit contained a black silicon membrane attached to the rear of the cage in which females could insert their ovipositor to lay eggs. Sex-specific survival of males and females and egg laying of females were recorded daily.

Data Analysis

Event life history graphs and response surfaces were generated using DeltaGraph 5 software (Red Rock Software, Inc, Salt Lake City, UT). LogRank analyses were performed for the data obtained with the population cages using the SAS PROC LIFETEST program (Cary, NC). The logistic model analysis for the fertility rate data was performed using the R Project for Statistical Computing program (http://www.r-project.org/). Analyses of variance (ANOVA) and Life-span and egg-laying data from the assays with individual flies using Statview Version 5.0 (SAS, Cary, NC). p < .0014 after Bonferroni adjustment for multiple comparisons was considered statistically significant for the experiments using individual flies.

RESULTS

We initially surveyed 14 compounds and botanical extracts for their potential longevity activity using population cages with approximately 2,000 mixed males and females per cage fed a standard diet with sugar: yeast at a 9:1 ratio. Median life span of flies under these treatments is shown in the Supplementary Tables 1 and 2. A treatment was considered to induce a significant life-span extension if the p value is less than .00083 after Bonferroni adjustment and median life span of both males and females under that condition is 2 SDs more than median life span of the corresponding control. Using these criteria, OC, an oregano and cranberry mixture, was found to extend median life span of both males and females at the highest concentration tested (1.6% OC in food) in this screening (Figure 1). No significant extension was observed with other supplements (online Supplementary Tables 1 and 2). OC has been described previously as a potent antimicrobial botanical extract containing 75% water-soluble oregano extract and 25% water-soluble cranberry extract (43). OC contains 15%–19% total phenolics as measured by the Folin–Ciocalteu assay and has antioxidant activity of 3,250 m mol Trolox per gram based on the oxygen radical absorbance capacity assay (44,45).
To further investigate aging-related effects of OC, we utilized virgin males and females housed individually in plexiglass chambers, which we refer to as “condominium” cages. Flies were fed a standard laboratory diet containing SY at a ratio of 9:1. Under this condition, the OC-supplemented diet significantly extended mean life span in females, but not males, in a dose-dependent manner (Figure 2). Specifically, the mean life span of females was increased by 20.5% by supplementation of 2% OC compared with the control, whereas 1% supplementation had no statistically significant effect (Table 1, see sugar: yeast 9:1 ratio). Maximum life span was not significantly affected in any treatment condition (Table 1). These results indicate that OC extends mean life span in a dose- and gender-specific fashion in mexflies under a standard diet condition.

Dietary customs vary dramatically across geographical regions and ethnic backgrounds (39), and accordingly, it would be important to consider the potential modulatory influence of diet when evaluating the prolongevity effects of any promising supplement. To this end, we assessed whether diet composition, defined by the relative balance of carbohydrate and protein, affects the prolongevity effect of OC. Separate groups of mexflies were provided different compositions of SY, representing carbohydrate and protein, respectively, at one of three ratios: 24:1, 9:1, or 3:1. This evaluation was conducted using the condominium cages, in which individual flies received fresh food daily. Results from the standard diet (SY 9:1) are described previously (Figure 2). In comparison with those findings, increasing the ratio of yeast or decreasing the ratio of sugar (SY 3:1) completely blocked the prolongevity effect of 2% OC relative to nonsupplemented controls maintaining on the same diet (Figure 3A and B and Table 1). In the lower yeast or higher sugar condition (SY 24:1), however, 2% OC significantly increased both mean and maximum life span of females and males (p < .0001; Figure 3C and D and Table 1). Although a numerical benefit of 1% OC was also apparent (Figure 3C and D and Table 1), this effect did not reach statistical significance after adjustment for multiple comparisons. These findings suggest that, besides gender, the prolongevity activity of OC is influenced by the diet composition and is potentiated by a low protein or high sugar level in the food.

The interactive influence of diet composition and botanical supplementation on life span is further illustrated in Figure 4 using surface response maps, providing a two-dimensional representation of longevity as a function of dietary yeast and OC concentrations. The interaction between OC and yeast extract on life span was also analyzed (Supplementary Table 3). Mean life span of females in general increased with higher concentrations of OC and reached a peak when yeast represented roughly 10% of the available carbohydrates.
diet (Figure 4A), whereas mean life span of males increased with increasing concentration of OC or yeast under the conditions tested in this study (Figure 4B). The effect of OC on mean life span of both males and females depended on diet composition represented by the percentage yeast as revealed by two-way ANOVA (Supplementary Table 3). The overall pattern of maximum life span in response to yeast and the OC supplement was similar to that for mean life span of females with some differences (Figure 4A and C). There is no significant interaction between OC and yeast concentrations.

