IJHSR
ijhighschoolresearch.org
■ RESEARCH ARTICLE

The Effects of Artemisinin, Berberine, and Sulforaphane Upon Ocular Tumor Formation in *Drosophila melanogaster*

Annalise H. Garrido

Westminster Christian School, 6855 SW 152 Street, Palmetto Bay, Florida, 33157, USA; annalisegarrido@gmail.com

ABSTRACT: Cancer is the second leading cause of death worldwide. Many patients do not have access to quality treatment, which is unfortunate since cancer survival rates are better when detected early. Some individuals elect to attempt to supplement their treatment with natural substances. This paper focuses on three substances: artemisinin, berberine, and sulforaphane, and their effects on tumor formation in *Drosophila melanogaster*. A cross of Yki *Drosophila* with GAL4 flies was performed to induce ocular tumors. Flies were then exposed to 0, 1, 2, and 4 uM of berberine and artemisinin and 0, .02, .01, and .005 ug/uL of sulforaphane in their media for three weeks, at which time flies were analyzed for tumor formation. There was a negative correlation between the concentration of all three substances and the percentage of flies that exhibited tumors (r=-.94, -.73, and-.61). Berberine and sulforaphane only significantly reduced tumor formation at the highest concentrations used ($p<.05$). Artemisinin significantly reduced tumor formation at all concentrations ($p<.001$). Artemisinin was found to have the best anticancer properties.

KEYWORDS: Cancer; Berberine; Artemisinin; Sulforaphane; Ocular Tumor; Yki; *Drosophila*.

■ Introduction

Cancer is the second leading cause of death worldwide and was responsible for 10 million deaths in 2020 alone. It is a disease of the cell cycle in which cells grow uncontrollably and beyond their usual boundaries, metastasizing to different parts of the body and spreading to various organs and tissues other than those in which they originated. Most cancer deaths are linked to its metastasis, which interferes with the functions of normal cells and bodily functions. The most common types of cancer in men are prostate, lung, stomach, and liver cancers, while in women, it is breast, lung, cervical, and thyroid cancer. Many patients do not have access to quality treatment and diagnosis, which is unfortunate since cancer survival rates are better when detected early and given effective treatment.[1] Therefore, this disease places an enormous physical, emotional, and economic strain on communities, families, and individuals.

The three most common treatment methods used for cancer today are surgery, radiation, and chemotherapy. Surgery removes localized masses of cancerous tissue, while radiation involves exposing the body to large amounts of radiation that induce mutations in the DNA of actively dividing cells if the cancerous cells are localized.[2] Chemotherapy is employed when there is a possibility of metastasis. It involves the administration of a chemical that inhibits the cell cycle in some way and stops mitosis, preventing the continued growth of cancerous cells.[3] Other forms of treatment include biomarker testing, hormone therapy, immunotherapy, and stem cell transplants.[4]

Although there are many treatments, they are not without side effects, including hair loss, nausea and vomiting, pain, exhaustion, depression, "chemo brain," neutropenia, and lymphedema.[5] There are also long-term effects, including dental issues, hearing loss, heart damage, infertility, loss of taste, blood clots, and osteoporosis.[6] Furthermore, there are issues with accessibility to treatment, such as the high cost of these methods, lack of education of individuals to know what is available to them, and limited treatment accessed by minorities.[7] Consequently, cancer is not only devastating because of the heavy loss of life but also in the quality of life of those who endure it because its treatments at times might even seem worse than the disease itself.

