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Article

Comparing the effects of different natural products on reducing tumor growth in a *Drosophila* model

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SUMMARY

Cancer is one of the main causes of death in the present day and due to side-effects on current chemotherapies there is a need for testing natural products in cancer studies. In this work, we compared the effects of common natural products, including sesame, cinnamon, garlic, moringa and turmeric. The tumor model was a comparison of "rough-eye" versus "smooth-eye" studied in *Drosophila* eye, which provides easy detection of the phenotypes via simple microscopy. We measured the size of the eye, the number of flies with a smooth-eye phenotype, the number of flies with uniform ommatidia, and the lifespan of the flies. Our data showed that these natural products cannot be used to reduce tumors once it has completely formed. However, our data confirmed that these natural products can be used to reduce cancer cell growth when treated early. Moringa and sesame helped reduce tumor size when used in right concentrations. Our study also revealed a 15% moringa solution is the best candidate, of the ones tested, to prevent tumors. Flies treated with moringa showed longer eye lengths and widths compared to the control sample and close to healthy wild flies. For the flies treated with moringa, the number of flies with smooth-eye and uniform ommatidia increased with natural product concentration until 15% and decreased at 20%. Garlic, turmeric and cinnamon products did not show much efficacy on the tumor reduction. Though turmeric treatment did not prevent cancer, it helped increase the lifespan compared to control samples and other natural products.

INTRODUCTION

Cancer is one of the most prevalent diseases in the world and is one of the main causes of death in the present day. It has been estimated that deaths due to cancer will rise from 8.2 million to 14.6 million annually in 20 years (1). In 2018, there were around 1.8 million cancer cases in the United States, while 0.6 million cases led to death, resulting in a case mortality rate of ~ 30%. Among females, lung cancer is the leading cause of death followed by breast cancer. Among males, lung cancer is the primary reason for most deaths followed by prostate, colon, and pancreas cancer (2).

Chemotherapy is effective in many cases in the initial stages of many types of cancer, but the side effects are a major concern amongst patients. Patients' discomfort sometimes increases greatly due to side-effects. Both medical providers and patients are hoping to find a better and easier solution to treat cancer and above all prevent cancer if possible (3). One of the popular chemotherapy drugs called Cytoxan (cyclophosphamide) has several major side-effects like decreased blood cell counts, reversible hair loss, bladder damage, fertility impairment, and lung or heart damage at high doses (4).

Cancer occurs when cells do not go through the process of apoptosis, which is the death of cells which occurs as a normal and controlled part of an organism's growth or development (5). Instead of dying, the cells multiply uncontrollably. In the process of multiplying, the cells consume a lot of energy (6). A potential anticancer agent should cause selective apoptosis of tumor cells, with minimal damage to the normal cells. Natural products that are readily available such as garlic (*Allium sativum*), sesame (*Sesamum indicum*), moringa (*Moringa oleifera*), turmeric (*Curcuma*), and cinnamon (*Cinnamomum verum*) are natural antioxidants and potential options for cancer therapy (7-11). The harmful effects of chemotherapeutic agents, which can be toxic to non-cancerous and healthy cells, can perhaps be avoided with the use of natural products (12-13).

Garlic is usually used in cooking, not only as a flavor component but also for its medicinal properties. These properties are due to sulfur compounds which alter tumor growth, have cancer preventative potential, help improve immune function, and have many other therapeutic effects in treating infection among other properties to cover the list (14). Recently, Dong *et al* (2014) studied the ability of garlic to inhibit colon cancer growth via the inhibition of PI3K/Akt signaling pathway (15).

Sesame is one of the oldest and most important oilseeds (16). The medicinal properties of the leaves and the seed oil were identified four decades ago and hence sesame products are used as ingredients in several cooked dishes to leverage these benefits (17). The medicinal properties include anticancer, antioxidative, anti-immunoregulation and anti-hypersensitivity effects. Sesame consists of many macronutrients such as proteins, carbohydrates, antioxidants, lignans, and tocopherols. (18). Many sesame compounds like sesamol, sesamin exert inhibitory effects on NF-κB and ERK/p38 MAPK signaling pathways and thereby suppress the inflammatory responses (19).

Moringa oleifera's leaves and bark have been a very popular food item since the 18th century (20). Several different studies suggest moringa leaves have antiviral, anti-heart disease, pro-immune, and anti-inflammatory properties (21-26). Moringa oleifera extract decreases the inflammatory mediators (NF-κB) but increases anti-inflammatory cytokines, IL-10 and IκB-α (27).

Turmeric root, which belongs to the ginger family, is widely used to add flavor to food in Asia and India. The main part of turmeric is curcumin, a yellow compound which purportedly has anti-cancer properties because of its ability to inhibit microsomal PGE2 synthase 1 (28). It has been shown to have several beneficial medicinal properties including anticarcinogenic, antioxidant, anti-inflammatory, antifertility, antidiabetic, antimicrobial, and antifibrotic effects. Due to turmeric's great natural antiseptic, disinfectant, anti-inflammatory, and analgesic properties it is also used in Ayurvedics, an alternative medicine involving a holistic ("whole-body") healing system (29). Administering the right dosages of curcumin to rodents showed great promise in preventing many types of cancer (30).

