

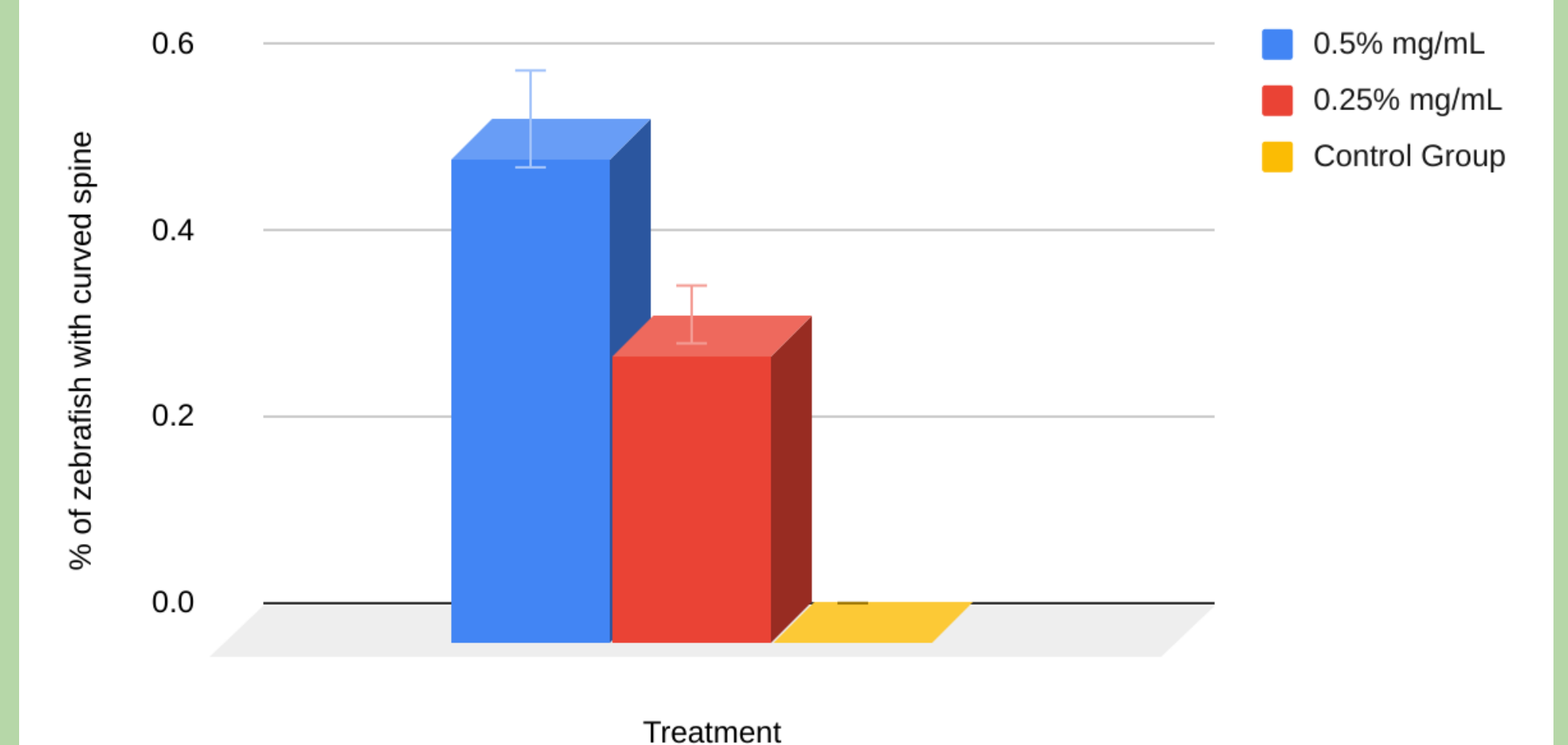
Abstract

Caffeine is a common substance found in many beverages around the world. This experiment is important because it shows if it is unhealthy for pregnant women to consume large amounts of caffeine and what it will do to the baby. The experiment showed how caffeine affects the development of a zebrafish embryo. Zebrafish were used in this experiment because their eggs develop outside of their body. They also have the same major organs and have transparent embryos, making it easier to see development. For the experiment, we used a plate with wells and divided it into different sections, with each containing a different amount of caffeine concentration, and one containing no caffeine. We placed around 10 embryos in each well and left them there for 72 hours, checking on them once a day. The results showed that the embryos placed in the higher concentration (0.5 mg/mL) had an average of 52% zebrafish with a curved spine, while the lower concentration (0.25 mg/mL), and the control treatment had no zebrafish with a curved spine. In conclusion, caffeine does have a negative effect zebrafish development and more experiments to prove this could be done.