![Figure 3. Life-span curves of female and male mexflies individually fed a 3:1 sugar and yeast extract (SY) diet (A and B) or a 24:1 diet (C and D) supplemented with 0%, 1%, and 2% oregano and cranberry (OC) mixture. SY ratios, concentrations of OC in the food as percentages, and mean life span of flies in days from each treatment are depicted in graphs.](https://academic.oup.com/biomedgerontology/article-abstract/65A/1/41/715322)
for maximum life span of females. Maximum life span of males in general increased with increasing concentration of OC, but not with the concentration of yeast extract (Figures 4D and Supplementary Table 3). Surface response mapping thereby complements the preceding analyses, consistent with the interpretation that the prolongevity effect of OC is gender and diet dependent.

Reproduction has profound effects on life span, and under certain conditions, life-span extension is associated with reduced reproduction (46). To evaluate whether the prolongevity effect of OC is coupled with suppressed reproduction, daily egg laying was recorded for females maintained in condominium cages. Event life history maps are shown in Figure 5, representing daily egg laying and life span for individual flies. Consistent with previously published results (41), fertility rate and egg laying were significantly reduced in flies as a function of relative dietary protein content (SY 24:1 < SY 9:1 < SY 3:1; Figure 5A, Table 1, and Supplementary Table 4). Within dietary conditions, however, fecundity of fertile females was similar across OC-supplemented and control subjects (Figure 5A and B). Of particular note, OC supplementation did not decrease lifetime egg laying per fertile fly under any concentration tested here (Figure 5A and Table 1). Indeed, the data trended in the opposite direction, and in one condition (SY 9:1, 1% OC), supplementation was associated with increased egg production relative to values for nonsupplemented controls. Fertility rate was significantly increased by 2% OC supplementation (Supplementary Table 4). Together, these findings indicate that OC can have prolongevity effects without compromising reproductive output, thus suggesting this effect is largely independent of processes regulating the reproductive pathway.

**Discussion**

After screening 14 compounds and botanicals for prolongevity effects using large groups of mexfl ies housed in population cages, we have found one botanical product that has robust effects on life span. This product is a blend of OC extracts, which increases median life span of both males and females in this initial screening with mixed population. The prolongevity effect of OC has been further examined within the context of diet composition in individually housed virgin flies. Using the standard 9:1 SY diet, we note a significant effect of OC on the mean life span of females. No effect is detected on the mean life span of males or on the maximum life span of either gender. With the higher protein or lower sugar diet (SY 3:1), OC supplementation produces no significant effect on life span. However, with the lower protein or higher sugar diet (SY 24:1), OC significantly increases mean and maximum life span of both males and females. This indicates that the higher sugar or lower protein content in food, the greater the effect of OC on life span, and the prolongevity effect of OC is sex dependent.

Using egg laying as a measure of healthspan, it is clear that OC has no detrimental effects on this measure, further supporting the health benefits of the OC supplementation.
Diet has an enormous impact on health and life span in almost all organisms including humans (47–49). Dietary restriction without causing malnutrition has been shown to extend life span and delay onset of age-related functional decline and diseases in diverse model organisms (46, 49, 50). Diet composition is also critical and can significantly influence health and aging. Nutritional geometry studies related to aging in *D. melanogaster* and *A. ludens* have indicated that optimal life span depends on a balanced level of protein and carbohydrates (41, 51, 52). How a supplement interacts with protein and carbohydrate in food to modulate life span has not been well addressed. The effects of dietary supplements on life span may vary significantly depending on gender and diet composition. Indeed, we have found that the prolongevity effect of OC is more prominent when the mexflies are on diets with relatively higher carbohydrate or lower protein content. OC does not extend life span of mexflies fed a high protein or low carbohydrate diet. Although the exact role of carbohydrate and protein in affecting the prolongevity effect of OC remains to be determined, results from this study suggest that one should take into consideration the physiological difference of gender and dietary composition when evaluating candidate prolongevity agents. In addition, we have manipulated only dietary carbohydrate and protein in this study. The standard fly diet contains only a small percentage of fat from the yeast extract. Given the importance of fat in human diets, it would be necessary in the future to evaluate the interaction of dietary fat and prolongevity supplements using modified fly diets.