Furthermore, *Drosophila melanogaster* is a model organism in many scientific disciplines (see Figure 1 below) and has been involved in countless experiments over the years. They have become a model of choice for several reasons. They have short generation times, low maintenance costs, easily visible characteristics, and various tools to study their complex pathways. Moreover, *Drosophila* shares 60% of its genome with humans and has 75% similarity in genes responsible for human diseases, making them well-suited for a study in cancer.[8] Various strains of fly stocks have been created to study human diseases and are stored in collections such as those of Bloomington Stock Center. Many models require crossing two different strains of flies to activate genes that will cause disease. One such example of this process is the cross between the Yorkie (mutated Yki S168A) fly and that of the GMR-GAL4, which only expresses GAL4 in the developing eye. GAL4/UAS system is a common mechanism to activate the expression of a gene of choice.[9] UAS-activated Yki flies will be called 'Yki' flies, and GMR-GAL4 flies will be called 'GAL4' flies for the rest of the paper. This system produces a protein known as a transcription factor in which the Gal4 protein binds to an upstream activation sequence (UAS) to enhance the transcription of the desired gene. In this particular case, GAL4 is used to activate the Yki gene, which, when turned on, induces tumorigenesis in the eye of the fly. The Yki gene inhibits the Hippo signaling cascade, resulting in increased growth and differentiation in the eye of the fruit fly.[10] The Hippo cascade is a conserved sequence found in many organisms involved in diseases such as cancer. This, in-

© 2023 Terra Science and Education

DOI: 10.36838/v5i2.3

turn, causes excessive growth and darkening of the eye that is visible not only under a microscope but, in advanced cases, can even be visualized by the naked eye.

In addition, berberine is an alkaloid that has been used in Chinese medicines for centuries and was found to have anti-inflammatory and anti-diabetic properties.[11] In a study by Wang, Tan, Li, Yuen, and Feng, it was suggested that berberine "may repress tumor progression by regressing abnormal cell proliferation, arresting cell cycle, and inducing cell death."[12] Its anti-tumor properties include inhibiting cell proliferation and angiogenesis and inducing apoptosis in cancerous cells. Previous studies by Eom and Kim have noted that berberine alters the cell cycle by arresting the cell at various stages of interphase.[13] Further, p53 is a tumor suppressor gene that is essential in the apoptosis of cancerous cells. According to Shukla *et al.*, berberine upregulates the p53 protein and aids in the arrest of the cell cycle in the G2/M phase.[14] Berberine has also demonstrated the ability to overcome multidrug resistance, which would make it ideal for tumor chemotherapy, as noted by Zhao *et al.*, who found that berberine increased DNA damage, which increases apoptosis more than what occurred with the injectable chemotherapy medication, cisplatin by itself.[15] Furthermore, as noted by Ji *et al.*, berberine is also a part of the AMPK pathway, which regulates metabolism and metabolic diseases.[16] This pathway is also a key player in the apoptosis of various human cancer cell types. Finally, it is also effective in suppressing metastasis by inhibiting the release of MMP-2 from tumor cells which prevents the tumor from de-degrading the tissue barrier, thus preventing the spread of cancer.[17] Therefore, due to berberine's antimitotic and anticarcinogenic properties, this naturally occurring substance could offer promising results in oncology.

Artemisinin is an antimalarial drug derived from *Artemisia annua* that has been used in Chinese medicine for generations. It has exhibited antifungal, antibacterial, antiviral, apoptotic, and antiangiogenic properties. In a study by Chen *et al.*, they determined that artemisinin induces ferroptosis in cancerous cells.[18] This property makes artemisinin a good candidate as an anticancer medication because it is attracted to heme groups and promotes cell death in cells with high iron concentrations. Cancerous tissues have incredibly high concentrations of iron, which aid in the absorption of artemisinin into those cells. This, therefore, suggests that artemisinin may have cytotoxic effects on cancerous cells with great ease of delivery. Furthermore, according to Waseem, Hasan, and Ahmed, it is well tolerated by people with no adverse effects, can be consumed orally, and has a low cost to produce and purchase. Furthermore, they suggested that countries such as Pakistan that have an abundance of artemisinin should use it as a potential treatment for cancer and help reduce the financial burden the treatment is placing on those in their low-income population.[19]

