JOURNAL OF
EMERGING INVESTIGATORS

Article

The effect of an anthocyanin on the gut permeability of a Type 2 Diabetic *Drosophila melanogaster*

Shriya Dale[1], Keerthi Maddipatla[1], Jessica Eliason[1]
[1] Academy of Science, Academies of Loudoun, Leesburg, Virginia

SUMMARY

Type 2 diabetes mellitus (T2DM) affected slightly less than half a billion patients globally in 2019 and is expected to affect 700 million by 2045. T2DM is characterized by the body's inability to respond to the body's circulating insulin, a phenomenon known as insulin resistance. A symptom of T2DM is increased gut permeability, which can lead to an increase in the toxins that enter the gut and bloodstream, resulting in complications such as nerve damage, heart disease, and kidney damage. The current treatment for T2DM, Metformin, can exacerbate gut permeability. One potential alternative to combat Metformin's negative effects is anthocyanins, like purple sweet potato extract (PSPE), which are water soluble plant pigments with antidiabetic effects like increased insulin secretion. Once anthocyanins reach the large intestine and are transformed by the gut microbiota, they form into metabolites, chemical compounds responsible for metabolism, resulting in decreased gut permeability. Therefore, we hypothesized that anthocyanins can be used as potential chemical mediators that decrease gut permeability. Our research aimed to find whether metabolites of purple sweet potato extract (PSPE) are associated with decreased gut permeability using a type 2 diabetic *Drosophila melanogaster* model. To determine the gut permeability, we used the Surf Assay and observed that the PSPE treatment drastically reduced gut permeability of the flies. Our research provides valuable knowledge about the relationship between PSPE, gut permeability, and T2DM, which may positively impact the lives of hundreds of millions of people.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) will affect approximately 700 million patients globally by 2045 (1). Unfortunately, T2DM diagnoses are predicted to increase exponentially due to the prevalence of obesity, hypertension, prediabetes, and alcohol consumption (2). Many of the common symptoms of T2DM include drastic weight change, frequent fatigue, and an increase in thirst. T2DM is a disease where the body cannot use and regulate glucose efficiently (1). One of the key characteristics of T2DM is insulin resistance (3). In a non-diabetic body, pancreatic beta cells produce insulin that allows glucose broken down from consumed carbohydrates from the blood to enter cells to contribute to cellular mechanisms (1). In T2DM patients, cells cannot respond properly to insulin leading to an over-abundance of glucose in the bloodstream, which is known as hyperglycemia (1, 3). To make matters worse, in response to low glucose levels in cells, the liver breaks down stored glycogen into glucose, creating more glucose within the T2DM patient, thus further exacerbating their hyperglycemia (3).

In a non-diabetic body, when glucose levels within cells are below the average level, the liver breaks down stored glycogen, which is a readily mobilized form of glucose (3). Glycogen is then converted into glucose to ensure that the glucose levels are within an acceptable range (1). A patient with T2DM cannot perform this same process, leading to hyperglycemia or high blood glucose levels.

One of the characteristics of T2DM is increased gut permeability, which is characterized by the negative translocation of microbial products into the blood and other integral parts of the body (4). In addition, gut permeability directly impacts the number of toxins that can enter the gut and the bloodstream (5). Gowd *et al.* have proposed that an increase in the gut or intestinal permeability further perpetuates the damage of pancreatic β-cells due to the increased number of toxins entering the gut (6). An increase in the number of toxins that can enter the gut and bloodstream results in the increase of other complications caused by T2DM, which include nerve damage, heart disease, kidney damage, and eye damage (1). It must be noted that increased gut permeability is further perpetuated by T2DM-combating drugs such as Metformin (3). Unfortunately, Metformin has various negative impacts on the body, prominently regarding the gut (2). For example, Metformin is known to cause lactic acidosis, which is the buildup of lactic acid in the bloodstream, which is directly related to increased gut permeability (3, 5).