Cinnamon is a spice with a sweet and spicy fragrance that is used in cooking and aromatherapy. Cinnamon primarily contains vital oils and other derivatives, such as cinnamaldehyde, cinnamic acid, and cinnamate. Cinnamon has several medicinal properties such as antioxidant, anti-inflammatory, antidiabetic, antibacterial, and anticancer effects (31). Recent studies have shown that cinnamon also has activities that aid in the treatment of neurological disorders, such as Parkinson's and Alzheimer's diseases (32). Ongoing research aims to study the anti-cancer properties of cuminaldehyde as this compound activates the Nrf2 pathway, which is involved in strengthening the cellular response against stressors such as carcinogen exposure (33-34).

Drosophila melanogaster has been used as a model system for several decades to study the effects of various drugs (35-36). *Drosophila* have been used extensively in animal studies because they are invertebrates and can be easily studied. In addition, Drosophila are useful for studying human disease since nearly 75% of human disease-causing genes are present in the *Drosophila* genome (37).

An ideal system in which cancer can be studied is in the *Drosophila* eye, since tumors in this location do not impact the viability of the flies and can easily be detected and measured via use of simple microscopes. The oncogene RET in *Drosophila* eyes is linked to thyroid and lung cancers in humans. There are many ways in which tumors can be induced in the fly eye, the easiest being the overexpression of an oncogene using the GAL4/UAS (upstream activation sequence) flies which are heterozygous for the given driver and responder. GAL4 is a transcriptional activator derived from yeast, GAL4 binds UAS and turns on expression of the downstream gene. This system requires a cross between male flies carrying a GAL4 only expressed in the eyes (such as GMR-GAL4) and virgin females that carry the UAS-"oncogene" (for example UAS-RasV12 or UAS-RetMEN) (38-39). In this study we used the GMR-GAL4 cross with RetMEN system. The eyes of the offspring express tumors in the eye photoreceptors and have what is called a "rough eye" (an irregular and uneven, usually smaller eye).

Our work addresses several unanswered questions in the field, especially since there are currently few studies on the anti-cancer properties of moringa oleifera. While there are several studies using natural products to treat cancer, there is no systematic study comparing the effects of these specific natural products in a cancer model. We hypothesize that the common natural products sesame, cinnamon, garlic, moringa and turmeric will have an anti-cancer impact on *Drosophila*. Our data showed that these natural products cannot be used to reduce tumors once it has completely formed. However, our data confirmed that these natural products can be used to reduce cancer cell growth when treated early.

RESULTS

Testing the effects of 100% concentrated natural products on tumor growth and fly viability

Our goal was to compare the effects of each of the natural products at 100% concentration (i.e. garlic, sesame, moringa, turmeric and cinnamon) on tumor growth using the GMR-GAL4 cross with RetMEN system that expresses oncogenic RET in the drosophila eye, causing cancer. We placed 12-15 flies with eye tumors in one of five test vials, each containing one of the five natural products. We also set up two control vials with no natural product, containing either flies with eye tumors or wild type healthy flies. Four to five days after the experiment was set up, all the flies in the test vials died whereas control and reference samples were alive.

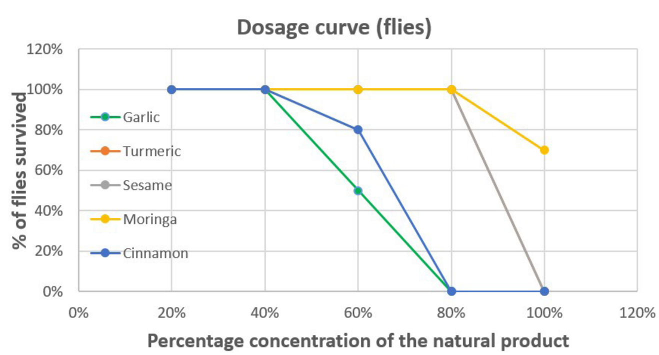

Figure 1. The number of flies survived in each of the vials vs the percentage concentration of each of the natural products (garlic, sesame, moringa, turmeric, and cinnamon)**.** All flies died in the garlic and cinnamon vials at concentrations above 40%. With turmeric and sesame, all flies died in the vials with concentrations greater than 80%. With moringa, flies in all vials survived except for those exposed to moringa at a 100% concentration. Note that the data for turmeric and sesame are overlapping. The plot for turmeric is hidden behind sesame plot in the graphs.