Finally, sulforaphane is obtained from cruciferous vegetables such as broccoli and has garnered attention for its ability to regulate tumor suppressor genes.[20] In a study performed by Li *et al.*, it was determined that sulforaphane inhibited breast cancer in stem cells and merited further investigation anticancer properties.[21] Furthermore, a study performed by Zhang, Su *et al.* found that sulforaphane exhibits epigenetic effects upon prostate cancer cells and reduces tumor formation by removing acetyl groups and methylating regions of the genome and regulating signal transduction pathways that control the cell cycle.[22] Methylation can block the expression of oncogenes to prevent cancer from occurring, and it may also slow the reproduction of cells. Moreover, Gills *et al.* discovered that sulforaphane inhibits the growth of tumors, hinders the cell cycle, and promotes apoptosis in mouse skin.[23] In addition to its effectiveness as an anticarcinogenic substance, it is non-toxic, safe, low cost, and readily available, making it a suitable candidate to test its anticancer properties further.[22]

Although there have been some studies performed on each of the three substances mentioned above concerning their efficacy against cancer, this study aims to do a comparative analysis of the three to determine which is the most effective applied using the methodology on the same type of organism. This way, those interested in choosing one natural alternative to their treatment program can feel confident in selecting the most efficacious of the three. Cancer treatment can be grueling, costly, and result in various side effects; by ascertaining the best natural supplement, a patient can have greater control over their treatment program and quality of life. In addition, most studies performed on sulforaphane are done using dietary cruciferous vegetables, and this one will use a commercially available extract instead to determine whether it is effective. Therefore, the author of this study plans to determine: What are the effects of artemisinin, berberine, and sulforaphane upon ocular tumor formation in *Drosophila melanogaster*?

Methods

Determination of Timing Required for Tumor Formation:

To determine when tumor formation becomes observable in the Yki/GAL4 flies, *Drosophila melanogaster* stocks expressing the Yki (RRID:BDSC_28818) and GAL4 (RRID:BDSC_1104) were obtained from Bloomington Drosophila Stock Center. Genotypes of the flies were as follows: 28818 w[*]; P{UAS-yki.S168A.V5}attP2, 1104 w[*]; P{GAL4-ninaE.GMR}12 and crossed flies were w[*]; P{-GAL4-ninaE.GMR}12/+;P{UAS-yki.S168A.V5}attP2/+. A cross was performed between six virgin Yki females with six male GAL4 first bred, per Carolina's Care Guide for Drosophila.[24] Finally, these flies were also selected because the tumor was readily visible in the eye[10] and could even be seen without a microscope, thus making it an excellent choice for this study.

Virgin female Yki flies used for the cross were obtained by clearing adults from vials and then collecting females within six-hour windows to ensure they had not mated with other adult flies (females will not breed for the first 8 hours or so after eclosion). To collect flies, Yki stock vials were frozen for 10 minutes so flies would not fly off. The flies were shaken onto a petri dish, and the females were collected. GAL4 flies were then frozen for 10 minutes, shaken out into a petri dish, and males were collected. Six of each fly type were placed into

the three vials. After seven days, adults were removed from the three vials. When the second-generation adults emerged, they were moved into new vials that had been prepared, as mentioned above. This was done to ensure that only the initial generation is observed for eye abnormalities, not their offspring.

Adult flies were observed each week until tumors could be detected. Flies were added to new vials and kept for altered phenotypes for another week. It was then determined that eye abnormalities became visible at three weeks.

Controls:

For the controls, 4 grams of *Drosophila* media obtained from Carolina Science Company were added to 16mL of distilled water into each vial. A plastic mesh was added to increase the surface area for larval attachment, and a pinch of yeast was added on top of the media. The vial was then plugged with flies inside and repeated for five controls.

Berberine Concentrations:

Five vials each of berberine concentrations (0, 2, and 4 mM) were set up. To obtain two mM and four mM berberine, 160uL and 320uL of berberine (300mg/mL, Hawaiian Pharm) were added to the 16 mL water used to make the fly media.

