Zebrafish Research: The Effects of 1x and 4x Aspirin Solution on Zebrafish Embryos

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Abstract

This experiment is designed to help determine an appropriate amount of aspirin that can be consumed without experiencing any harm. On the first day, 10 Zebrafish were placed into 4 different wells 3 different times, with 1x aspirin, 4x aspirin, and our control, instant ocean. Each day, data was collected and microscopes were used to observe and record the number of deaths, deformities, and hatchings. Detailed pictures were able to be taken with the use of a microscope, so one could further observe the deformities. Also, everyday the concentrations were drained and replaced with new solution to ensure a sterile environment for the Zebrafish to live in. So, throughout the 96 hours-post-fertilization that these embryos were exposed to 1x aspirin and 4x aspirin, it can be concluded that exposing developing Zebrafish embryos to 4x aspirin is unsafe, and unhealthy. It is unsafe and unhealthy, because every developing Zebrafish embryo that was exposed to the 4x solution experienced deformities, whether it be a curved and bent spine or a yolk sac edema. These Zebrafish would all go on to live a short life, and die in the course of 96 hours, helping to support why it is unsafe to endanger these organisms with such a heavy dosage. These findings are especially significant, because of the information it presents for human health. Since Zebrafish are so strikingly similar to the human embryonic development this knowledge is helpful in determining what is a safe and healthy dose of aspirin for humans to consume when pregnant.

Introduction

Zebrafish(Danio rerio) are an intricate part of the research done on the effects of various substances on human health. Zebrafish have been on the up and up in recent years to become one of the most important organisms used in studying the effects of some toxicants, related to human embryos (Kumar et al., 2012). Human embryonic development is strikingly similar in signaling process, anatomy, and physiology as to that of Zebrafish embryos (Kumar et al., 2012). With zebrafish being fertilized and their eggs being laid externally, it makes observing them much easier (Petering et al., 2007). Also, giant clutches allow researchers to look at the effects of their toxic compound on the Zebrafish in a rather timely manner. Zebrafish reproduce rapidly and develop quickly making them a perfect researching tool (Kumar et al., 2012).

Acetylsalicylic acid, more commonly known as aspirin can be used to treat aches or pains throughout the body, and is also used as an anti-inflammatory drug (Nordqvist, 2017). Many doctors prescribe aspirin not only for pain, but also for the reduced risk of blood clots and heart attacks (Kumar et al., 2012). Aspirin has been proven to increase the likelihood of bursting a blood vessel, causing a stroke. Also, gastrointestinal bleeding with ulcerations and severe allergic reactions have also been proven to more likely occur if aspirin is consumed (Mayo Clinic Staff, 2011). Overall, aspirin is not considered lethal, however with higher doses being consumed during pregnancy, this increases the chance of bleeding on the brain in premature infants or premature closure of a vessel on the heart of the fetus which could lead to fatalities (Rana et al., 2010).

The question this experiment proposes is, How does aspirin impact or effect the development of Zebrafish embryos? Through this week long experiment, many embryonic Zebrafish will be tested with different amounts of aspirin, enabling the scientist to identify the effects this toxicant has on the development of these embryos. It is hypothesized that exposing the Zebrafish embryos to 1x and 4x concentrations aspirin over the course of several days will result in defects, and death.

Materials and Methods

Materials

Quantity	Item
1 bottle	Solutions of 1x and 4x Aspirin
1	Beaker for dead embryos and liquid disposal
1	Sharpie
1	Disposable pipette
As many as needed	Disposable pipette, 1 mL
1	Plate with wells
1	28.5 degrees celsius incubator
1	Depression slide with slip cover
1	Dissecting and compound microscope.
As much as needed	Methyl Blue

Procedure

Day 1

a. Obtain rinsed embryos from the teacher.
b. Label the plate with group name and class hour. Label the aspirin concentration of each well using the Sharpie provided.
c. Fill the one well of the plate with 1 mL of Instant Ocean/Embryo Media solution using the disposable pipette. Fill the remaining wells with the appropriate aspirin with the appropriate aspirin stock solutions. Divide the embryos so there are approximately 10 embryos in each well. Label the plate on the student data sheet.
d. Record exact numbers of live embryos on student data sheet.
e. Observe the embryos under the dissecting microscope. Record observations on student data sheet.
f. Place each plate in the 28.5 C incubator overnight.