Introduction

Caffeine is a stimulant that increases alertness for a short amount of time (Cox). It is most commonly found in tea and coffee. It increases blood adrenaline levels and brain activity of neurotransmitters norepinephrine and dopamine (Petre). Pregnant women who drink 50 - 149 mg of caffeine daily have a 13% higher risk of low birth weight, and pregnant women who drink more than 200 mg of caffeine have a higher chance of miscarriage (Meixner). There are many similarities between a zebrafish embryo and a human fetus, which is why they will be used in this experiment. (Close). In this investigation, zebrafish will be exposed to different amounts of caffeine. The first group of fish was placed in a solution containing no caffeine, the second group was placed into a solution containing 0.25 mg/mL caffeine, and the third group was placed into a solution containing 0.5 mg/mL caffeine. The zebrafish stayed in the solutions for 96 hours and were checked daily to replace the solution and the amount of alive fish was recorded. Zebrafish exposed to higher concentrations of caffeine will show more spine curvature, while zebrafish exposed to no caffeine won't show any.

The Effect of Caffeine on Average Percent of Zebrafish With Curved Spine After 72 Hours



This bar graph shows that there is a higher average of zebrafish with a curved spine in the treatment group than in the control group. The 0.5% mg/mL treatment had a higher average of zebrafish with curved spine than the 0.25% mg/mL treatment. The control group showed no spine curvature.

Materials and Methods

- Bottle of stock solutions of caffeine (0.25 ml and 0.5 ml)
- Beaker for liquid disposal and dead embryos
- Bottle of instant ocean
- Sharpie
- 2 disposable pipettes
- Plate with wells
- 28.5 degrees celsius incubator
- Microscope
- Phone for pictures

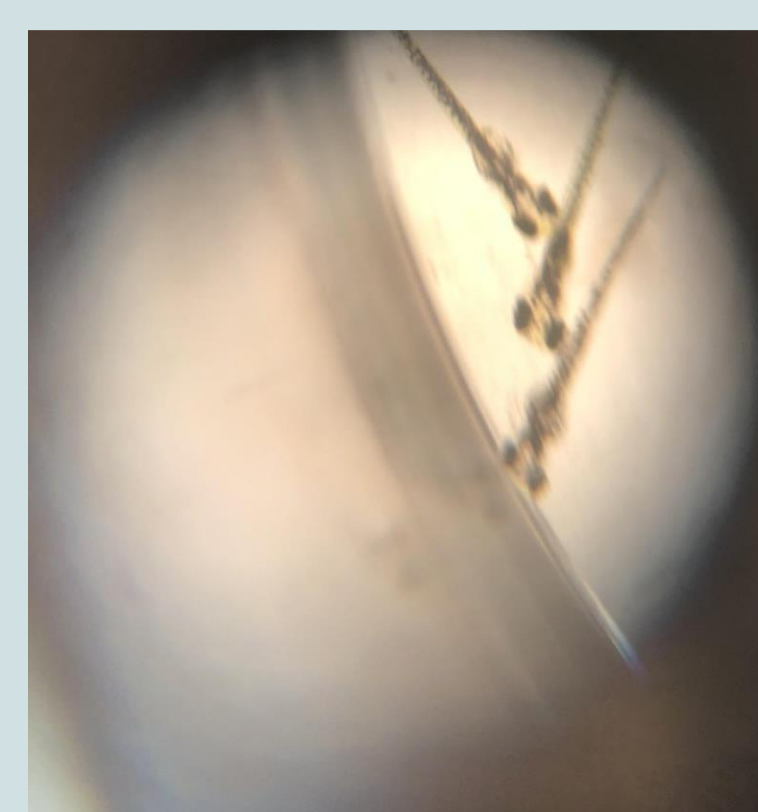
First Day: We received the zebrafish embryos for the experiment. We received a plate with wells and labeled it with our names. Next we divided the plate into 4 sections. The 2 solutions of 0.25% and 0.5% caffeine concentration were created. Using a disposable pipette, around 10 zebrafish eggs were added to each well.