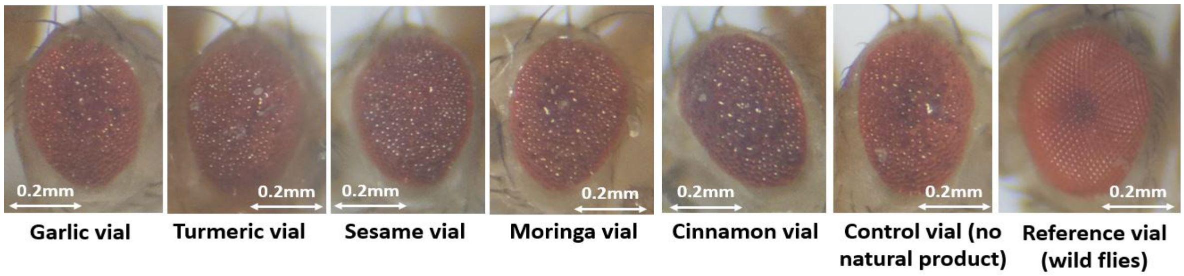

Figure 2. Representative images of the eyes of the flies from each of the natural products. From left to right: eyes of flies in vials with garlic, turmeric, sesame, moringa, cinnamon, Control sample (no natural products), and wild flies (healthy flies eyes).

This showed that a 100% concentration is lethal to the flies. Then we decided to determine the concentrations at which the flies would survive.

Dose-response curves can be used to determine the concentrations for each natural product at which flies remain viable. We sought to generate dose-response curves and use these concentrations for the experiment using the cancer flies. The number of flies that survived in each of the vials was plotted against the percentage concentration of each of the natural products (**Figure 1**). All the flies died in the garlic and cinnamon vials at concentrations above 40%. With turmeric and sesame, all the flies died in the vials with concentrations greater than 80%. With moringa, flies in all vials survived except for those exposed to moringa at a 100% concentration. Based on our results, we selected concentrations of 10%, 25%, and 40% for garlic and cinnamon, 25%, 50%, and 75% for turmeric and sesame, and 30%, 60%, and 90% for moringa.

Testing if we can treat cancer using dosage curve concentrations (on adult flies)

Next, we aimed to compare the effects of each of the various concentrations of the natural products (i.e. garlic, sesame, moringa, turmeric and cinnamon) on tumor characteristics. Vials with different concentrations (from the dosage curve) were prepared for each of the natural products (added 12-15 cancer flies) along with control sample vials, one with GAL4/UAS flies (without any natural product) and other healthy flies (without any natural product). Four to five days after the experiment, we examined the eyes of the flies in each vial under the microscope. Unfortunately, all the flies in all the test sample vials and concentrations showed blurry eyes with no improvement. Representative images of the eyes from each of the natural products are shown (**Figure 2**). This implies that the natural products may not be able to cure cancer once it is formed in the adult stages.

Imaginal discs of *Drosophila* eyes are formed during the initial larval stage (40). Most of the food that the flies eat during their life is consumed during the larvae stage (41**)**. So, we hypothesized that these natural products can help prevent cancer if the larvae feed on the experimental food.

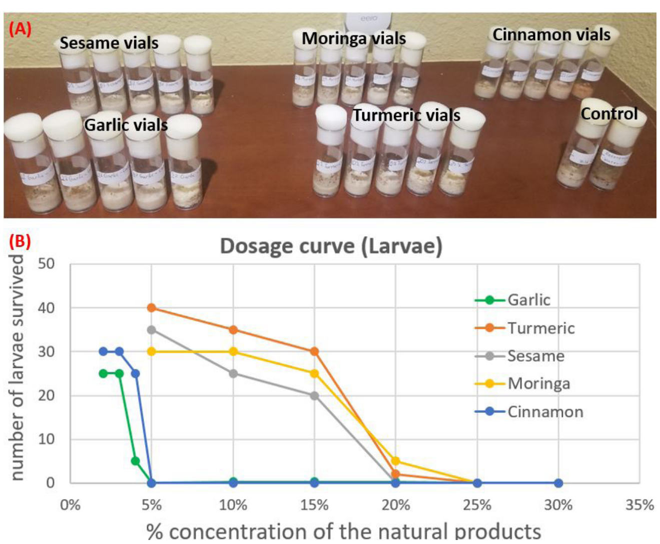

Figure 3. A) Experimental setup with flies in vials containing garlic, turmeric, sesame, moringa, cinnamon, and no product (control). **B)** Number of surviving flies in each vial plotted against the percentage concentration of each of the natural product.

Testing if we can reduce the tumors using dosage curve concentrations (on larvae)

We set up a cross between UAS-RetMEN males and GMR-GAL4 females and the larvae but after two weeks none of the experimental vials with natural products had any larvae in them. The control and reference vials had a normal number of larvae. This shows that the dosage curve for adult flies is different from that of the larvae. Hence, we sought to generate a dosage curve for the larvae.

One week after the larvae were exposed to various dosages of natural products, most of the vials had viable larvae except for vials with high concentrations of each of the natural products (**Figure 3A)**. We counted the larvae in each of the vials, and we plotted the survival rate of the larvae in each vial against the natural product concentration, generating dose-response curves (**Figure 3B**). Based on the results of the experiment, we selected concentrations of 1%, 2%, 3%, 4% and 5% for garlic and cinnamon, and 5%, 10%, 15%, 20% and 25% for sesame, moringa and turmeric.

