

Investigating the Effects of Aspartame, Sugar, and Stevia On *Lumbriculus Variegatus* Pulsation Rates

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ABSTRACT

The effect of sugar on our health has become well recognized, and this is often substituted by artificial sweeteners such as aspartame or stevia (at times regarded as a more natural product). Although the effects of sugar on our bodies continue to be well researched, there aren't as many studies testing the safety of these substitutes on our bodies. To understand this to a greater extent, California Blackworms (*Lumbriculus variegatus*) were utilized as the blackworms share certain systems with humans, including their circulatory system, which relates the Blackworm's pulsation rates and human's heart rate. This project used specific concentrations based on the experimental group solution type and placed 3 Blackworms, each for approximately 25 minutes, and compared each average of the pulsation rates with each other. The four groups recorded water, 3 sugar concentrations, 3 aspartame concentrations, and 3 stevia concentrations. After conducting these tests, it was found that by comparing the average pulsation rates of the baseline group to the other groups, the experimental group's average was higher. However, statistically, there was not a significant difference in pulsation rate between all of the groups. There are many reasons why this conclusion may have been reached, one being that there weren't enough replicates to fully test the hypothesis. Another limitation of this experiment was the higher concentrations, comparable to those consumed in the human diet, could not be tested due to its small scale. Therefore, we cannot thoroughly determine the physiological change regarding heart rates in human bodies through this.

Introduction

The impact of sugar on health is widely acknowledged, eventually leading up to the substitution of using artificial sweeteners, such as aspartame or stevia. The effects of sugar is frequently researched regarding its effects on the body, however, the safety of these substitutes are not as commonly discussed. This study aims to fill this gap by examining the effects of these sweeteners on a model organism, California Blackworms.

The main purpose of this experiment is to determine how sugar and artificial sweeteners impact heart rate in humans by comparing these effects to the pulsation rates in California Blackworms. These organisms were chosen specifically due to their similar circulatory system to the human body and transparent bodies allowing for an accurate measurement of pulsation rates. By testing specific concentrations of sugar, aspartame, and stevia on the Blackworms, this study will potentially provide insights on whether these common artificial sweeteners are more beneficial or harmful in comparison to sugar.

Comparisons

For this study, it was crucial to first determine what the connection between the California Blackworms and humans was. Through research, it was uncovered that the two share a closed circulatory system, meaning that their blood or hemolymph was contained in vessels. A large reason as to why California Blackworms were

chosen for this project was due to the dorsal blood vessel and ventral blood vessels being shown through the transparency of the worm's body wall. The blood is able to easily flow through the circulatory system majorly because of the pulsations along the dorsal blood vessel. As the body wall is transparent, the dorsal blood vessel is shown, meaning that it is easier to count the pulsation near the vessel. Another reason to why Blackworms are a model organism for this experiment is because of their simple body, meaning any substance would have an immediate impact on the worm, specifically their pulsation rate, due to quick diffusion in the skin. Just as any substance can control the worm's pulsation rate, a human's heart rate is controlled by their nervous system and endocrine system.

Specifically, *Lumbriculus variegatus*, commonly known as California blackworms, are known to be distant relatives of earthworms, living in shallow marshes, swamps, and ponds in Europe and North America. They mainly tend to feed on microorganisms and debris in their natural habitats. Blackworms are especially popular in various studies due to their translucent skin, giving researchers free access to view their systems and physiological changes. This can be extremely helpful for studies related to human cardiovascular health.

Sugar Substitutes

The two substitutes being used in this specific project include aspartame, which is commonly found in products such as diet drinks, and stevia in baked goods in order to "reduce" sugar intake. Sweeteners such as these are commonly used in society by certain individuals who wish to reduce their sugar intake, whether it be for weight loss or various health concerns associated with excessive sugar consumption. Aspartame, a low-calorie sweetener, is often marketed with products said to be "sugar-free" or diet-related. It is often made through two naturally occurring amino acids, aspartic and phenylalanine acid, also known to be parts of protein often seen in our food and bodies. On the other hand, stevia is obtained from *Stevia rebaudiana* plant, which is dried and then extracted through a certain process including hot water. Stevia is often used for products that wish for natural sweetening without the same calories of sugar.

The usage of sweeteners such as aspartame, stevia, and sugar has been shown to have significant consequences on human health as well as the environment. Sugar consumption is largely known to be linked to a wide range of health issues, leading to the promotion of alternatives such as stevia. As for aspartame, while it does provide sweetness without the same amount of calories as sugar, there has been recent debates regarding its safety on health conditions. Although, stevia is more known for its health benefits being a natural alternative, there are many questions regarding its environmental impact due to the large-scale cultivation of the actual plant.