To measure gut permeability, the Smurf Assay is ideal. The Smurf Assay gets its name from its most important aspect: the blue color of the dye that is used. The higher the gut permeability is, the more blue dye permeates throughout the body, as the gut barrier is not working efficiently to stop the spread of blue dye. Darker blue hues indicate that the gut barrier is not separating pathogens and other microorganisms effectively. If most of the blue dye is retained within the flies' guts, those flies will be classified as Non-Smurfs. However, if the dye was vividly outside the gut, those flies will be categorized as Smurfs. The following formula will be used to determine the proportion of Smurfs in the population, which

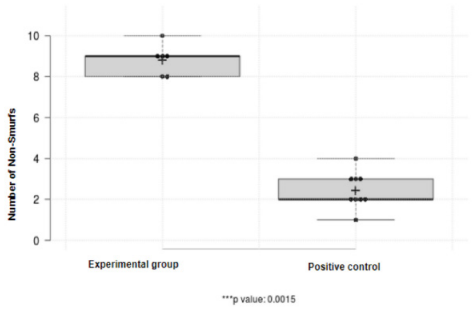

Figure 1. Diabetic flies with and without PSPE treatment. Box plot showing the number of Non-Smurfs in the positive and experimental groups (the most important relationship) with Spear whiskers extending to the minimum and maximum values of each set (N = 8 and N = 6, respectively). High-glucose diets were used to induce T2DM, PSPE diets were used for treatments, and Smurf Assays measured gut permeability. Positive and experimental groups were statistically significant. Mann-Whitney statistical test, ***p = 0.0015.

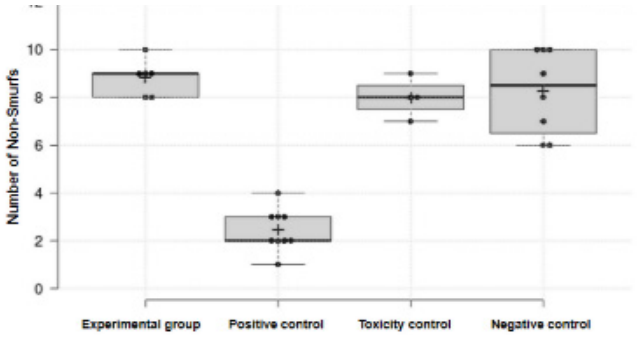

Figure 2. Flies with T2DM, PSPE, or a combination of both. Box plot showing the amount of Non-Smurfs in the positive, experimental, negative, and toxicity groups with Spear whiskers extending to the minimum and maximum values of each set (N = 8, N = 6, N = 8, and N = 4, respectively). The experimental group and the toxicity control flies had the same amount of PSPE supplementation. High-glucose diets were used to induce T2DM, PSPE diets were used for treatments, and Smurf Assays measured gut permeability. Kruskal-Wallis, Dunn's Multiple Comparison Test between the experimental group and positive control (statistically significant) and the positive and the negative controls (statistically significant), ***p=0.0004

will then be used to determine if PSPE influenced reducing gut permeability:

$$S = \frac{Smurfs}{Smurfs + Non-Smurfs} \quad (1)$$

Anthocyanins are the main focus of this research. They are water-soluble plant pigments with various antidiabetic effects, such as insulin secretion (7). Furthermore, anthocyanins can reach the large intestine and can be transformed by the gut microbiota into secondary metabolites, which are chemical compounds responsible for metabolism (1,8). These secondary metabolites are produced by organisms as a result of environmental stimuli. The metabolites are not necessary for growth but allow organisms to gain an advantage in their environment, which can be used for acquiring necessary nutrients for metabolism (9). Metabolites can decrease gut permeability, so this process indicates that anthocyanins can be used as a potential mechanism to decrease gut permeability (8).