The phenotype of rough versus smooth eyes can be visibly seen under the microscope (**Figure 4**). The *Drosophila* compound eye contains approximately 700-750-unit eyes known as 'ommatidia' (42). Ommatidia can be seen under the microscope and can be evaluated for uniformity qualitatively.

A decrease in the size of the eye is one of the symptoms of cancer. The size of the eye is compared to that of the control and healthy flies' eyes based on the measurements of length and width of the eyes. The average length and width of the flies' eyes in each of the vials of the natural products was plotted compared to each other and the control and healthy flies' sample (**Figure 5A-B**). Around 10 flies for each Turmeric and sesame treatment groups were analyzed and the average length and width for each sample was calculated. Flies treated with turmeric and sesame showed smaller lengths compared to the control sample. Garlic- and cinnamon-treated samples showed the same lengths compared to the control sample. mMringa-treated samples showed longer lengths than the control sample and close to healthy wild flies. Turmeric- and sesame-treated samples showed smaller widths compared to the control sample. Garlic- and cinnamon-treated samples showed the same widths compared to the control sample. moringa-treated samples showed widths longer than the control sample and close to healthy wild flies. When compared the size among the different natural treatments, sesame- and turmeric-treated flies showed the smallest sizes (both length and width), moringa-treated flies showed the largest size and cinnamon- and garlic-treated samples had intermediate sizes.

We also performed a simple t-test to compare each of the natural treatment conditions to the control sample to determine if the treatment had a significant effect on the length and width of the flies' eyes. For the length of the eyes, 10%, 15%, 20% moringa samples were significantly higher than the control samples with the p-values <0.05. For the samples 10%, 15%, 20% turmeric, 5%, 10%, 15%, 20% sesame are significantly lower than the control samples with the p-values <0.05. Rest of all the samples are not significantly different to the control samples with the p-values >0.05.

For the width of the eyes, 15%, 20% moringa samples showed significantly higher than the control samples with the p-values <0.05. For the samples 5%, 10%, 15%, 20% turmeric, 10%, 20% sesame are significantly lower than the control samples with the p-values <0.05. Rest of all the samples are not significantly different to the control samples with the p-values >0.05.

We plotted the lifespan (or survival rate) of flies for each of the samples including the control sample (**Figure 6A-E**). As the number of days increased, the percent of flies that survived decreased after around 30 days. The natural products fall under three distributions as shown in the plots. Garlic falls in one distribution where all the flies survived until 30 days and all of them died around 80 days. Flies treated with garlic distribution matches to that of the control sample distribution. The average lifespan for garlic and control sample was around 50 days. sesame, cinnamon and moringa fall in a 2nd distribution where all the flies survived until 35-40 days and all of them died around 95-100 days. The average lifespan for sesame, cinnamon and moringa samples was around 65-70 days. Turmeric falls in the 3rd distribution where

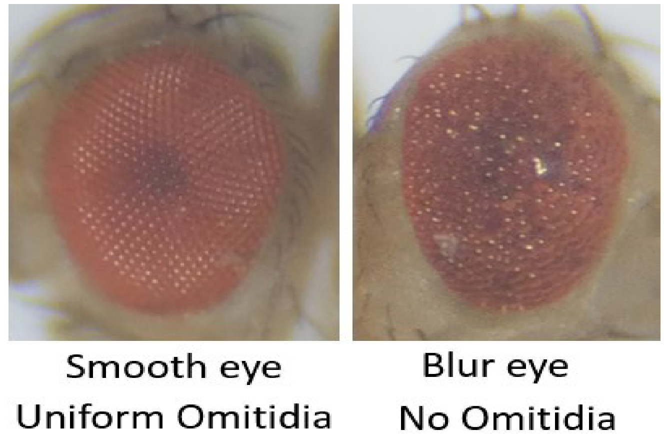

Figure 4. Comparison of Drosophila eye phenotypes visible under the microscope. Left image shows smooth eye and uniform ommatidia in healthy wild fly eye and right image shows rough eye and non-uniform ommatidia in cancer fly eye.