Artemisinin Concentrations:

Five vials of artemisinin (0, 1, 2 uM) were formed in the following manner. Two more vials of the two mM would be made to verify the results. We used the identical five vials of 0 as above for the 0 uM. To prepare 1uM and 2uM artemisinin, 80uL and 160 uL of artemisinin (250 mg/mL, Herb Pharm) were added to the 16 mL of water used in media preparation.

Sulforaphane Concentrations:

Create five vials of sulforaphane (.02ug/uL, .01ug/uL, .005 ug/uL). Since sulforaphane is not water soluble, the controls were created by putting 4 g of Drosophila media, adding 70 uL of alcohol to a graduated cylinder, and then pouring distilled water into the cylinder until it reached a total volume of 16mL. The solution was then added to the media and placed into a vial. These control vials were placed in a refrigerator for 24 hours for ethanol to diffuse through the media. Ethanol was also used in the control to ensure that it was not adding any other variable to the outcome of the fly study. Mesh was added, and then a pinch of yeast and plug. It was repeated four more times.

A serial dilution of 33.4 mg/mL sulforaphane obtained from Healthy Drops was conducted in ethanol to obtain 0.02ug/uL, 0.01 ug/uL, and 0.005 ug/uL solutions. For each fly vial, 70 uL of each solution was added to the 16mL water used for media preparation. Vials were placed in a refrigerator for 24 hours for ethanol to diffuse through the media.

The concentrations chosen were based on studies performed on humans, mice, or flies and scaled according to the size of the model organism to avoid overdosing on the flies. Any comments about the highest concentrations applied were also a limiting factor in the chosen concentration range. Ethanol was used in the sulforaphane trial because the liposomal version of sulforaphane was liposomal and needed a substance to help uniformly dilute it.

Crosses:

Once concentrations were created, the following was performed for each vial. For each cross, 2 Yki virgin females (RRID:BDSC_28818) with two male GAL4 flies (RRID:BDSC_1104). Adults were removed after seven days to ensure that only the initial generation was observed. Once these flies reached three weeks of maturity, their eyes were observed for abnormalities. Adult flies were transferred to new vials every two weeks to ensure that the correct generation was analyzed in the cross.

Results and Discussion

After crosses were performed, flies were observed for discoloration and overgrowths to determine the effectiveness of the natural substance with t-tests and correlation coefficients run to assess statistical significance. Negative correlations obtained between the concentration amount and tumor formation showed a relationship between increasing the amounts of the natural substance and decreasing tumor formation.

Figure 1: Normal Eyed Fly (top) Fly with Overgrowth due to Yki expression (bottom).

Table 1: Concentrations of Berberine and Eye Tumor Occurrence

Concentration of Berberine (uM)	0	2	4
Percent of Eye Abnormalities out of 50 flies per vial	0.97	0.7	1
	0.97	0.77	0.53
	0.98		0.63
	1		0.38
	1		0.45
Average	98%	74%	77%
Standard Deviation	0.02	0.05	0.24
Ttest	0.08	0.29	0.02

While exposure to berberine at 2uM did not significantly decrease tumor formation, flies exposed to 4uM berberine developed significantly fewer tumors than control flies ($p<.05$; Table 1). There was a statistically significant negative correlation between tumor formation and the amount of berberine the flies were exposed to ($r=-.73$).

DOI: 10.36838/v5i2.3

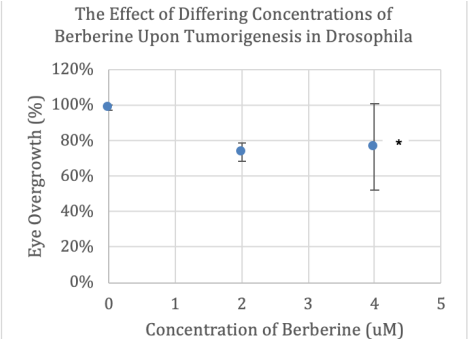

Figure 2: Berberine Concentration and Percent Eye Abnormality.