Studies that correlate anthocyanins with animal models have been completed in the past (10 - 14). Six anthocyanin supplements, including blackberries and Concord grapes, in mice diets positively modulated the gut microbiome, creating relative abundances of healthy, necessary microbial bacterial populations (10,11). One unexplored aspect of this research is the effects of anthocyanins on the gut permeability of those affected by T2DM.

In another study, bilberries, which have a high anthocyanin content, reduced the overexpression of insulin from 60% to 35% in *Drosophila melanogaster* with T2DM (12,13). The implementation of the bilberries suggests that anthocyanins reduce the overexpression of insulin, supporting the claim that anthocyanins have a positive effect on T2DM and its main characteristic (14).

Among the various anthocyanins available, our study focused on purple sweet potato extract (PSPE). PSPE is a vegetable known to have a large anthocyanin content, and it is easily accessible to the population (15). Out of the various anthocyanins present, purple sweet potato extract is less studied, and is rich in anthocyanins as it is almost double the content of anthocyanin in blueberries, which are known to have a high anthocyanin content (7). Furthermore, PSPE is highly accessible, allowing people to incorporate it into their daily diets easily (15 - 17).

Due to the relationship between T2DM, its treatments, and increased gut permeability, our proposed research aims to find the effectiveness of anthocyanins on reducing the gut permeability of a type 2 diabetic *Drosophila melanogaster* model. Because T2DM is not present naturally in flies and the Drosophila genome has a 60% similarity to humans, there will be some disparities between the effectiveness of this PSPE treatment on T2DM in humans (15, 16). However, it is important to note that compared to other invertebrate models, fruit flies have homologs of 75% of the genes that lead to diseases in humans, making them the most accessible and efficient model for testing (16). The relationship between anthocyanins and decreased gut permeability is important because it could eliminate the negative side effects of T2DM and increased gut permeability.

We hypothesized that having a diet with PSPE would result in decreased gut permeability because anthocyanins modulate gut microbiota composition through metabolite production (7, 9). This novel area of research could generate a naturopathic treatment for gut permeability, decreasing the number of toxins entering the intestine and, therefore, reducing the potential development of other diseases induced through T2DM.

RESULTS

We hypothesized that implementing PSPE into flies' diets with T2DM would reduce gut permeability. Anthocyanins,

which are present in PSPE, are transformed into metabolites that play a crucial role in decreasing gut permeability (1). To test whether PSPE would decrease the gut permeability of T2DM flies, we divided normal fly food diets into either PSPE or no PSPE. The toxicity assay, which is exemplified in the Experimentation section of the Materials and Methods, provided the ideal concentration of PSPE that would optimize the health of the *Drosophila*, which was 10 mg per mL of fly food. We analyzed the gut permeabilities of the flies through the Smurf Assay, as explained in the Materials and Methods section.

The positive control had the greatest average number of Smurfs, which was eight for every ten flies, whereas the experimental group had a significantly lower average number of Smurfs: one for every ten flies (**Table 1**). We observed a relationship between the implementation of purple sweet potato and the Smurfs and Non-Smurfs in the experimental group (**Table 1 and Figure 1**). It must also be noted that the Non-Smurfs was three-fold in the experimental group, compared to the positive control (Experimental S-value: 8.83, Positive S-value: 2.444). The statistical analysis of the positive control and experimental group yielded a p-value of 0.0015, which is extremely significant compared to the alpha level of 0.05 (Mann-Whitney test, **Figure 1)**.

We used a Kruskal-Wallis test to compare all four groups (experimental group, positive control, toxicity control, and negative control). The results from this Kruskal-Wallis test were statistically significant, yielding a p-value of 0.0004, indicating that there were statistically significant differences between the gut permeabilities of flies with T2DM, PSPE supplementation, or a combination of both (Kruskal-Wallis test, **Figure 2**). To further corroborate these claims, we conducted a Dunn's Multiple Comparison Test between the experimental group and positive control and the positive and negative controls. Both of these tests had statistically significant results (Dunn's Multiple Comparison Test, **Figure 2**).

