THE EFFECTS OF ASPIRIN ON ZEBRAFISH EMBRYO DEVELOPMENT

Landon Osterhaus
Pardeeville High School

Abstract:
Now days when you get a cold or a headache the simple solution is to just take some medicine such as aspirin. Aspirin is a very common medicine taken to relieve pain. Is this medicine safe? Do the benefits outweigh the risks involved? Most people wouldn't think about what negative effects a medicine prescribed by the doctor could have on you or your offspring. This experiment tested to see if aspirin affected mortality rate, hatch rate and growth rate along with the potential to cause deformities in zebrafish embryos. This experiment required a well plate with ten zebrafish embryos in each of the twelve wells. The wells were filled with either a solution of aspirin or filled with the embryo media. Everyday the solutions were changed along with any dead fish or floating debris. Each day data was collected and recorded. This experiment showed that the high concentration of aspirin affected the mortality rate, the medium and high concentrations both were statistically significant and impacted the hatch rate of the embryos. There were also two deformities found in the low concentration and, one dead fry with bent spine. The results of this experiment can be used to help better understand the effects aspirin can have on human embryological development.

Introduction:
There are many chemicals in the world that are harmful to the human body and a lot of them are consumed at a high rate, even daily, without the consumer knowing the effects it may have on their body. This experiment was performed to show if aspirin had a negative effect on embryos. The fields of science that this experiment relates to are toxicology and pharmacology. The reason that this may be a problem is because aspirin is one of the most common pain killers in the United States. Aspirin is used to treat moderate pain like headaches and toothaches; it is also commonly prescribed by some doctors as a light blood thinner according (WebMD, 2015). “Studies with animals show aspirin may interfere with blood clotting in the fetus with potential for brain damage. Regular aspirin takers had an increased incidence of anemia, excessive bleeding before birth and after, longer pregnancies, and a higher frequency of complicated deliveries” (Kassuba, 2015-2016).

This experiment tested the effects of aspirin on zebrafish embryos. The reasons that scientist use these embryos include the fact that they develop outside the fish, the embryos are clear, they develop and hatch within 4 to 5 days of being laid, and finally, the embryos are also laid in large quantities. The zebrafish embryos are a close comparison to what a human is like in its early developmental stages. They will react similarly to how a human embryo would act to the chemical. Therefore, if the zebrafish have deformities, it is probable that the same thing would happen to human embryos as well.

The independent variable in this study was the amount of aspirin that was put in each well. The dependent variables that were measured in this study included developmental delays, the number of dead zebrafish, and the number of hatched fry at the end of the experiment. Another major thing the group looked for was if there was any deformities that were noticeable
in the embryos/fry. The hypothesis stated that the aspirin would have a negative effect on the embryological development on the zebrafish embryos.

Methods and Materials:
The materials used in this experiment were the following: microscope, minimum and large bore pipettes, 50 mL beakers, 12-well plate, Instant ocean/embryo solution, the three different solutions of aspirin which were 0.175 mg/mL, 1.75 mg/mL and, 17.5 mg/mL. The embryos were also placed in an incubator every night. Gloves were worn to protect against the chemical solutions. 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.

For the experiment the zebrafish had to be fed brine shrimp to prepare them. The second day there were 120 embryos collected. After placing 10 embryos into each well all containing the Embryo solution, the team changed the solution with the new ocean embryo solution to get all material and debris out of the water. Next, for wells A1, B1, C1 the solution was changed because this set of wells is the control. The next set of wells known as the low concentration is wells A2, B2, C2, with these wells take the low concentration of aspirin and swap the embryo solution with the new aspirin solution, The solution used in this experiment was 0.175 mg of aspirin per every mL of water. For the Medium concentration wells the group used a concentration of 1.75 mg/mL, change the water the same as the control and low only using the medium concentration solution. Repeat this process for the high concentration (17.5 mg/mL) in wells A4, B4, and, C4.

For this experiment the results were measured using statistics. The software used to find the statistics is the online GraphPad software. The measurement used to tell if the results were significant or if they were not statistically significant was if the probability was less than 0.05 that means it was statistically significant and if the probability was higher than 0.05 then it was not statistically significant.

Results:
This experiment was conducted over a period of 5 days. The first day was putting and exposing the independent variable (aspirin) to the sample of embryos. The 5 days were used to collect and record data and observations. This data was used to tell if the aspirin had an impact on the embryological development of the zebrafish embryos. The controls in this experiment were filled with embryo media solution. The independent variable in this study was the amount of aspirin that was put in each well. The dependent variables for this study was if aspirin caused slowed development, affected the number of dead zebrafish along with the number of hatched fry at the end of the experiment. The group also observed any types of deformities and or abnormalities.