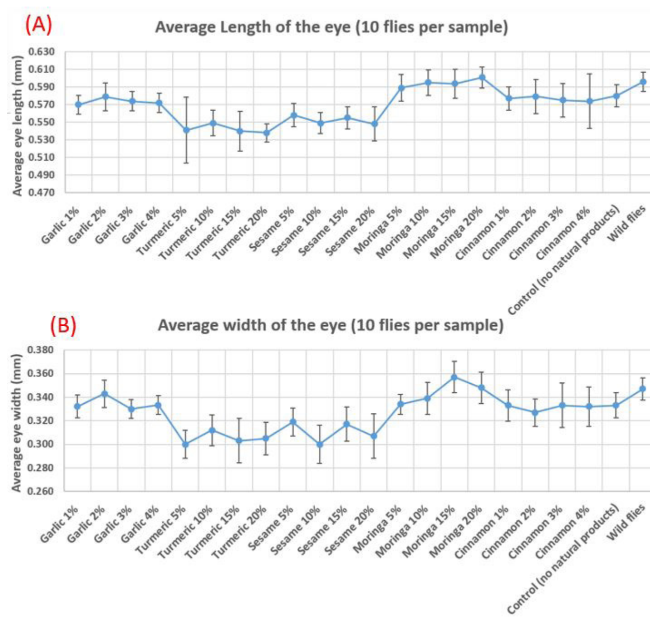

Figure 5. The average length and width of the flies' eyes in each of the vials of the natural products compared to each other and the control sample. A) For the length of the eyes, 10%, 15%, 20% moringa samples were significantly higher than the control samples with the p-values 0.034, 0.04, 0.014 respectively. For the samples 10%, 15%, 20% turmeric, 5%, 10%, 15%, 20% sesame are significantly lower than the control samples with the p-values 0.015, 0.038, <0.001, 0.049, 0.01, 0.022, 0.044 respectively. For the samples 5% moringa, 5% turmeric, 1%, 2%, 3%, 4% garlic, 1%, 2%, 3%, 4% cinnamon are not significantly different from the control samples with the p-values 0.23, 0.82, 0.68. 0.56, 0.62, 0.59, 0.33, 0.38, 0.45, 0.66 respectively.
B) For the width of the eyes, 15%, 20% moringa samples showed significantly higher than the control samples with the p-values 0.009, 0.039 respectively. For the samples 5%, 10%, 15%, 20% turmeric, 10%, 20% sesame are significantly lower than the control samples with the p-values 0.003, 0.038, 0.021, 0.028, 0.01, 0.041 respectively. For the samples 5%, 15% sesame, 5%, 10% moringa, 1%, 2%, 3%, 4% garlic, 1%, 2%, 3%, 4% cinnamon are not significantly different to the control samples with the p-values 0.69, 0.72, 0.27, 0.31, 0.36. 0.24, 0.33, 0.35, 0.33, 0.52, 0.39, 0.34 respectively.