Group	Smurfs	Non-Smurfs	Smurf Assay S-value	Average S-value
Experimental Group (T2DM + PSPE Treatment)	1	9	0.1	0.1
	2	8	0.2	
	0	10	0.0	
	1	9	0.1	
	2	8	0.2	
	1	9	0.1	
Positive Control (T2DM + No PSPE Treatment)	7	3	0.7	0.8
	8	2	0.8	
	8	2	0.8	
	7	3	0.7	
	9	1	0.9	
	8	2	0.8	
	7	3	0.7	
	8	2	0.8	
	6	4	0.6	
Negative Control (No T2DM + No PSPE)	0	10	0.0	0.2
	1	9	0.1	
	0	10	0.0	
	0	10	0.0	
	4	6	0.4	
	3	7	0.3	
	2	8	0.2	
	4	6	0.4	
Toxicity Control (No T2DM + PSPE Treatment)	2	8	0.2	0.2
	3	7	0.3	
	2	8	0.2	
	1	9	0.1	

Table 1. Smurf Assay on experimental, positive, negative, and toxicity groups. Data table showing the amounts of Smurfs and Non-Smurfs, along with the S-values of each trial and the average S-value for the entire group. High-glucose diets were used to induce T2DM, PSPE diets were used for treatments, and Smurf Assays measured gut permeability.

DISCUSSION

In our statistical analysis, our alternate hypothesis, which is that the S-value for the positive control would be greater than the S-value of the experimental group, failed to be rejected. In other words, the null hypothesis was rejected. Thus, the significant difference supports the initial hypothesis as implementing purple sweet potato in the fly's diet led to decreased gut permeability. The number of Non-Smurfs essentially tripled when purple sweet potato was added as a treatment (**Figure 2**). To conclude, purple sweet potato dramatically aided the flies' gut permeability, which aligns with the anti-diabetic properties of anthocyanins as expected. We originally hypothesized that implementing PSPE would decrease the gut permeability of flies that have induced T2DM, as anthocyanins positively modulate the gut microbiome, hence decreasing gut permeability through increased metabolite production. PSPE had a major positive effect on the flies with T2DM as the number of Smurfs in the population decreased when purple sweet potato was added to their diets, and the number of Non-Smurfs increased by over three-fold (**Figure 1**). The S-values were eight-fold larger in the groups of flies with T2DM without PSPE supplementation (**Table 1**). There are a few important aspects to note about the data. The flies in the toxicity control, non-diabetic flies with PSPE supplementation, had the same average S-value as the flies in the negative control, non-diabetic flies without PSPE supplementation. This demonstrates that wild-type flies with and without PSPE naturally had strong guts. More toxicity control trials in the future would provide insight into if PSPE benefits the guts of already healthy flies. The gut permeability of the negative control, which represents wild-type flies without T2DM and without the PSPE supplementation, signifies how permeable Drosophila guts are in nature without the influence of external factors (**Table 1**). Furthermore, T2DM flies treated with PSPE, which are indicative of the experimental group, obtained lower gut permeability levels compared to the negative control, demonstrating how PSPE made Drosophila guts less permeable than they originally were (Figure 2). Despite these results, there are limitations that impede our research.

The main limitation of our research is the similarity between the *Drosophila* model and humans. T2DM can naturally occur in humans and not flies. Additionally, fruit flies are 60% similar to humans, with homologs for 75% of the genes that cause diseases in humans, so there will

be some discrepancies between the effectiveness of the PSPE treatment on T2DM in humans in comparison with the effect it had on Drosophila with T2DM (16). It is crucial to consider this factor when applying the results to humans. Furthermore, the relationship between anthocyanin content and decreasing gut permeability would be a logistic curve instead of an exponential one. Consequently, there will be an "ideal" concentration of anthocyanins that would best alleviate symptoms of increased gut permeability.

