**Does Pseudoephedrine Stunt Growth In *Caenorhabditis Elegans*?**

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**Abstract**

Attention Deficit Hyperactivity Disorder (ADHD) is a disorder commonly diagnosed in children. Stimulants are the most common medicine used to treat ADHD. Stimulant medication used to treat ADHD in children has been suspected to stunt growth. Pseudoephedrine is a decongestant used to treat colds, but is also a stimulant similar to the medicines used to treat ADHD. Using *Caenorhabditis elegans (C. elegans)* as a model for humans, tests were done to determine if growth was affected by the use of pseudoephedrine. The tests consisted of administering pseudoephedrine to the *C. elegans* once daily, observing their movement and growth, measuring them, and recording the data collected from those trials. The data was run through statistical analysis and comparisons were made. The average length of the group treated with pseudoephedrine was 7.06% shorter than the control group. Overall the data comparisons were cohesive with the hypothesis that the use of pseudoephedrine negatively affects growth.

**Keywords**

*Pseudoephedrine, Caenorhabditis elegans, Attention Deficit Hyperactivity Disorder, Stimulant.*

**Introduction**

Some symptoms that are observable in children with Attention Deficit Hyperactivity Disorder (ADHD) are fidgeting, trouble focusing, incomplete tasks, forgetfulness, and trouble with organization. Having more than one of those symptoms is usually an indication of ADHD. Children and adults are affected by ADHD, but are usually diagnosed at a younger age. There are multiple treatments for ADHD, but stimulant drugs, such as methylphenidate, are the most popular (Safren, *et al.*, 2005).

Ever since the medical community developed medication to help children with ADHD there have been questions of how it affects a child's health, more specifically their growth. Many parents have noted that their children seem lethargic, have a low appetite, and seem to have less energy (Powell, *et al.*, 2015). The question being asked was, does ADHD medication stunt growth (Harstad, *et al.,* 2014)? If this is the case, children who have ADHD, and are taking stimulant medication, would benefit from information gathered from this study.

Methylphenidate is a stimulant medication specifically used to treat ADHD. Similar to methylphenidate, pseudoephedrine is a stimulant medication, but it is commonly used to treat head colds. Regardless of what the medication is treating, stimulants are

medications that stimulate the central nervous system and enhance neural activity, producing increased alertness and awareness. As a result, stimulant medications have proved to be successful in treating children with ADHD, who have a lack of focus, by improving their ability to focus (Wilens & Spencer, 2000). In this project, pseudoephedrine will be replacing methylphenidate as a stimulant. Since methylphenidate requires a prescription and could therefore not be obtained, pseudoephedrine was used instead.

*Caenorhabditis elegans* are small nematode worms that are commonly used as a model organism for humans. *C. elegans* are frequently used as model organisms due to the fact that they are easily observable under a microscope, and they have a life cycle of two to three weeks. In addition, many of the molecular signals within the *C. elegans* are similar to ones found in humans, and the genes found in the *C. elegans* are comparable to genes in the human body (Sommer & McGaughran, 2013).

This study will focus on how pseudoephedrine affects the growth of *C. elegans* over time. The impact of this study will be determining the effects the pseudoephedrine has on growth. If the impact is positive, negative, or null, the results can give more insight into how pseudoephedrine impacts the human body, specifically pertaining to how stimulants affect the growth of children with ADHD. The overarching question for this study is: Does pseudoephedrine stunt growth in *Caenorhabditis elegans*? The hypothesis was that pseudoephedrine will have a negative effect on the growth of *Caenorhabditis elegans* by stunting their growth and making them 5% shorter than those not given the pseudoephedrine.

**Methodology**

This study was conducted in a BSL 1 lab at a southwest Missouri high school. Thirty petri dishes were set up for the *C. elegans*. They were made with nematode growth agar and *Escherichia coli* (*E. coli*) K12 broth culture was added to serve as a food source. When preparing these plates, the correct safety gear was worn: gloves, goggles, and a face mask. The growth agar was poured into petri dishes after being heated on a hot plate. The *E. coli* K12 broth was administered to the petri dishes with a pipette. Proper disposal methods for the pipettes were used. The *C. elegans* were transferred onto the petri dishes by being cut from their original agar with a sterile disposable scalpel. The *C. elegans* were then put into incubation at a temperature of 16° C for each day of experimentation. Each newly prepared petri dish received a 1cm by 1cm block of agar with an estimated amount of four to five *C. elegans*. Once the *C. elegans*, *E. coli* K12 broth, and growth agar were present on the petri dish, the petri dishes were moved into

incubation for five days before the other variables were introduced.