The observation that females have a more robust response to OC treatment than males when they were maintained in condominium cages but not when they were housed in population cages suggests that the sex-specific effects of OC are affected by mating status as well as by density. Both of these factors have age- and sex-specific effects on the Mediterranean fruit fly (53–55) and thus it is likely they have similar effects on the mexfly. For example, mated flies tend to have shorter life span compared with virgins but the effects differ between the sexes. The cost of reproduction in laying females can also explain the difference in life span of males and
females because their reproductive responses differ qualitatively but both depend on dietary, mating, and/or density conditions. Both sexes maintained in population cages tend to live shorter than those individually housed in condominium cages due to heightened stress from high densities, competition for food and mates, and exposure to pheromones that elicit different behaviors. Most or all of these stress factors are avoided when flies are maintained in solitary confinement. In general, the finding that the sex-specific outcome of OC-supplemented diet is conditional on cage and mating conditions underscores the complex life table dynamics involved in the longevity outcomes due to different dietary restriction treatments. Together, our findings point to the challenges of identifying effective aging interventions and the importance of including comprehensive dietary manipulations when evaluating potential prolongevity supplements.

What are the potential mechanisms of life-span extension by OC? Egg laying is not reduced by OC and is even increased by one concentration of OC, suggesting that OC does not extend life span through reproduction-related pathways. Cranberry and oregano are rich in polyphenolics (8,16), and a number of polyphenolics can act as antioxidants or modulators of signaling pathways, such as the JNK and NFκB pathways (3–5). Shetty and colleagues found in their studies on antimicrobial activities of OC that proline could suppress the inhibitory activities of OC on microbial growth (30,43). Based on these observations, they proposed that phenolics in OC can disrupt the flow of the electrons along the bacterial membrane by either quenching free electrons or by inhibiting proline dehydrogenase in bacteria, which, in turn, would affect ATP production and consequently microbial growth (56,57). Considering the prokaryotic origin of mitochondria and the fact that a reduction of expression of mitochondrial genes, such as those involved in oxidative phosphorylation, can modulate life span in C. elegans (58–60), polyphenolics in OC may modulate life span by influencing mitochondrial function, especially ATP production in eukaryotes. Consistent with this hypothesis, we have found that OC is more effective for life-span extension in mexflies under a higher glucose or a lower protein diet. OC may suppress mitochondrial respiration induced by sugar through the inhibitory effects of its polyphenolics on the electron flow and/or proline dehydrogenase. This suppression may result in lower production of ATP and/or oxidants and lead to longer life span in animals fed OC (61,62). Further experiments need to be conducted to confirm this hypothesis and dissect potential molecular mechanisms responsible for the prolongevity effect of OC in genetically tractable organisms, such as D. melanogaster.

In many cell-based and whole animal experiments, the beneficial effects of supplementation of one fruit or herb become apparent only at fairly high concentrations, which is either hard to achieve or has unpleasant side effects when applied to humans (26,27,43). Failure to detect robust prolongevity effects for compounds or botanicals other than OC in our study may be due to the possibility that the concentrations of supplements are not optimal. In clinical trials to evaluate the effects of cranberry juice on preventing UTIs, a high percentage of dropouts occurred, mostly due to the fact that cranberry often causes too much acidity in the digestive system and upsets the stomach (19,20). Therefore, regimens with a lower dose of botanical extracts are desirable to achieve both effective concentrations and to reduce side effects.

In this study, we have found that a mixture of oregano and cranberry at 2% in food has a prolongevity effect. This mixture is more potent in terms of antimicrobial activity compared with either cranberry or oregano extract alone (30,43). Our findings suggest that the prolongevity effect of OC may be at least partially due to the synergy of two botanical extracts. However, it remains to be determined whether an oregano or cranberry extract alone at the concentrations tested in this study produces the prolongevity effect. Although it is hard to estimate effective dosages for human consumption based on our current study due to physiological differences between flies and humans, this study supports health benefits of consuming fruit-enriched diets.

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SUPPLEMENTARY MATERIAL
Supplementary material can be found at: http://biomedgerontologyjournals.org/

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REFERENCES


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