Other natural variables caused limitations and sources of error. The data displays that the latter half of the negative control groups have a significantly higher number of Smurfs (0.325 on average) compared to the first half (0.025 on average) (**Table 1**). This inconsistency is likely due to the effects of seasonal changes on the flies' lifestyles, such as their diets or mating patterns. Additionally, the age and amount of food each fly ate after being starved could have slightly varied within the various groups and affected the results, but this was not a major source of error. Since males have darker abdomens, it was more difficult to analyze gut permeability because the blue dye was hard to observe and quantify; consequently, females were used because of their light abdomen, making them easier to analyze with the Smurf Assay. Overall, only female flies were used in data collection due to the ease of observing their abdomens. Although there are physiological differences between the two, male flies are expected to have similar results, but using male flies would make the results more applicable to the entire population. Furthermore, there were varying trials between each group, and having an equal number of trials for all the groups would also strengthen the claims made (**Table 1**). It is crucial to remember that this research targets a major diabetic symptom.

The problem with T2DM that we aim to resolve is not the fact that there is increased insulin resistance but common anti-diabetic drugs increase gut permeability. Although anti-diabetic drugs are improving a major aspect of T2DM, they are also hurting patients by increasing the dispersion of toxins throughout the body as toxins can easily pass through the gut's loose intestinal walls (3). Purple sweet potato would provide an easily accessible way of reducing this increased gut permeability to accelerate the treatment process. Because of the positive impacts of purple sweet potato on flies with increased gut permeability induced through a diabetic diet, this research can help foster other research which can further analyze these effects into vertebrate species so that humans can benefit. Ideally, purple sweet potato would be used to supplement Metformin and other diabetic drugs for humans with T2DM.

Future work includes investigating the exact proportion of PSPE supplementation in human diets that is required to improve gut permeability, as it did in this study with flies. Additionally, analyzing foods with lower anthocyanin contents is another goal for the future. For example, dried fruits have substantially lower anthocyanins than fresh fruits and vegetables, and studying them would provide further insight into how many types of fruits and vegetables can be consumed to reduce gut permeability (18). This would dramatically improve the widespread nature of our research, as people would have more accessibility to natural treatments.

Furthermore, to increase the relevant audience of this study, this study can be expanded to type 1 diabetes as well, as gut permeability because of type 1 diabetes is also a major problem for patients (18). Additionally, this research served as a baseline to understand the effects of anthocyanins on increased gut permeability when it is part of a disease; however, a major goal is to implement antidiabetic drugs such as Metformin and testing the anthocyanin treatment on that to see how gut permeability is positively, or negatively, affected by anthocyanins.

Overall, our initial hypothesis was supported as implementing PSPE in the flies' diet led to decreased gut permeability. To conclude, purple sweet potato aided the T2DM flies' gut permeability dramatically, aligning with anthocyanins' anti-diabetic properties and current literature. Furthermore, other diseases, such as inflammatory bowel disease, irritable bowel syndrome, and severe acute pancreatitis, are all characterized by increased gut permeability (19). Therefore, a baseline for future research is offered regarding the utilization of anthocyanins to combat other overarching complications of diseases, such as T2DM.

MATERIALS AND METHODS

Fly Maintenance

Culture and Maintenance

Half of the vials had normal fly food, whereas the other vials had a high-glucose diet. Adult Canton S. wild-type *Drosophila* (Bloomington, Stock# 64349) were placed in enough vials for 10 flies per vial. The aim was to get at least four trials per group, leading us to test at least 40 flies per group. The toxicity control had 40 flies tested, the negative control had 80 flies tested, the positive control had 90 flies tested, and the experimental group had 60 flies tested.