Beyond the potential health risks these sweeteners may have, the production of all three sweeteners contribute to large environmental concerns. The production of sugar often involves thorough agricultural practices that can lead to issues such as deforestation, water pollution from pesticides, and habitat loss. Creating aspartame involves different chemical processes that produce emissions and waste that are harmful to ecosystems. Lastly, stevia, which is considered to be more environmentally friendly due to its connection to the plant, would still require a vast amount of land and water to grow the plant, creating challenges for sustainable agriculture practices.

Research Question and Hypothesis

The research question is whether normal sugar, also known as sucrose, would have a higher pulsation rate in comparison to the alternate sweeteners, aspartame, and stevia. It is hypothesized that sucrose would be the result of the highest pulsation rate of the California Blackworms, followed by aspartame, and lastly, stevia. The metabolism of regular sugar could release a possible influence of glucose and fructose on neurotransmitters, which

is correlated with neural activity. Compared to this, aspartame and stevia are both low in calories, showing that they may not increase the pulsation rates as high due to the minimal impact of metabolic processes and neurotransmitter release.

Literature Review

Previous studies and pieces of literature have examined the physiological impacts of substances, including caffeine and alcohol, yet the research specifically examining impacts of artificial sweeteners and sugar on biological systems still remains limited.

Experiments with the worms and caffeine have shown the blackworms becoming extremely active and alert due to stimulatory effects on the central nervous system. Similarly, research with alcohol, the worms begin to become inactive as concentrations increase. Even till current times, both of these substances continue to be studied in detail due to the large consumption of the two products.

However, research regarding the physiological effects of sweeteners has not been as popular, despite their widespread usage in food and beverages. For current studies for aspartame, its gained attention for potential health issues, yet experiments on its physiological effects often lacks the depth of research in comparison substances like caffeine and alcohol. Likewise, projects regarding stevia have been focused on taste perception and safety of consumption rather than the impacts on their cardiovascular health in specific.

Methodology

The experimental setup began with maintaining environmental conditions: keeping the worms in low-light areas and room-temperature habitats. The water in which the blackworms are kept should be below 20 degrees Celsius. Three different sets of test tubes were prepared, containing a wide range of concentrations of sugar (0.5%, 2%, and 5%), aspartame (0.05%, 0.2%, and 0.5%), and stevia (0.02%, 0.1%, and 0.5%), alongside a control group with distilled water.

To determine a baseline pulsation rate, the pulsation rate of 3 to 4 blackworms was measured to establish a reference for the other groups. Subsequently, approximately 10 blackworms were placed into each test tube and exposed to the concentrated solutions for 25 minutes, during which a timer was set, and observations were noted. After 25 minutes, individually transfer 3 to 4 blackworms from the different concentrated solutions into the capillary tubes. These tubes were used to limit the movements of the worms in order to measure the pulsation rates under a dissecting microscope. The data collected included various pulsation rates from blackworms across experimental groups, aiming to determine any significant differences influenced by the different sweetener concentrations.

Throughout the experiment, detailed records were kept of any type of blackworm behavior and any specific changes in their physiological responses during the time of exposure. Statistical analysis of the data collected largely focused on comparing the average pulsation rates of each concentrated groups to each other, including the control group. Through this, the aim is to identify trends and potential impacts of each sweetener type on cardiovascular-related responses in the model organism.

Results and Discussion

After the exposure of California Blackworms to the various concentrations of sugar, aspartame, and stevia, significant observations were made in regards to their pulsation rates. Firstly, comparing the average pulsation rates of the blackworms exposed to sugar, aspartame, and stevia were significantly higher compared to the control group. Specifically, the average pulsation rates ranged from 10.67 beats per minute (bpm) in the control

group to 18.67 bpm for the sugar group, 17.67 bpm for the aspartame group, and 19.50 bpm for the stevia group. The averages increase from the control group of water to aspartame, sugar, and then stevia. While these results suggest an apparent increase in pulsation rates with exposure to sweeteners, statistical analysis revealed that the differences were not statistically significant when considering the standard error of the experiment.

The observed increases in pulsation rates across the experimental groups may suggest a potential physiological response of the blackworms in the presence of the sweeteners. However, the overlap in standard error bars in the data indicates that the variations observed may actually not be that significantly different from each other or from the control group.

Limitations

Some of the limitations in this study included the fact that human diets tend to contain much higher concentrations of sugar in comparison to the concentrations the blackworms were given. This is because blackworms can only withstand a certain amount of sugar before they pass away, therefore, the low concentration given. Due to this, the wrong conclusion may have been reached, as humans wouldn't just consume that little amount of concentrated sugar. It is only when you consume large amounts of the sweeteners that your heart rate or pulsation rate would be affected.

Conclusion

As seen through the graph provided above, the actual difference between the control group and experimental group was not that spread apart; instead they were quite close. This conclusion may not be factually correct as it could have been the outcome of many experimental errors. One cause may have been the lack of enough replicates of the experiment, resulting to an incorrect outcome. There were also many challenges and limitations along the way, which led to this final conclusion. So although the averages of the pulsation rates claim that the rates grew drastically, in reality, it is discovered that not much of a difference is made.

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