THE EFFECT OF CAFFEINE ON THE MORTALITY AND HATCH RATE OF ZEBRAFISH

Madelyn Chapman
Pardeeville High School

Abstract:
This experiment was done to test the effects of caffeine on developing zebrafish. Zebrafish are model organisms commonly used in scientific research because the embryological development of this species and humans are very similar. For five days, zebrafish were placed in wells of three different columns. Column one contained the Embryo Media Solution and was used as the control. Columns two through four contained different concentrations of caffeine. The zebrafish were observed over the five days and the number hatched and number dead were recorded. The results showed that high and low concentrations of caffeine proved to have a statistically significant effect on the hatch rate. There were many birth deformities present within all the concentrations. Knowing what caffeine can do to developing zebrafish embryos, humans can understand the effects of consuming caffeine while pregnant and its affect on developing human embryos.

Introduction:
There are many people that have a dependence and an increased liking of coffee. Coffee along with many other liquids such as soda and tea have caffeine. There is a major misconception when discussing caffeine: individuals feel caffeine has more beneficial effects than harmful effects. In reality, caffeine, if consumed frequently in large portions, can cause many health problems such as insomnia, an upset stomach, irritability, anxiety, a fast heartbeat, and even muscle tremors. These health effects, caused by consumption of caffeine, can be even more harmful to a human embryo during pregnancy. In a recent study reported on by Medical News Today, researchers supported the statement that if pregnant women consume 300 mg of caffeine a day it may increase the risk of low birth weight babies and early death rate (Whiteman, 2015). Caffeine was tested to try to understand the effects of this drug on human embryos and their development by using Danio rerio, conventionally known as zebrafish.

Zebrafish are freshwater fish that are commonly used in scientific research. These model organisms are used to study diseases among humans as these organisms share similarities. 70% of genes found in humans are also found in zebrafish and 84% of human disease-causing genes are also found in zebrafish (Animals in research: zebrafish, 2013). The embryological development of zebrafish is very similar to that of humans, making these organisms an ideal species to test and compare a drug’s effect on the development of embryos. With the research gained one can then infer the effects of a drug on human embryo development. Zebrafish also provide a surplus of embryos that can be used for scientific testing. The developmental rate of zebrafish is favorable as one test can be completed in a week, instead of months. This
experiment along with many others help further explain the need for public health and medical research.

It was hypothesized that if embryos were exposed to caffeine in the early developmental stages, then the drug would cause birth deformities and an increase in hatch rate among zebrafish.

**Materials and Methods:**

The materials used in this experiment were: zebrafish embryos, a beaker for liquid disposal and dead embryos, a sharpie, embryo media solution, stock solutions of caffeine (0.05 mg/mL, 0.25 mg/mL, 1 mg/mL), large bore transfer pipette (1.5 mm), well plate, and a dissecting microscope. Throughout this experiment, gloves and goggles should be used when handling caffeine. All of the materials were provided by the Wisconsin Inquiry-based Scientist-Teacher Education Partnership (WInSTEP) Program, which is part of the NIH Science Education Partnership Award (SEPA) Program administered by the University of Wisconsin–Milwaukee and the Children's Environmental Health Sciences Core Center.

This experiment was done over a week’s time. The first day the spawning tank was set up in preparation for this experiment. The rinsed embryos were received on day 2 and the well plate was labeled. Column 1 was filled with the embryo media solution using a transfer pipette. In A1 there were 8 embryos, in B1 there were 9 embryos, and in C1 there 10 embryos. This step was repeated in column 2, but instead the 0.05 mg/mL concentration of caffeine was used. There were 9 embryos in A2, 10 embryos in B2 and 10 embryos in C2. Column 3 was filled with 0.25 mg/mL of caffeine and there were 10 embryos in A3 and B3, but there were 9 embryos in C3. Then, column 4 was filled with 1 mg/mL of caffeine and there were 10 embryos in A4, while there were only 9 embryos in B4 and C4. The next day (Day 3), the embryos were looked at under a microscope to acquire qualitative and quantitative data to understand the drug’s effect. Each day, the dead embryos were counted, removed and placed in a beaker for disposal. The number of hatched embryos and living embryos were recorded. The embryos were examined under a dissecting microscope. The embryo media solution along with the different caffeine solutions were replaced by new embryo media solution and caffeine solutions using a transfer pipette. These steps were repeated for the remainder of the week.

To analyze the data, a statistical t-test was used (GraphPad software) to understand the statistical significance of the results.
Results:

In this experiment, the independent variable was the caffeine and the dependent variable was the hatch rate and mortality rate of the zebrafish embryos. The control was in column 1 of the well plate and was only filled with Embryo-Media Solution. The lowest concentration (0.05 mg/mL) was in column 2, the medium concentration (0.25 mg/mL) was in column 3, and the highest concentration (1 mg/mL) was in column 4. The hypothesis was that if developing zebrafish embryos were exposed to caffeine, then the zebrafish would have a slower hatch rate and would acquire birth de.