Normal Fly Food

To prepare food for *Drosophila melanogaster*, the following ingredients were added to a 500 mL beaker. Using a 100 mL graduated cylinder and a pipette, 390 mL of distilled water was measured. A weight boat, scoopula, and the balance were used to weigh 6.75 g of yeast, 3.90 g of soy flour, 28.50 g of yellow cornmeal, 30 mL of light corn syrup, and 2.25 g of agar. Glass stir rods were used to mix these ingredients thoroughly. The mixture was boiled three times with periodic stirring to prevent overflowing. After that, 1.88 mL of propionic acid (mold inhibitor) was added when the mixture was 70°C. The mixture was poured into the necessary number of vials at 10 mL increments. The vials were then covered with cheesecloth, rubber bands, and a box to cool the food. After becoming solid and reaching room temperature, the vials were capped with the *Drosophila* Identi-Plug. For storage, the

vials were placed in the fridge until use.

Disposal of Drosophila

To dispose of the Drosophila, the vials were stored at -79°C for 2 minutes. After all the flies had died, they were thrown in the trash with the plug pushed into the vial tightly.

Diet

Induced T2DM - High-Glucose Diet

Of the total volume in the mixture, 20% was determined and converted to grams of glucose (20). The food was prepared using the recipe given in Section Normal Fly Food, but the glucose was added right after all other ingredients were added and before microwaving. Then, the remainder of the high-glucose diet was created according to the rest of the protocol Section Normal Fly Food. To confirm that the flies obtained diabetes and the glucose was reacting in their bodies as we expected it to, we ensured that they were more energized and jittery, along with being on the chubbier side.

PSPE Diet

The PSPE diet was prepared following the Normal Fly Food recipe, with a few modifications. After all the preliminary materials were added and stirred, the mixture was poured into a graduated cylinder to measure the volume in millimeters. Before microwaving the mixture, 10 mg of PSPE powder (Amazon, ASIN: B007Z91Q2A) was added. The concentration of PSPE per mL was determined by the toxicity assay that is described in Section Toxicity Assay. After microwaving, the remainder of the protocol was adapted from Section Normal Fly Food.

Diet with Bromophenol Blue Dye

To make the blue stock solution, 0.5 g of Bromophenol blue dye (Sigma-Aldrich, SKU B0126-25G) was measured with a scoopula, weigh boat, and balance (20). The dye was added to a flask with 75 mL of distilled water and combined with a stirring rod.

To prepare the food with the blue dye, a 100.00 mL graduated cylinder and a pipette were used to measure 359.58 mL of distilled water. The water was added to a 500 mL beaker. The normal fly food recipe from Section Normal Fly Food was followed, starting with the addition of yeast. After microwaving the food, the blue stock solution was added to the mixture. The remainder of the protocol was adapted from Section Normal Fly Food.

Experimentation

Toxicity Assay

A toxicity assay was done on various concentrations of PSPE to determine the safest and highest concentration that can be given to flies. The flies were first exposed to 1 mg of PSPE per mL of food. After the first exposure, 10-fold increments were used to increase the amount of PSPE. The flies had continued exposure to increasing amounts of PSPE until the amount of PSPE used killed 50% of the flies. Once this amount was reached, 10-fold less of that amount of PSPE was utilized in the diet as advised by our research mentor. For our toxicity assay, we started with 1 mg/mL of PSPE in the vial of toxicity assay flies. Then, we used a new vial with new flies exposed to 10mg/mL and, finally, a new vial with new flies with 100 mg/mL PSPE exposure, which is when 50% of the flies were killed.

Smurf Assay

At the start of the Smurf Assay data collection, all the flies were four days old. All flies were given their corresponding diet up until 17 hours before experimentation (21). Flies would start fasting at 3:30 PM, and at 8:30 AM (on the day of experimentation), the flies were given a diet with Bromophenol blue dye. These fasting times remained constant during experimentation. The flies were allowed to eat the new diet until data collection, which occurred between 9:15 AM and 10:45 AM.