As seen in Figure 2, a statistically significant negative correlation was obtained (r=-.73). There was considerable variance in the data for the 4 uM concentrations, evidenced by the large error bars. This outcome occurred because one vial experienced 100% of the flies in one vial having eye phenotypes. However, the other samples at this concentration showed a marked difference in the amount of eye tumor formation.

Table 2: Concentrations of Sulforaphane and Eye Tumor Occurrence.

Amount of Sulforaphane (ug/uL)	0	0.005	0.01	0.02
Sulforaphane (uM)	0	0.028202	0.056405	0.11281
Percent Overgrown out of 50 flies per vial	0.97	1	0.86	0.88
	0.97	0.91	0.98	0.5
	1	1	0.91	0.6
	1	1	0.8	0.56
	1	0.45	0.64	0.84
Average	0.988	0.872	0.838	0.676
Standard Deviation	0.02	0.24	0.13	0.17
Ttest	0.34	0.79	0.13	0.02

At 0.02 ug/uL, sulforaphane significantly reduced tumor formation ($p<0.05$), while lower concentrations did not have a significant effect (see Table 2 above). However, there was a statistically significant negative correlation between the amount of sulforaphane applied and ocular tumor formation (r=-.61).

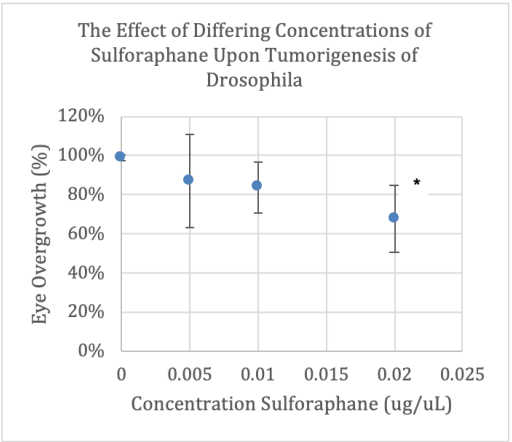

Figure 3: Sulforaphane Concentration and Percent Eye Abnormality.

All concentrations of artemisinin tested significantly reduced tumor formation ($p<0.05$; see Table 3 below). In the first three trials, no overgrowths were detected in flies given 2 uM artemisinin. To confirm this result, two additional trials were performed at this concentration.

Table 3: Concentrations of Artemisinin and Eye Tumor Occurrence.

Concentration Artemisinin (uM)	0	1	2
Percent of Eyes Overgrown out of 50 flies per vial	0.97	0.38	0
	0.97	0.33	0
	0.98	0.23	0.088
	1	0.46	0.097
	1	0.05	0
			0.037
			0.072
Average	0.984	0.29	0.042
Standard Deviation	0.02	0.16	0.04
Ttest	0.00	0.02	2.5E-11
	* 0 to 1	*1 to 2	*0 to 2

However, even after the extra trials, all vials were indeed statistically different from each other. There was also another negative correlation coefficient run. This trial had the most marked statistically significant inverse relationship between the amount of artemisinin applied and the appearance of ocular tumors (r= -.91, Figure 4). Figure 7 shows flies exposed to 2uM artemisinin, and the eyes show no evidence of growth.

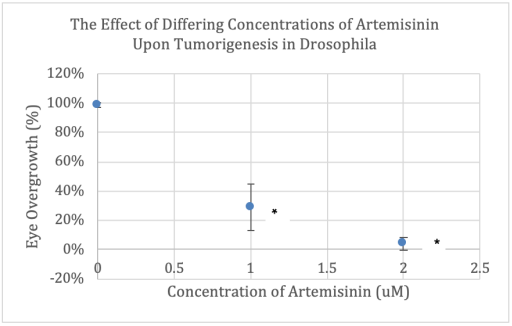

Figure 4: Artemisinin Concentration and Percent Eye Abnormality.