The pseudoephedrine water dilution solution was then made. Under a fume hood, while using a respirator to avoid breathing in any harmful substances, pseudoephedrine was crushed with a mortar and pestle and transferred to a sterilized weigh dish. The crushed, singular 30 mg pseudoephedrine tablet was then mixed into six gallons (22.71247 L) of spring water, in a sterilized tank. Once the medication was dissolved, 10 ml was taken and put into two sterilized test tubes, 5 ml per tube. These were labeled and placed aside for later use.

For the five days after the initial incubation, the *C. elegans* were observed throughout their growth period to determine when the medication should be introduced (Sommer & McGaughran, 2013). The plates, after the first 2-3 initial days of observation, had notable amounts of *C. elegans*; they had rapidly multiplied. The study was looking at the length of the individual *C. elegans*. To make the individual *C. elegans* more easily observable the number of *C. elegans* per plate was lowered using sterilized plastic inoculating loops. After reducing the number of *C. elegans* on the plates to 1-3 per plate, they were observed and the initial measurements were taken.

The plates containing *C. elegans* were placed into an incubator at a temperature of 16° C every day after the initial measurement. Placing the *C. elegans* in an environment that is 16° C slowed the growth period, so that there was a longer period of observation (Girard, *et al.*, 2007). The *C. elegans* were divided into two groups: The control group and the group that would receive the pseudoephedrine and water dilution. There were 30 total plates- 15 control and 15 with the pseudoephedrine and water dilution. The *C. elegans* were treated with the pseudoephedrine and water dilution, once daily for five days. Before the dilution was administered every day, the *C. elegans* were observed and measured (**Figure 1**). The *C. elegans* initially were 0.50 mm or slightly less in length.

**Figure 1**. Examples of nematode worms on petri dishes prior to study (Photo taken by Isabella Sotlar).

The observation, measuring, and administration of the pseudoephedrine dilution continued for four days. The observation of the *C. elegans* was conducted under a compound light microscope, using a transparent metric ruler. The plates were taken out of the incubator and uncovered, placed under the microscope, and observed. *C. elegans* were identified on the plates each day, and were measured (**Figure 2**). This process was repeated for four days. For each of the four days, after the observation and measurement, the pseudoephedrine dilution was added using a pipette to the 15 plates of the drug trial group. After receiving the treatment, the *C. elegans* were then placed back into incubation.

Once the five total days of experimentation were completed, the *C. elegans* were autoclaved. All surfaces that came into contact with the *C. elegans* throughout the study were cleaned with 70% ethanol. The pipettes themselves were cleaned with 70% ethanol as well. The *E. coli* K12 broth was also autoclaved to prevent contamination.

**Results**

As mentioned, there were thirty total petri dishes. Fifteen plates containing three to four *C. elegans* each were in the control group. Fifteen plates containing between three to four *C. elegans* were also in the group that was treated with the pseudoephedrine and water dilution. There are thirty measurements, although there were more worms. The worms that were measured were all taken from a plate with newly hatched eggs. They were all initially 0.50 mm and below in length. The other *C. elegans* that were left on each plate were much larger and therefore were not included in the study.

The *C. elegans* left tracks in the agar while they moved in the dish. During daily observation, the tracks they left were traceable to each *C. elegans*. The measurements were recorded daily for four days. After factoring in the initial measurements, there was a total of five days' worth of data. All data was recorded in a table represented in **Figure 2**.