Second Day: After 24 hours the number of alive embryos was recorded using a microscope. The instant ocean solution that was in each well was removed, and then replaced by the correct solution. The solution in column 1 contained 0.25% caffeine concentration, and column 2 contained 0.5% caffeine concentration. Rows 3 and 4 contained no caffeine and only instant ocean solution. The old solution was put into a beaker for disposal.

Third and Fourth Day: The plate was put into a 28.5 degrees celsius incubator and was taken out 48 hours post fertilization and 72 hours post fertilization. During the time it was out, the solution was replaced and the number of alive zebrafish was recorded. Pictures were also taken using a phone and microscope.

Results

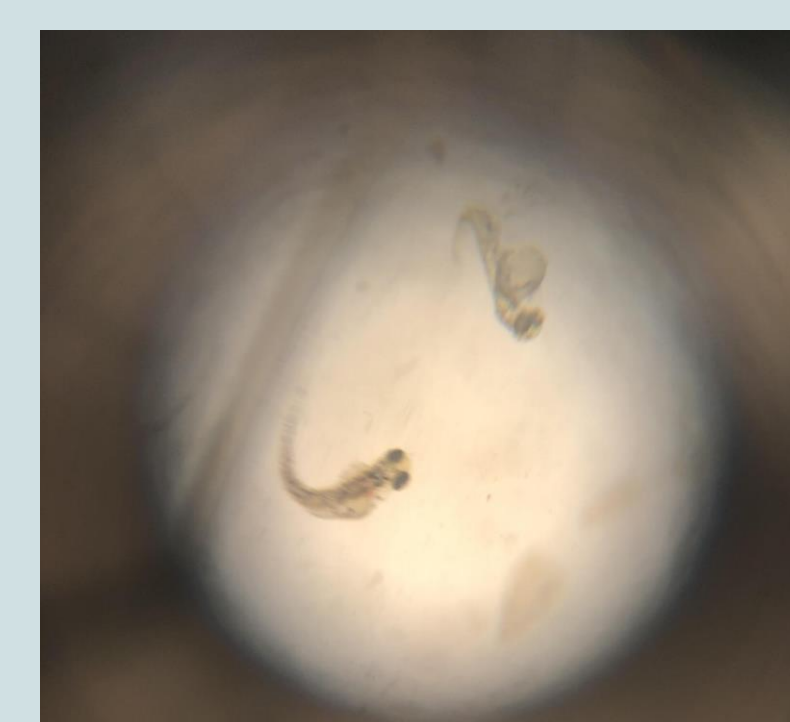
The results showed that zebrafish placed in an experimental group would have a higher chance of getting a curved spine than a zebrafish in the control group. To check my statistical significance, I performed a t-test. The p- value was 0.0065 and showed that the difference between the control group and 0.5% mg/mL treatment was statistically significant. The results indicate that caffeine does have an effect on the development of zebrafish. The independent variable was the amount of caffeine that te zebrafish were exposed to and the dependent variable was the amount of zebrafish that had a curved spine. The control group was the zebrafish not exposed to any caffeine.



No caffeine



0.25 mg/mL caffeine



0.5 mg/mL caffeine

No zebrafish had spine curvature in the control group. 31% of zebrafish had spine curvature in the 0.25 mg/mL treatment after 72 hours. 52.22% of zebrafish had spine curvature in the 0.5 mg/mL treatment after 72 hours

Discussion

This experiment was done to show how caffeine can affect the development of zebrafish embryo. The data collected from the experiment supports the hypothesis. After 72 hours, zebrafish that were exposed to caffeine showed some amount of spine curvature. 52.22% of zebrafish placed in the 0.5% treatment had spine curvature and 31% of zebrafish placed in the 0.25% treatment had spine curvature. Caffeine affects the development of zebrafish embryos, therefore human fetuses can also be affected by caffeine.

The findings are important because it shows a correlation between caffeine and embryo development. The results from my experiment were similar to other experiments, one experiment tested different drugs, including caffeine, on zebrafish and also ended up with spine curvature in the zebrafish. (Lantz-McPeak) The limitations and errors in my project were that some zebrafish would be accidentally disposed when replacing the solution.

Reference

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