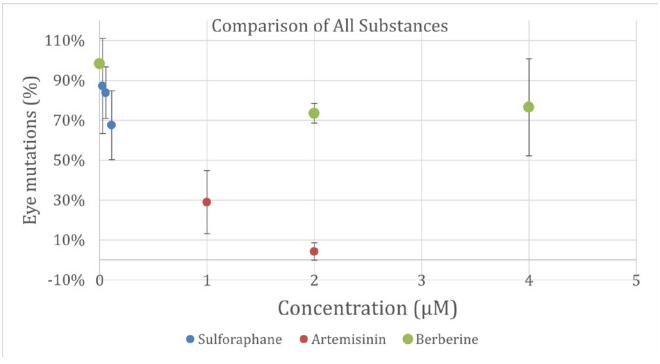

Figure 5: Comparison of Berberine, Sulforaphane, and Artemisinin Upon Percent Eye Tumor Formation.

Figure 5 overlays all three trials to compare the three natural substances against one another. The marked negative correlation of the artemisinin trials can readily be seen in this image (r=.91). This, therefore, leads one to conclude that if looking for a natural supplement while undergoing cancer treatment, artemisinin might be the best option to pursue.

Figure 6: Controls.

Figure 6 is a sample from the control and shows overgrowth.

Figure 7: Artemisinin at 2 uM.

Figure 7 depicts flies exposed to artemisinin (2uM).

Conclusion

All three substances demonstrated a negative correlation between the amount of substance given and the percentage of mutants that formed. The berberine exhibited a statistically significant difference between the control and 4 uM ($p<.05$). The sulforaphane showed a statistically significant difference between the control and 0.11 uM ($p<.05$). Artemisinin demonstrated a statistically significant difference between each amount ($p<.05$) and had the most significant negative correlation ($r=-.94$; $p<.001$).

Based on the results of these trials, artemisinin possessed the most significant negative correlation ($r=-.94$; $p<.001$) compared to sulforaphane and berberine. All of the substances yielded a significant negative correlation (berberine $r=-.73$; sulforaphane $r=-.61$) and demonstrated a statistically significant difference between the control and their highest relative molar concentration ($p<.05$). However, artemisinin, on the other hand, demonstrated statistically significant data when comparing each concentration amount. Therefore, all substances appear to have some antitumorigenic properties; nevertheless, artemisinin was the most efficacious.

It should be noted that different cancers are caused by varying gene mutations and, in some cases, multiple mutations. These three substances may have different efficacies on cancer types due to the mutations causing cancer. For example, artemisinin works best on cancers caused by Hippo pathway mutations, while berberine will work better on p53 mutations.

These findings imply that it first informs that more studies should be done on varying types of cancer and different model organisms to see if the outcome is the same. For example, it would be good to perform this test on both mouse models and *in vitro* human cell lines. Mice are mammals within the same class as humans, rather than *Drosophila*, which are invertebrates. One such human cell line that would be interesting to apply these substances to would be Hela cells. Hela cells are a cancer cell line in which many studies have been performed, and much is understood about their nature and genome. The findings of this study provide an excellent comparative analysis for those seeking to use a natural supplement as they battle cancer to choose the most effective method without wasting valuable money and time. These results suggest that artemisinin is the most effective natural combatant to reduce cancer formation.

Naturally, there are some limitations to the application of these findings. Even though *Drosophila* shares many genes, specifically disease genes, with humans, trials of the efficacy of these substances in humans must be performed to conclusively state that they would be helpful and applicable to humans; another impediment that arose while engaging in this experiment was that of having a lipid-based sulforaphane which required the use of alcohol to dissolve the concentrations effectively. Although those trials were compared to an alcohol control that had similar results to the other controls performed in this study, it might be worthwhile to try using a different solvent of sulforaphane to see if that would produce different or more significant results. Another limitation of this study is the sheer amount of virgin female flies that needed to be obtained to perform the crosses. Although there were indeed over 50 crosses done in this study, it would be better to attempt these trials in cell lines to make findings more applicable to humans.