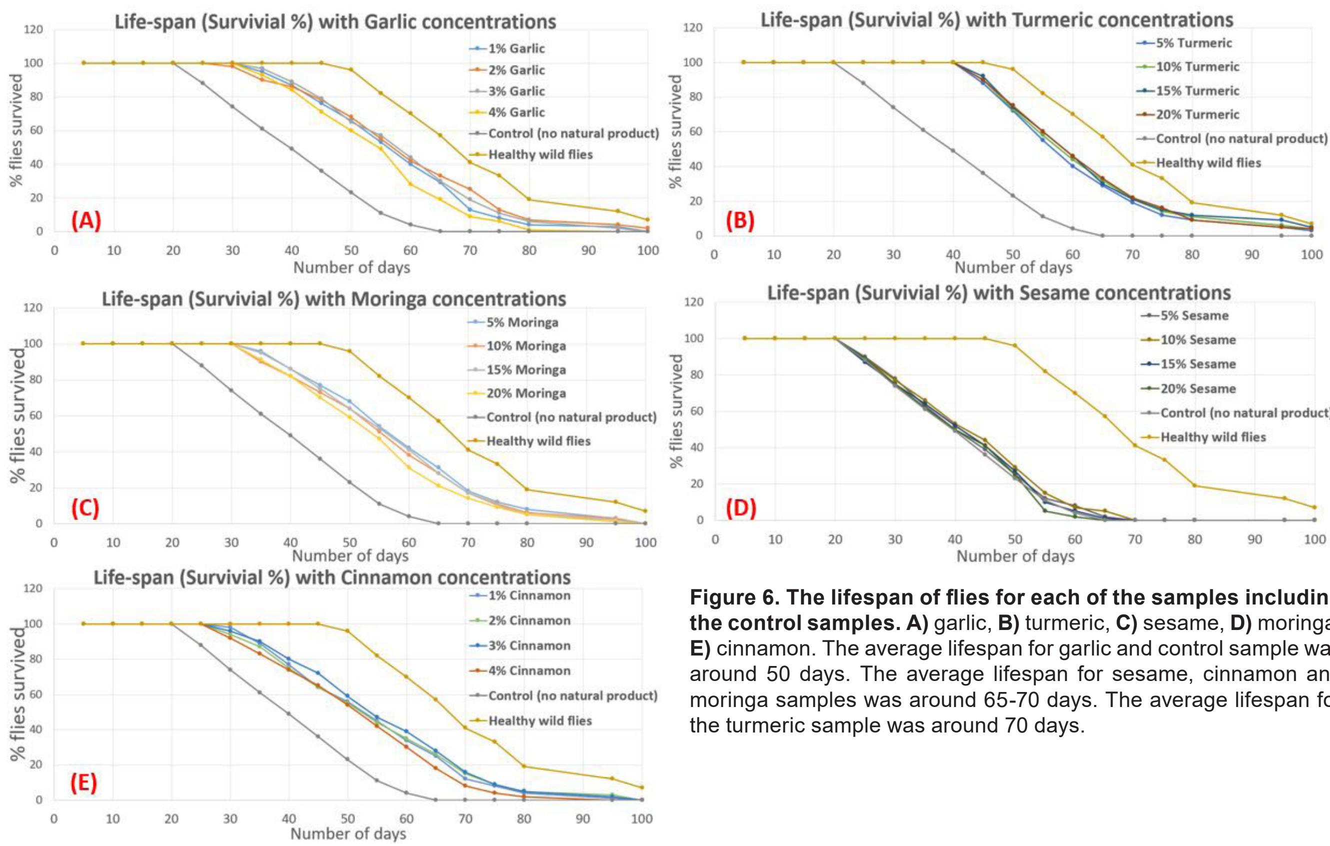

Figure 6. The lifespan of flies for each of the samples including the control samples. A) garlic, **B)** turmeric, **C)** sesame, **D)** moringa, **E)** cinnamon. The average lifespan for garlic and control sample was around 50 days. The average lifespan for sesame, cinnamon and moringa samples was around 65-70 days. The average lifespan for the turmeric sample was around 70 days.

all the flies survived until 50 days and all of them died around 100 days. The average lifespan for the turmeric sample was around 70 days. All the natural products showed increased lifespan (except for garlic which was similar to the control sample) compared to the control sample. Turmeric showed the longer lifespan compared to all the other natural products. The lifespan for the healthy wild flies is longer than all the natural products including the control sample, as expected.

No concentrations of garlic, turmeric, sesame, and cinnamon led to uniform ommatidia for any of the flies, and so did not prevent cancer (**Figure 7A-E**). All concentrations of moringa showed some flies with uniform ommatidia. For moringa, the number of flies with uniform ommatidia increased with natural product concentration until 15% and at 20% it decreased because the number of flies that survived were less. So, moringa showed better performance for tumor characteristics. Our study revealed that 15% moringa is the best candidate, among those tested in our study, to prevent tumors of the eye in this fly model of cancer. The number of flies with smooth eyes and the number of flies with uniform ommatidia for each of the vials including the control sample were plotted (**Figure 8A-B**). 5% and 10% sesame showed some flies with smooth eyes but all the concentrations of moringa showed flies with smooth eyes. For moringa the number of flies with smooth eyes increased with natural product concentration until 15% and at 20% it decreased because the number of flies that survived at 20% concentration were less. So, moringa showed better performance for tumor inhibitory characteristics.

DISCUSSION

We hypothesize that the common natural products sesame, cinnamon, garlic, moringa and turmeric will have an anti-cancer impact on *Drosophila*. This study showed that natural products (sesame, garlic, moringa, turmeric, cinnamon) cannot be used to treat cancer once it is completely formed during the adult stage of a fly's life cycle. During the larval stages, imaginal discs of the *Drosophila* fly's eyes are formed and during the pupal stages, eyes are developed (40). As a result, by the time we treated the adult flies with the experimental food, the tumors in their eyes were already formed. We therefore concluded that these natural products cannot cause tumor regression in adult flies. Since most of the food consumption in the flies is done during the larval stages, we hypothesized that treating flies with natural compounds before the larval stages could prevent cancer (41).

The larval lethal dose for each of the natural products, as defined by the dosage response curves, was lower than for adult flies. Since the larvae generally consume a large dose of food, they may not be able to survive at high concentrations as the adult flies do. Garlic and cinnamon lethal doses needed were considerably lower than other natural products for both larvae and the adult flies. It may be due to either the color or the odor of the two natural products. Cinnamon and turmeric were noted to have a strong odor and garlic was observed to have a strong odor that is persistent.

Though these natural products may not slow down or reverse tumor growth, they could potentially be used for preventing cancer. Sesame showed some improvement in the tumor incidence in *Drosophila* eyes at 5% and 10% concentrations. At 10% sesame concentration, about 30% of the flies that were examined showed smooth eyes with no visible tumors. Although the flies in the sesame test samples demonstrated decreased tumor incidence, they did not show uniform ommatidia in the eyes. Moreover, the average length and width of the flies' eyes in the sesame samples were smaller compared to the control sample and most of the other natural products. Smaller eyes generally are a symptom of disease. This showed that sesame alone cannot prevent the cancer activity completely in these flies. This showed that sesame treated flies had decreased tumor incidence, they still had diseased eyes, meaning that sesame alone cannot prevent tumor formation.

Moringa showed better results in most of the concentrations. Interestingly, as the concentration of the moringa increased, the anti-cancer performance (tumor in the eyes and size of the eyes) improved, showing the best performance at 15% and decreased at 20%. At 20% concentration, the sample size was less than at 15% concentration, because only eight larvae survived at the higher concentration. Moringa showed bigger eye sizes, both lengths and widths, compared to the control sample and were very close to healthy wild flies. One thing to note is even in the moringa-treated flies, none of the vials showed 100% of the flies free of tumors. Some of the larvae may not have consumed or absorbed the moringa fully and hence when those larvae turned into adult flies still showed tumor symptoms.