Experimental Sorting

To collect the females for data collection, cold sorting was performed. Flies were left in their vials and placed in a bucket that was 75% full of ice. The flies were checked every 2 minutes to ensure they fell asleep. Afterward, they were taken out of the ice bucket. After turning the cold sorter (TECA LHP-1800CPV Liquid Cooled Thermoelectric Cold Plate, manufactured by ThermoElectric Cooling America Corporation) on and clearing the surroundings, the temperature of the sorter was adjusted to 1.5°C. Weigh paper was placed on the cold sorter with a small batch of flies on it. A magnifying glass and feather were used to sort the flies based on gender. After sorting one batch, the weigh paper was exchanged with a new one, and the sorting process was repeated until all batches were sorted. The sorted female flies could recover for 24-48 hours before data collection. No noticeable difference was observed between flies that recovered for 24 hours versus ones that recovered for longer. After the flies were sorted into vials, they were randomly assigned to testing groups.

Statistical Analysis

To test the significance of the effect of the implementation of PSPE on the gut permeabilities of a fly with T2DM, we performed nonparametric statistical tests. We performed Mann-Whitney tests on the positive control that had flies with T2DM without PSPE and the experimental group that exhibited flies with T2DM and PSPE (**Figure 1**). We performed a Kruskal-Wallis test on all groups (**Figure 2**). GraphPad InStat, version 3, software by Dotmatics allowed for the completion of the Mann-Whitney and Kruskal-Wallis tests. We used nonparametric tests instead of its corresponding parametric tests (two-tailed t-test and ANOVA test) due to the limited sample size. Furthermore, these tests use medians instead of means, meaning they are less affected by skewed

data as there were a few outliers present in the data.

ACKNOWLEDGMENTS

The authors wish to thank the Academies of Loudoun for giving us the necessary funding, equipment, and laboratory space to research.

Received: July 18, 2022
Accepted: August 23, 2022
Published: March 23, 2023

REFERENCES

1. Galicia-Garcia, Unai, *et al.* "Pathophysiology of Type 2 Diabetes Mellitus." *International Journal of Molecular Sciences*, vol. 21, no. 17, 2020, p. 6275. doi:10.3390/ijms21176275

2.Graham, P., and L. Pick. "Drosophila as a Model for Diabetes and Diseases of Insulin Resistance." *Current Topics in Developmental Biology*, 2017, pp. 397–419. doi: 10.1016/bs.ctdb.2016.07.011.

3. Borse, Swapnil P., *et al.* "Management of Type 2 Diabetes: Current Strategies, Unfocussed Aspects, Challenges, and Alternatives." *Medical Principles and Practice*, vol. 30, no. 2, 2020, pp. 109–21. doi: 10.1159/000511002.

4. Aw, Wanping, and Shinji, Fukuda. "Understanding the Role of the Gut Ecosystem in Diabetes Mellitus." J*ournal of Diabetes Investigation*, vol. 9, no. 1, 2017, pp. 5–12. doi: 10.1111/jdi.12673.

5. Gurung, Manoj, *et al.* "Role of Gut Microbiota in Type 2 Diabetes Pathophysiology." *EBioMedicine*, vol. 51, 2020, p. 102590. doi: 10.1016/j.ebiom.2019.11.051.

6. Gowd, Vemana, *et al.* "Anthocyanins as Promising Molecules and Dietary Bioactive Components against Diabetes – A Review of Recent Advances." *Trends in Food Science & Technology*, vol. 68, 2017, pp. 1–13. doi: 10.1016/j.tifs.2017.07.015.

7. Hair, Rachel, *et al.* "Anthocyanins, Microbiome and Health Benefits in Aging." *Molecules*, vol. 26, no. 3, 2021, p. 537. doi: 10.3390/molecules26030537.