This study compared three natural substances in similar conditions. It concluded that artemisinin would be the best substance for anyone to use should they desire to add a natural substance to their treatment. It also tested a form of sulforaphane that was not directly from broccoli to determine whether other forms of sulforaphane would also exhibit antitumorigenic properties. This also was supported by this study since the liposomal sulforaphane also negatively correlated with ocular tumor formation.

Acknowledgments

I would like to thank Lisa Garrido, my science research mentor, and Ms. Hannah North, my AP Research Instructor, for their support throughout my scientific research. I would also like to thank Ms. Annette Parks from Bloomington Stock Center for suggesting a cancer model organism.

References

1. World Health Organization. (21 September 2021). Cancer. World Health Organization. Retrieved November 8, 2021, from https://www.who.int/news-room/factsheets/detail/cancer.
2. Radiation therapy for cancer. National Cancer Institute. (n.d.).

DOI: 10.36838/v5i2.3

Retrieved November 17, 2021, from https://www.cancer.gov/about-cancer/treatment/types/radiation-therapy.
3. Chemotherapy to treat cancer. National Cancer Institute. (n.d.). Retrieved November 17, 2021, from https://www.cancer.gov/about-cancer/treatment/types/chemotherapy.
4. Types of cancer treatment. National Cancer Institute. (n.d.). Retrieved November 8, 2021, from https://www.cancer.gov/about-cancer/treatment/types.
5. Centers for Disease Control and Prevention. (2021, May 12). Side effects of cancer treatment. Centers for Disease Control and Prevention. Retrieved November 8, 2021, from https://www.cdc.gov/cancer/survivors/patients/side-effects-of-treatment.htm.
6. Mayo Foundation for Medical Education and Research. (2020, October 6). Managing the lingering side effects of cancer treatment. Mayo Clinic. Retrieved November 8, 2021, from https://www.mayoclinic.org/diseases-conditions/cancer/in-depth/cancer-survivor/art20045524.
7. McDowell, S. (2019, December 5). Researchers set goals for improving access to cancer care. American Cancer Society. Retrieved November 8, 2021, from https://www.cancer.org/latest-news/researchers-set-goals-for-improving-access-to-cancercare.html.
8. Mirzoyan, Z., Sollazzo, M., Allocca, M., Valenza, A. M., Grifoni, D., & Bellosta, P. (1AD, January 1). Drosophila melanogaster: A model organism to study cancer. Frontiers. Retrieved November 8, 2021, from https://www.frontiersin.org/articles/10.3389/fgene.2019.00051/full.
9. Xu, T., Kumar, S., & Denton, D. (2016, November 23). Characterization of autophagic responses in drosophila melanogaster. Methods in Enzymology. Retrieved November 10, 2021, from https://www.sciencedirect.com/science/article/abs/pii/S0076687916303627.
10. Wittkorn, E., Sarkar, A., Garcia, K., Kango-Singh, M., & Singh, A. (2015). The Hippo pathway effector Yki downregulates Wg signaling to promote retinal differentiation in the Drosophila eye. Development (Cambridge, England), 142(11), 2002–2013.
11. Wang, K. Feng, X., Chai, L., Cao, S., Qiu, F. (2017) The metabolism of berberine and its contribution to the pharmacological effects. Drug Metabolism Reviews 49:2, pages 139-157.
12. Wang, N., Tan, H., Yuen M., & Feng Y. (2015, October 28). Recent updates and future perspectives include berberine and coptidis rhizoma as. Journal of Ethnopharmacology. Retrieved November 5, 2021, from Berberine and Coptidis Rhizoma as potential anticancer agents: Recent updates and future perspectives - Science Direct.