**Treatment vs *C. elegans* Length**

| | Initial Worm Length (mm) | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|---|
| Worm 1 | 0.5 | 1 | 1.25 | 1.5 | 1.5 |
| Worm 2 | 0.5 | 1 | 1.25 | 1.5 | 1.5 |
| Worm 3 | 0.5 | 0.75 | 1 | 1 | 1.25 |
| Worm 4 | 0.5 | 0.75 | 1 | 1.25 | 1.5 |
| Worm 5 | 0.5 | 1 | 1.25 | 1.5 | 2 |
| Worm 6 | 0.5 | 0.5 | 0.75 | 0.75 | 1 |
| Worm 7 | 0.5 | 0.75 | 1 | 1 | 1.5 |
| Worm 8 | 0.5 | 0.75 | 0.75 | 1 | 1.5 |
| Worm 9 | 0.5 | 0.75 | 1 | 1 | 1 |
| Worm 10 | 0.5 | 1 | 1 | 1.25 | 1.5 |
| Worm 11 | 0.5 | 1 | 1.5 | 1.75 | 2 |
| Worm 12 | 0.5 | 0.75 | 1 | 1.5 | 1.5 |
| Worm 13 | 0.5 | 0.75 | 0.75 | 1 | 1 |
| Worm 14 | 0.5 | 1 | 1 | 1.5 | 1.5 |
| Worm 15 | 0.5 | 0.75 | 0.75 | 1 | 1 |
| Worm 16 | 0.5 | 0.75 | 1 | 1.25 | 1.5 |
| Worm 17 | 0.5 | 0.75 | 1 | 1 | 1.25 |
| Worm 18 | 0.5 | 0.75 | 0.75 | 1 | 1 |
| Worm 19 | 0.5 | 1 | 1.25 | 1.5 | 1.5 |
| Worm 20 | 0.5 | 0.75 | 0.75 | 1 | 1 |
| Worm 21 | 0.5 | 0.75 | 1 | 1 | 1 |
| Worm 22 | 0.5 | 1 | 1 | 1.25 | 1.5 |
| Worm 23 | 0.5 | 0.75 | 1 | 1 | 1 |
| Worm 24 | 0.5 | 0.75 | 1 | 1.25 | 1.5 |
| Worm 25 | 0.5 | 1 | 1.25 | 1.5 | 1.5 |
| Worm 26 | 0.5 | 0.75 | 1 | 1 | 1.25 |
| Worm 27 | 0.5 | 1 | 1.25 | 1.5 | 1.5 |
| Worm 28 | 0.5 | 1 | 1.25 | 1.5 | 1.5 |
| Worm 29 | 0.5 | 0.75 | 1 | 1.25 | 1.25 |
| Worm 30 | 0.5 | 1 | 1.25 | 1.5 | 1.5 |

**Figure 2.** All the *C. elegans* measurements were recorded in this table. The numbers 1-15 represent the control group, and the numbers 16-30 are the group that was treated with the pseudoephedrine water dilution (Figure created by Isabella Sotlar).

The average length of *C. elegans* in the control group was 1.4167 mm. The average length of the group treated with pseudoephedrine was 1.3167 mm. The comparison between the averages is demonstrated in **Figure 3**. The group that was treated with pseudoephedrine was 7.06% shorter than the control group. This supports the hypothesis of a projected 5% decrease in size. The median for the control and the pseudoephedrine treated group was the same (1.50mm), and the mode was 1.50 mm. The control had a range of 1.00 mm and the group treated with pseudoephedrine had a range of 0.50mm. A T-test was also conducted on the final collected data. The p-value was 0.05. The p-value in this study shows whether or not the results were significant enough to have had a true impact. Error bars are also graphed and shown in **Figure 3**.

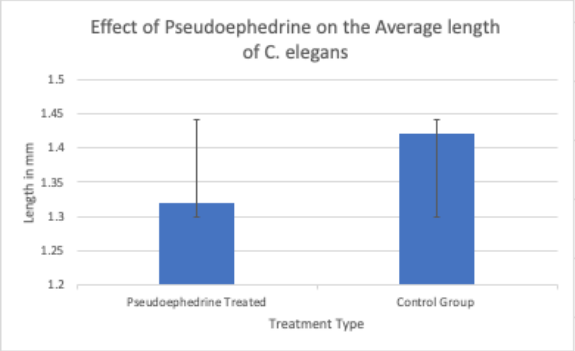


**Figure 3.** The average for the control group of *C. elegans* was compared to the average for the group of *C. elegans* treated with pseudoephedrine. The error bars represent the variability or possible error in the data (Graph created by Isabella Sotlar).

**Discussion**

The most important finding in this study was that the pseudoephedrine did in fact stunt the growth of the *C. elegans*. The average length of the *C. elegans* that were treated with the pseudoephedrine was 7.06% shorter than the

group that had no added treatment. However, the p-value for this study was 0.05, 5% shorter. To be statistically significant the p-value would have needed to be 16% or higher. Therefore, even though there was a 7.06% difference in length, statistically, that was not a large enough variance to deem that the pseudoephedrine made a large impact on the growth of the *C. elegans*.