8. Bonesi, Marco, *et al.* "The Role of Anthocyanins in Drug Discovery: Recent Developments." *Current Drug Discovery Technology,* vol. 17, no. 3, 2020, pp. 286-298. doi: 10.2174/1570163816666190125152931

9. Pang, Zhiqiang, *et al.* "Linking Plant Secondary Metabolites and Plant Microbiomes: A Review." *Frontier Plant Sciences,* vol 12, 2021, p. 621276. doi: 10.3389/fpls.2021.621276

10. Song, Haizhao, *et al.* "Black Currant Anthocyanins Improve Lipid Metabolism and Modulate Gut Microbiota in High-Fat Diet-Induced Obese Mice." *Molecular Nutrition & Food Research*, vol. 65, no. 6, 2021, p. 2001090. doi: 10.1002/mnfr.202001090.

11. Cao, Lei, *et al.* "Long-Term Blackcurrant Supplementation Modified Gut Microbiome Profiles in Mice in an Age-Dependent Manner: An Exploratory Study." *Nutrients*, vol. 12, no. 2, 2020, p. 290. doi: 10.3390/nu12020290.

12. Igwe, E. O., *et al.* "A Systematic Literature Review of the Effect of Anthocyanins on Gut Microbiota Populations." *Journal of Human Nutrition and Dietetics*, vol. 32, no. 1, 2018, pp. 53–62. doi: 10.1111/jhn.12582.

13. Neamtu, Andreea-Adriana, *et al.* "Bilberry (Vaccinium Myrtillus L.) Extracts Comparative Analysis Regarding Their Phytonutrient Profiles, Antioxidant Capacity along with the In Vivo Rescue Effects Tested on a *Drosophila melanogaster* High-Sugar Diet Model." *Antioxidants*, vol. 9, no. 11, 2020, p. 1067. doi: 10.3390/antiox9111067.

14. Bell, L., *et al.* "A Study of Glycaemic Effects Following Acute Anthocyanin-Rich Blueberry Supplementation in Healthy Young Adults." *Food & Function*, vol. 8, no. 9, 2017, pp. 3104–10. doi: 10.1039/c7fo00724h.

15. Han, Ying, *et al.* "Purple Sweet Potato Extract Extends Lifespan by Activating Autophagy Pathway in Male *Drosophila melanogaster*." *Experimental Gerontology,* vol. 144, 2021, p. 111190. doi: 10.1016/j.exger.2020.111190.

16. Mirzoyan, Zhasmine, *et al.* "*Drosophila melanogaster*: A Model Organism to Study Cancer." *Frontier Genetics,* vol 10, no 51, doi: 10.3389/fgene.2019.00051

17. Lohachoompol, Virachnee, *et al.* "The Change of Total Anthocyanins in Blueberries and Their Antioxidant Effect After Drying and Freezing." *Journal of Biomedicine and Biotechnology*, vol 2004, no 5, p. 248-252, doi: 10.1155/S1110724304406123

18. Zhou, He, *et al.* "Evaluating the Causal Role of Gut Microbiota in Type 1 Diabetes and Its Possible Pathogenic Mechanisms." *Frontier Endocrinology*, vol 11, 2020, doi: 10.3389/fendo.2020.00125

19. Fukui, Hiroshi. "Increased Intestinal Permeability and Decreased Barrier Function: Does It Really Influence the Risk of Inflammation?" *Inflammatory Intestinal Diseases*, vol. 1, no. 3, 2016, doi: 10.1159/000447252

20. Wang, Lijun, *et al.* "Purple Sweet Potato Anthocyanin Attenuates Fat-Induced Mortality in *Drosophila melanogaster*." *Experimental Gerontology*, vol. 82, 2016, pp. 95–103., doi: 10.1016/j.exger.2016.06.006.

21. Martins, Raquel, *et al.* "How to Catch a Smurf? – Ageing and Beyond…In Vivo Assessment of Intestinal Permeability in Multiple Model Organisms." *BIO-PROTOCOL*, vol. 8, no. 3, 2018. doi: 10.21769/bioprotoc.2722.