13 Eom K. S., Hong J. M., Youn M. J., So H. S., Park R., Kim J. M., *et al*. (2008). Berberine induces G1 arrest and apoptosis in human glioblastoma T98G cells through mitochondrial/caspases pathway. Biol. Pharm. Bull. 31, 558–562. 10.1248/bpb.31.558
14. Shukla S., Sharma A., Pandey V. K., Raisuddin S., Kakkar P. (2016). Concurrent acetylation of FoxO1/3a and p53 due to sirtuins inhibition elicit Bim/PUMA mediated mitochondrial dysfunction and apoptosis in berberine-treated HepG2 cells. Toxicol. Applied Pharmacol. 291, 70–83. 10.1016/j. taap.2015.12.006
15. Zhao Y. W., Jing Z. L., Li Y., Mao W. F. (2016). Berberine in combination with cisplatin suppresses breast cancer cell growth through induction of DNA breaks and caspase-3- dependent apoptosis. Oncol. Rep. 36, 567–572. 10.3892/or.2016.4785
16. Xu, T., Kumar, S., & Denton, D. (2016, November 23). Characterization of autophagic responses in drosophila melanogaster. Methods in Enzymology. Retrieved November 10, 2021, from https://www.sciencedirect.com/science/article/abs/pii/S0076687916303627.
17. Zhang, C., Sheng, J., Li, G., Zhao, L., Wang, Y., Yang, W., Yao, X. , Sun, L., Zhang, Z., & Cui, R. (2020, January 15). Effects of berberine and its derivatives on cancer: A Systems Pharmacology Review. Frontiers in pharmacology. Retrieved November 5, 2021, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6974675/.
18. Chen, G.-Q., Benthani, F. A., Wu, J., Liang, D., Bian, Z.-X., & Jiang, X. (2019, May 21). Artemisinin compounds sensitize cancer cells to ferroptosis by regulating iron homeostasis. Nature News. Retrieved November 5, 2021, from https://www.nature.com/articles/s41418-019-0352-3.
19. Waseem, Y., Hasan, C. A., & Ahmed, F. (2018, November 23). Artemisinin: A promising adjunct for cancer therapy. Cureus. Retrieved November 5, 2021, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6347441/.
20. Baier, S. R., Zbasnik, R., Schlegel, V., & Zempleni, J. (2014, June). Off-target effects of sulforaphane include the derepression of long terminal repeats through histone acetylation events. The Journal of nutritional biochemistry. Retrieved November 5, 2021, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4019935/.
21. Li Y, Zhang T. Targeting cancer stem cells with sulforaphane, a dietary component from broccoli and broccoli sprouts. Future Oncol. 2013;9:1097–103.
22. Zhang, C., Su, Z. Y., Khor, T. O., Shu, L., & Kong, A. N. (2013). Sulforaphane enhances Nrf2 expression in prostate cancer TRAMP C1 cells through epigenetic regulation. Biochemical pharmacology, 85(9), 1398–1404. https://doi.org/10.1016/j. bcp.2013.02.010.
23. Gills J., Jeffery E., Matusheski, N., Moon R., Lantvit, D., and Pezzuto, J. Sulforaphane prevents mouse skin tumorigenesis during the stage of promotion. Cancer Lett 236: 72–79, 2006.
24. Care guide: Drosophila. Carolina.com. (n.d.). Retrieved September 28, 2022, from https://www.carolina.com/teacher-resources/Interactive/care-guide-drosophila/tr10495.tr

Author

Annalise Garrido is a senior at Westminster Christian School in Miami, Florida. She has performed research on *Drosophila melanogaster* for the past three years. She has placed at the state level and most recently competed at Regeneron's International Science and Engineering Fair. She hopes to pursue a chemistry degree.