Though sesame has better absorbance samples treated with sesame did not show the better performance compared to the samples treated with moringa, since moringa has an anti-tumor compound called niazimicin which helps prevent

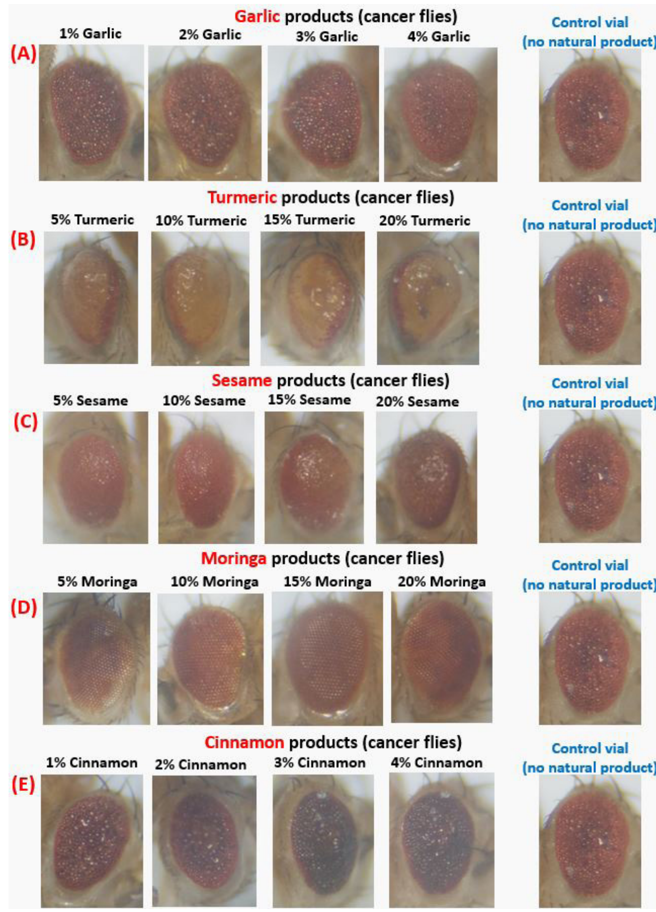

Figure 7. Representative pictures of the eye from each of the concentrations of the various extract vials compared to the control sample. A) garlic, **B)** turmeric, **C)** sesame, **D)** moringa, **E)** cinnamon.

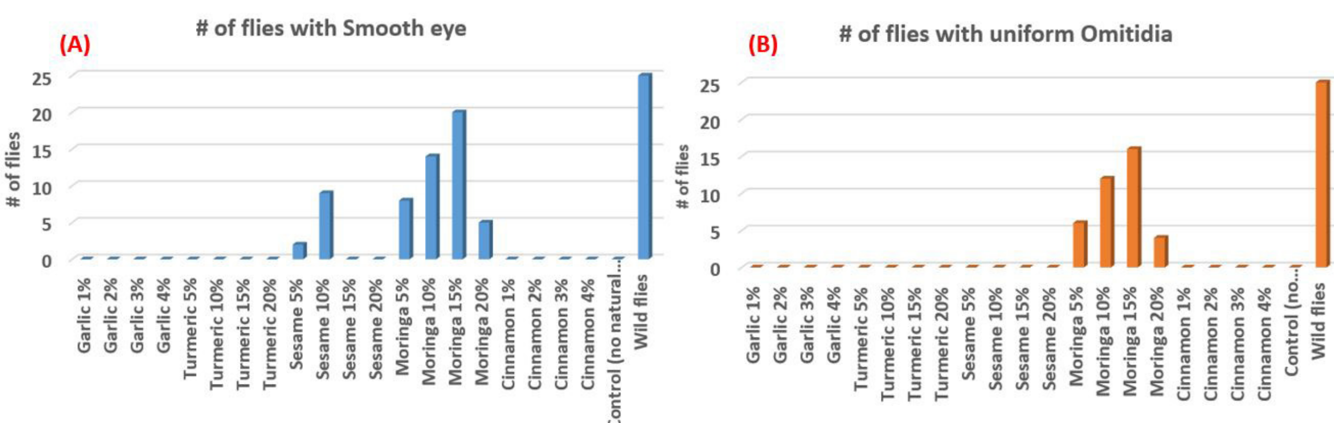

Figure 8. Flies with smooth eye and ommatidia. A) The number of flies with smooth eye for each of the test vials (out of 25 total flies taken for analysis), across five concentrations of each natural product, and the control sample. **B)** The number of flies with uniform ommatidia for each of the test vials (out of 25 total flies taken for analysis), across four concentrations of each natural product, and the control sample.

tumors, it showed better performance compared to other natural products (43-44). It is interesting to note that niazimicin is not present in any of these other natural products.

Though turmeric products did not prevent cancer, it helped increase the lifespan compared to control samples and other natural products. Curcumin impacts ageing and age-related diseases at the organismal and cellular level. On an organismal level, curcumin helps postpone the ageing symptoms and delays the on-set of diseases related to old-age (45). On cellular level, Curcumin enhances the activity of some of the anti-aging proteins and decreases the activity of some of the age-causing proteins (e.g., NF-κB, mTOR).