The study, *Effect of Stimulants on Growth of ADHD Children: A Critical Review* (Ptacek, *et al.*, 2009) produced results that showed that over time the children who were taking stimulant ADHD medication had decreased height and loss of appetite, which is what they suspected caused the growth deficit. Comparatively, this current study saw similar results. It is acknowledged that the eating habits of the *C. elegans* were not a variable that was measured. Their length was tracked and recorded. The length of the *C. elegans* was negatively affected by the pseudoephedrine. The results of the experiment did support the hypothesis of a negative change in growth, and although the numerical value was not exact to what was hypothesized, it was very close.

**Conclusion**

To obtain a greater knowledge of the effects of stimulants on the growth of children taking stimulant medication, multivariate and longitudinal data would need to be collected from those taking specific stimulants. For ethical compliance, the data would come from patients taking a stimulant medication as part of a normal course of treatment. Although initial studies show that children on stimulants eventually catch up with their peers in terms of growth, the National Institute of Health (NIH.gov) reports that this research is lacking due to insufficient follow-up on the children's final, adult heights.

**Limitations**

There were numerous limitations within this experiment, some of which were the *C. elegans* themselves. There was a large amount of *C. elegans,* which was unexpected. When reading about the *C. elegans* and looking over the order, there was a projected amount of between 25-50 in the initial plate. When those *C. elegans* were seeded, they reproduced within days, which in turn resulted in hundreds of *C. elegans*. This was an issue due to the fact that each plate needed to contain only 1-3 *C. elegans*. To amend this complication, the plates were swiped with inoculating loops, to lower the number of *C. elegans* on each plate. Another limitation was the inability to procure methylphenidate, a stimulant meant solely for treating ADHD. This would have allowed for a more solid comparison to the ADHD studies. I would also say that the inability to get smaller measurements was a limitation. The *C. elegans,* due to their small size, could only be measured to a certain size on the measurement tool used. A method to get smaller, more precise

measurements could have provided for a more accurate measurement.

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**References**

Girard, L. R., Fiedler, T. J., Harris, T. W., Carvalho, F., Antoshechkin, I., Han, M., … Chalfie, M. (2007). WormBook: the online review of Caenorhabditis elegans biology. *Nucleic Acids Research*, *35*(Database), D472–D475. https://doi.org/10.1093/nar/gkl894

Harstad, E. B., Weaver, A. L., Katusic, S. K., Colligan, R. C., Kumar, S., Chan, E., … Barbaresi, W. J. (2014). ADHD, Stimulant Treatment, and Growth: A Longitudinal Study. *PEDIATRICS*, *134*(4), e935–e944. https://doi.org/10.1542/peds.2014-0428

Powell, S. G., Frydenberg, M., & Thomsen, P. H. (2015). The effects of long-term medication on growth in children and adolescents with ADHD: an observational study of a large cohort of real-life patients. *Child and Adolescent Psychiatry and Mental Health*, *9*(1). https://doi.org/10.1186/s13034-015-0082-3

Ptacek, R., Kuzelova, H., & Paclt, I. (2009). Effect of Stimulants on Growth of ADHD Children: A Critical Review. *Activitas Nervosa Superior*, *51*(4), 140–146. https://doi.org/10.1007/bf03379556

Safren, Otto, Sprich, Winett, Wilens, Biederman, 2005, Cognitive-behavioral therapy for ADHD in medication-treated adults with

continued symptoms, Behaviour Research and Therapy, Volume 43, Issue 7 https://doi.org/10.1016/j.brat.2004.07.001.

Sommer, R. J., & McGaughran, A. (2013). The nematodePristionchus pacificus as a model system for integrative studies in evolutionary biology. *Molecular Ecology*, *22*(9), 2380–2393. https://doi.org/10.1111/mec.12286

Wilens, Spencer (2000), The Stimulants Revisited, Child and Adolescent Psychiatric Clinics of North America, Volume 9, Issue 3, Pages 573-603, https://doi.org/10.1016/S1056-4993(18)30108-1.

Wasantwisut, E. "Nutrition and Development: Other Micronutrients' Effect on Growth and Cognition." *The Southeast Asian Journal of Tropical Medicine and Public Health*, 199