Day 2

a. Remove plate from incubator.
b. Remove dead embryos from plate using the disposable pipette. Squirt dead embryos into waste beaker.
c. Count remaining embryos, hatched fish, and record in data table.
d. Remove aspirin stock solutions from each well of the plate.
e. Replace the aspirin stock solutions with the appropriate fresh aspirin stock solution using a clean pipette each time.
f. Place plate under dissecting microscope and record observations on student data sheet. Note/ describe any developmental markers and abnormalities. Repeat for all aspirin concentrations.

g. Remove 1-2 embryos and place on the depression slide with cover slip. Observe the embryos using the compound microscope. Record observations on student data sheet. Repeat for all aspirin concentrations.
h. Return the embryos to their well in the plate.
i. Return the plate to the appropriate incubator.

Day 3

a. Repeat Day 2 work and observations. Record all data.

Day 4

a. Repeat Day 3 work and observations. Record all data.
b. Place all embryos and fish in waste container. The teacher will properly dispose of the organisms. Procedure provided from SEPA - UW-Milwaukee

Data

Data Table 1:

Survival Rate of Zebrafish Embryos with Aspirin					
Concentration	# of starting embryos	# alive 24 hpf	# alive 48 hpf	# alive 72 hpf	# alive 96 hpf
Control	40	37	34	34	34
1x Aspirin	40	36	36	35	34
4x Aspirin	40	31	24	2	0

Data table 1 is used to show the number of zebrafish embryos that survived in the different concentrations of Aspirin over a 96 hour period. From hour 1 through hour 96, the number of Zebrafish still alive in the 1x and concentration solutions remains pretty consistent. In the concentration of 4x Aspirin, there is a significant number of lives lost everyday, which eventually dwindles down to 0 alive at the 96 hour mark.

Figure 1:

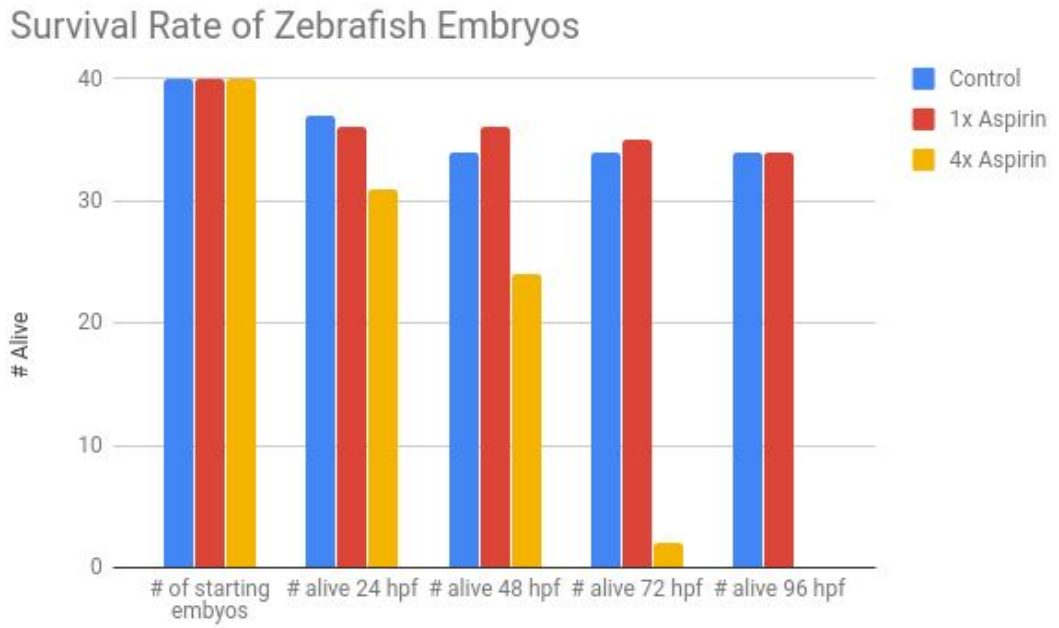

Figure 1 shows the total number of Zebrafish alive in all 4 wells of each solution at 24 hours, 48 hours, 72 hours, and 96 hours.

Data Table 2:

P-Values and Significance of Unpaired t-test Comparisons with Aspirin

	P-Value	Significance
Control vs 1x - 24 hpf living	0.7502	Not Significant
Control vs 4x - 24 hpf living	0.0686	Not Quite Significant
Control vs 1x - 48 hpf living	0.7796	Not Significant
Control vs 4x - 48 hpf living	0.0308	Significant
Control vs 1x - 72 hpf living	0.5370	Not Significant
Control vs 4x - 72 hpf living	0.0001	Extremely Significant
Control vs 1x - 96 hpf living	0.5560	Not Significant
Control vs 4x - 96 hpf living	0.0001	Extremely Significant

This data table is used to demonstrate how Aspirin affects survival rates.

Data Table 2 shows an unpaired T-test. This T-test shows that there was no significance in the Control vs. 1x Solution, but there was in Control vs. 4x Solution. A t-test was used with a p-value set at 0.05 to test statistical significance. An unpaired t-test is used to determine if two groups have different average values. This analysis allows predictions to be made from the data set. As time went on the Control vs. 1x Solution remained not significant, but as time went on in the Control vs 4x Solution the

P-Value became more and more significant, going from not quite significant at 24 hours to extremely significant at 96 hours. This data table is used to demonstrate how Aspirin affects survival rates.

Figure 2 **Figure 3** **Figure 4** **Figure 5** **Figure 6**

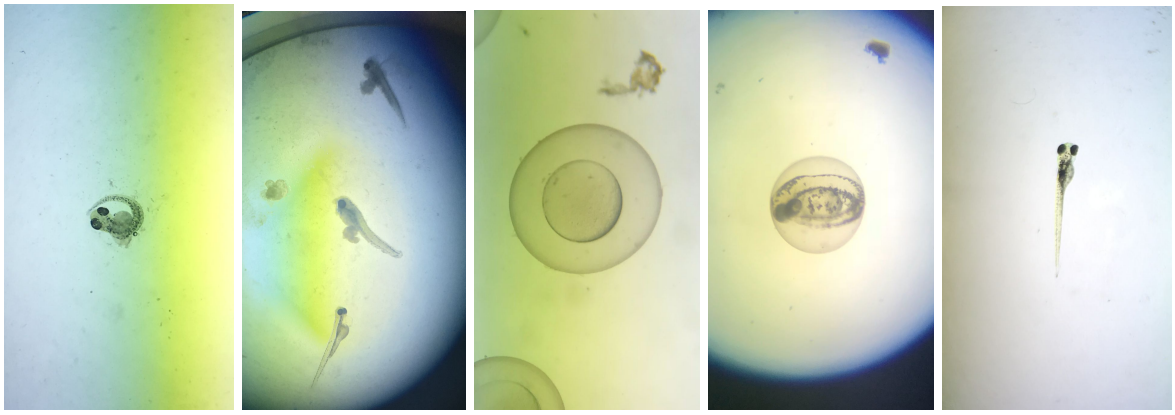

Figure 2 shows a Zebrafish with a curved spine in 4x aspirin. Figure 3 shows two Zebrafish with yolk sac edema in 4x aspirin. Figure 4 shows a normal embryo in the control. Figure 5 shows normal development of an embryo in the control. Figure 6 shows a normal Zebrafish completely developed in the control.