The results showed that the amount of caffeine had a statistically significant effect on the hatch rate of zebrafish, but not the mortality rate of zebrafish. All concentrations proved to not have a statistically significant effect on the mortality rate of zebrafish. The concentration (0.05 mg/mL) and the high concentration (1 mg/mL) were shown to slow hatch rate. The low and high concentrations allowed little to no hatchings. However, the highest concentrations had the greatest affect on the mortality and hatch rate of zebrafish. There were developmental deformities, especially deformed spines, that were caused by the exposure to different concentrations of caffeine. The images displayed explains the birth defects among zebrafish placed in high concentrations of caffeine.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Well 1</th>
<th>Well 2</th>
<th>Well 3</th>
<th>Average</th>
<th>Standard Deviation</th>
<th>Probability</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>9</td>
<td>0.82</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1 mg/mL</td>
<td>4</td>
<td>8</td>
<td>7</td>
<td>6.333</td>
<td>1.69 p=0.1161</td>
<td>Not Significant</td>
<td></td>
</tr>
<tr>
<td>0.25 mg/mL</td>
<td>10</td>
<td>10</td>
<td>9</td>
<td>9.667</td>
<td>0.47 p=0.3739</td>
<td>Not Significant</td>
<td></td>
</tr>
<tr>
<td>0.05 mg/mL</td>
<td>9</td>
<td>10</td>
<td>10</td>
<td>9.667</td>
<td>0.47 p=0.3739</td>
<td>Not Significant</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1:** Final Number of Live Fry. This table shows the number of live fry throughout the entire experiment in the control, low concentration, medium concentration, and high concentration.
Figure 1: Averages for Caffeine Exposures and Final Number of Live Fry. This graph shows the mortality rate among zebrafish exposed to caffeine. The graph also shows the increase in mortality rate for the high concentration (1 mg/mL).

Table 2: Final Number of Hatched Zebrafish. This table shows the number of hatched zebrafish throughout the entire experiment.
**Figure 2:** Average Caffeine Exposure and Final Number of Hatched Fish. This graph shows the number of hatched zebrafish. When compared to the control, the decrease in hatch rate is very noticeable for all concentrations, especially 1 mg/mL concentration.

![Average Caffeine Exposure and Final Number of Hatched Fish](image)

**Figure 3:** A Deformed Zebrafish in the Low Concentration of Caffeine. The figure shows that the spine of the zebrafish is curved and the fish is noticeably smaller/underdeveloped, compared to the controls.

![A Deformed Zebrafish in the Low Concentration of Caffeine](image)
Discussion:

The results supported the hypothesis that the zebrafish would acquire birth defects if exposed to caffeine, but did not support the hypothesis that caffeine would increase the hatch rate of zebrafish. Caffeine proved to only be statistically significant for the hatch rate in the low and high concentrations. When zebrafish were exposed to the low and high concentrations of caffeine the hatch rate greatly decreased, in comparison to the control. In the 1 mg/mL concentration of caffeine, only three zebrafish hatched out of a total of 28 embryos in the high concentration. In the 0.05 mg/mL concentration of caffeine, only eight zebrafish hatched out of a total of 29 total zebrafish in the low concentration. The 0.25 mg/mL was surprisingly healthier than the 0.05 mg/mL concentration. The 1 mg/mL concentration was the least healthy, which was expected.

There were deformities prevalent among the zebrafish in all concentrations. The zebrafish had bent spines and were underdeveloped, as seen in Figure 3. The deformity of bent spines was the most common deformity and was found in the low, medium and high concentrations of caffeine. Most of the zebrafish were underdeveloped. The caffeine has an affect on the way the zebrafish develop causing birth deformities.

This experiment had positive outcomes, but would have been more accurate if each well started with ten zebrafish and if the experiment lasted for more than five days. Possible errors within the experiment could have been the misjudgement of whether the embryos were dead or alive. Also, there is a possibility that live embryos were removed before thorough examination.

Caffeine can be consumed in many different dosages, depending on how much the user wants/needs. This experiment showed that caffeine can affect the embryological development of zebrafish, and is more than likely to affect the embryological development of humans. High dosages are much more dangerous to pregnant women than lower dosages. If a pregnant woman decreased the amount of caffeine she consumed while pregnant the embryo has a better chance of developing correctly. Possible future solutions to lessen the effects of caffeine, are to test medications that could prevent or decrease birth deformities in embryonic development. Another experiment that could be done is to study the effect caffeine has on teen or adult zebrafish to understand how caffeine affects not only embryos, but also developed humans.

References