However, there are some limitations in this study. The microscope used in this study had low resolution, and as such, limited the accuracy of the eye measurements. Although the flies were anesthetized for image acquisition, they still moved slightly, making it difficult to capture good quality images. The methodology used to estimate the size of the flies' eyes is not robust as it assumed the size of the wild flies and compared the test samples to that. Since we are comparing the size of the eyes among each of the samples the actual numerical values are not as important for tumor performance study.

In this study, we did not test the performance of a combination of different natural products. Since both sesame and moringa showed promising results in terms of decreasing tumor activity, we plan to combine these natural products in various proportions and test the various endpoints in future experiments. Also, for future study we would like to combine these natural products with medicines that are already FDA-approved for the treatment of cancer, such as chemotherapy.

MATERIALS AND METHODS

Summary of the procedure

To test the effectiveness of 100% concentration of natural compounds, we took five test vials with each of the natural products and 12-15 cancer flies (eye tumors) were added into all of them. A 6th vial was added without any natural products (just water) to compare with the test samples. We also added a 7th vial with wild flies (healthy flies without cancer) for reference to compare regular healthy flies' eyes. Natural Products that were used include commercially available pure garlic, turmeric, sesame, moringa, and cinnamon. It was noted that the pure cinnamon mixed poorly with the food. Flies were allowed to feed for 4-5 days. Pure natural products are denoted as 100% concentration.

In order to test the effectiveness of lower concentrations of natural compounds obtained using dosage curve (on adult flies), we took 25 vials (5 for each natural product) with varying concentration and 12-15 cancer flies (eye tumors) were added to the vials. All the natural products (except cinnamon) were diluted using water. Cinnamon, which was not soluble in water, was diluted using PEG-400 (poly-ethylene glycol). A 16th vial was added without any natural products (just water) to compare with the test samples. We also added a 17th vial with wild flies (healthy flies without cancer) for reference to compare regular healthy flies' eyes and waited for 4-5 days till the flies fed on the food.

To test the effectiveness of lower concentrations of natural compounds obtained using dosage curve (on larvae), we set up the 17 vials including control and reference vials. We then set up a cross between UAS-RetMEN males and GMR-GAL4 females by adding 6-8 flies each type. The flies were allowed to mate for 4-5 days.

All the parent flies were separated from the vials to prevent confusion with the progeny. The larvae were grown into flies and checked their eyes for the "rough eye" tumor/overgrowth. After 2 weeks all the larvae turned into adult flies. The eyes of all the flies were examined in the microscope and measurable endpoints such as length and width of the eye, number of flies with no blur, number of flies with uniform ommatidia, lifespan of the eyes were used to compare the performance of each of the natural products for cancer prevention.

Drosophila melanogaster care

Drosophila wildtype flies were purchased from Carolina Biological Supply Company. The mutant strains (RetMEN2B mutant gene and UAS-GAL4 driver gene) were purchased from Bloomington *Drosophila* Stock Centre. The *Drosophila melanogaster* were cared for using the guidelines of the Carolina ® *Drosophila* manual (46). The flies in all the vials were fed with Formula 4-24® Instant *Drosophila* Medium food (15ml) and water(15ml) 6-8 grains of yeast were added to each of the vials. The stock of cancer flies was prepared by taking several vials and setting up a cross between flies having RetMEN2B mutant gene and UAS-GAL4 driver gene.

Dosage curve

For the adult flies' dose response, we took 20 vials (4 vials for each natural product) with varying concentrations from 20-100% and added wild flies (assuming the survival conditions of the wild flies were similar to the cancer flies). Then 20%, 40%, 60% and 80% concentrations of each product were prepared and added to the vial. The total volume of the natural products and water was always maintained at 15ml. For example, to prepare 20% moringa concentration we added 3ml of moringa to 12ml of water and mixed it well. After 4-5 days, the total number of flies that survived in each vial were counted and plotted against the concentrations.

For the larvae dosage curve, twenty vials (four for each natural product) were prepared with varying concentrations. The 21st vial without any natural products (just water) and 22nd vial with wild flies was also included. Then in each of the 21 vials (including the control sample), 6-8 UAS-RetMEN and GMR-GAL4 flies were added.

Measuring size of the eyes, lifespan of the flies

The microscope used in this study was AmScope SE306R-PZ-LED Stereo Microscope, 20X/40X/80X Magnification. The size of the eyes was measured by comparing them with the known size of wild flies' eyes. The size of the wild flies' eyes was assumed as 0.6mm long and 0.35mm wide (47). Pictures of all the flies were taken under the microscope with the same zoom and lens. The eye sizes of the test samples are calculated by comparing the eye sizes of both the samples on the pictures. Around 10 flies were taken and averaged to get the length and width of eyes of flies in each vial. The lifespan of the flies was measured by counting the number of flies that died every 5 days (initially when few of them died) and eventually counted the number of flies that survived (when most of the flies died).

Statistical analysis

A simple two-sample t-test was used to test the difference between the lengths and width of the eyes of the various test samples against the control or healthy flies. We then obtained the *p*-value (the probability that the sample means are different) for each of the comparisons. If the p value was <0.05 we reject the null hypothesis that there is no difference between the means and conclude that a significant difference does exist.

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