Results

The purpose of this experiment was to study and identify the effects 1x and 4x aspirin had on Zebrafish embryos compared to the Zebrafish embryos not exposed to any aspirin. The independent variable in this experiment was the different concentrations of aspirin we exposed to the Zebrafish. The dependent variable of this experiment was the survival and hatch rates of the two different concentrations of the aspirin and the constant, of the Zebrafish embryos. It was clearly evident that the exposure of Zebrafish embryos to the 1x and 4x concentrations of aspirin affected the embryos in a great way. As shown in data table 1 and figure 1, the control and the 1x aspirin group, exposed to instant ocean solution and 1x aspirin concentration, stayed relatively healthy. But most importantly alive throughout the experiment. Only 6 lives were lost in each of the 1x and control groups throughout the entire experiment. This shows that a 1x dose is safe to expose the Zebrafish to. Also exhibited in data table 1 and figure 1, are the survival rates of the embryos in the 4x aspirin group when exposed to a 4x aspirin concentration. 9 Zebrafish were deceased after the first 24 hours, 16 after 48 hours, 38 after 72 hours, and all 40 after 96 hours. The t-test in data table 2 shows that after 96 hours-post-fertilization, 1x aspirin remained not significant compared to the control group, and 4x aspirin after 96 hpf was extremely significant. 4x aspirin became significant after 24 hpf, and as more Zebrafish died, significance grew greater and greater. Figures 2 and 3 show Zebrafish in the 4x concentration with a curved spine and a yolk sac edema. Figures 4, 5, and 6 demonstrate the normal developmental process going from an embryo, to the beginning stages of development, and eventually to a fully developed Zebrafish. In primarily the 4x concentration major changes and deformities were seen, impacting the way the Zebrafish were able to move and survive. This experiment was setup to see if different amounts of aspirin would have effects on the developmental and mortality rates of the Zebrafish embryos, and these results clearly prove there is a negative effect on these organisms when exposed to the 4x dose of aspirin.

Discussion

The experiment conducted to help gather information on whether or not aspirin has an effect on the development of Zebrafish embryos gave great insight, and provided a wonderful set of data to analyze. Data analysis supports the hypothesis of, "Exposing the Zebrafish embryos to 1x and 4x concentrations aspirin over the course of several days will result in defects, and death." In data table 2, the t-test displays that 1x dosage does not affect the survival rate, but greatly affects the survival rate with 4x dosage when compared to the survival rate of the control. Also, figure 1 along with data tables 1 and 2, demonstrate that the 4x concentration of aspirin has extremely significant effects on developing zebrafish embryo, and shows a high mortality rate of these zebrafish the longer they are exposed to such great amounts of aspirin. Now, when comparing figures 2 and 3, zebrafish with severe deformities, to figure 6, a normal, fully developed zebrafish, it is evident that those exposed to 4x solution of aspirin were clearly more likely to have malformations. Based on observations, it is apparent that exposing these embryos to such high doses of aspirin that many embryos either die before hatching, or hatch too soon and die right away. A potential limitation of this experiment is the lack of concentrations exposed to the embryos. Using only 4x and 1x concentrations made it possible to see that 4x clearly has a negative effect on the development of the Zebrafish embryos, but with this data one is unable to identify if 3x concentration or 2x concentration has effects as well. If so, which level is a safe level to give the embryos, and which starts to negatively affect them. Finally, a possible side effect of overdosing on aspirin is gastrointestinal bleeding. Throughout the experiment one could observe that many of the embryos exposed to 4x concentration seemed to have much larger stomachs, called a yolk sac edema. This is very similar, if not exactly the same as gastrointestinal bleeding. Using the evidence this data provides from this experiment, one could easily identify what is a safe dosage to consume without noticing negative side effects, such as gastrointestinal bleeding or perhaps premature closure of a vessel on the heart, and bleeding of the brain.

Works Cited

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