The data provided by the software showed that for the total number of living fry the low and medium concentrations were not statistically significant. However, the high concentration was extremely significant. The data for the total number of hatched fry said that the low concentration was not quite significant but the medium was significant and the high concentration was extremely significant.

Along with the data provided above there was also deformities found in two of the hatched fry that were exposed to the low concentration of aspirin and one dead fry that had a
spine deformity that was in one of the medium concentration wells. This could have been natural deformities and may have nothing to do with the experiment. With this information the data shows that aspirin dose effect the growth rate and the mortality rate of the embryos in a negative way. The team saw that the aspirin had an obvious effect on the embryos with the high concentration killing all of the embryos in a matter of hours, the other observation made was that the fish that were exposed to the aspirin grew slower and less of them hatched by day five.

The results of this experiment were supportive of the hypothesis. The experiment showed that the aspirin had a negative effect on the embryos. The high concentration killed the embryos within the first 24 hours thus, affecting the final hatch and final living rates. The medium concentration slowed the growth rate of the embryos and had on deformity in its wells. The low concentration had two deformities and had not quite enough of a result to be statistically significant in the growth rate. With this information the hatch rate and living rate is linked to the amount of aspirin in the solution. So the higher the concentration the worse off for the embryos, there is no plateau until the concentration is just too much and the all die off.

The results of this experiment may have been affected slightly by some small mistakes made while performing the experiment. One thing that may have been slightly off was the incubator temperature and the miss counting of the embryos. The results of this experiment will help further the study of aspirin and will support the hypothesis of aspirin negatively affecting the development of embryos.

Table 1.

<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>7</td>
<td>9</td>
<td>10</td>
<td>8.66</td>
<td>1.53</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>0.175 mg/mL</td>
<td>5</td>
<td>8</td>
<td>6</td>
<td>6.33</td>
<td>1.53 p= 0.1347</td>
<td>Not Significant</td>
<td></td>
</tr>
<tr>
<td>1.75 mg/mL</td>
<td>8</td>
<td>10</td>
<td>5</td>
<td>7.66</td>
<td>2.52 p=0.5879</td>
<td>Not Significant</td>
<td></td>
</tr>
<tr>
<td>17.5 mg/mL</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.00 p=0.0006</td>
<td>Extremely Significant</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: This table represents the data for the total number of living fish. The table shows the different results from the different solution amounts. This table shows that the higher solution concentration had a huge impact on the final number of living fry. The medium and low concentrations were found to not be statistically significant. This table also gives the standard deviation and the averages for the different concentrated solutions.
Table 2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Well 1</th>
<th>Well 2</th>
<th>Well 3</th>
<th>Average</th>
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<th>Probability</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>8</td>
<td>10</td>
<td>8.33</td>
<td>1.53</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>0.175 mg/mL</td>
<td>4</td>
<td>7</td>
<td>5</td>
<td>5.33</td>
<td>1.53</td>
<td>p=0.0739</td>
<td>Not Quite Significant</td>
</tr>
<tr>
<td>1.75 mg/mL</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>4.66</td>
<td>1.15</td>
<td>p=0.0295</td>
<td>Significant</td>
</tr>
<tr>
<td>17.5 mg/mL</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>p=0.0007</td>
<td>Extremely Significant</td>
</tr>
</tbody>
</table>

Table 2: This is a table that is representing the total number of hatched fry by the end of the experiment. The low concentration was not quite enough to be statistically significant. Although the medium and high concentration were significant. This shows that the aspirin dose have an impact on the growth rate of the embryos. This table also shows that the high concentration killed off the all the embryos after the first day. This table also gives the standard deviation and the averages for the different concentrated solutions.

Figure 1.

Figure 1: This graph shows the final number living fry in the different treatment types. This graph also shows the average number of living fry. This is helpful for comparing this to different graphs and charts. There is no fry that lived throughout the experiment in the high concentration. The lowest average number of living fry was the low concentration after the high concentration that is.
Figure 2: This graph includes the standard deviation of the final number of living fry. The standard deviation is a representation of the total difference of final number of fry for each of the wells in the different concentration type.

Figure 3: This graph represents the final number of hatched fry. Both the medium and the high concentration were statistically significant with lowering the hatch rate of the zebrafish. The low concentration was not quite statistically significant on effecting the hatch rate of the zebrafish. None of the embryos hatched in the high concentration.
Figure 4: This graph includes the standard deviation of the final number of hatched fry. The standard deviation is a representation of the total difference of final number of hatched fry for each of the wells in the different concentration type. There was a semi high standard deviation in all of the different concentration although, this is also true for the control so this may be normal.

Figure 5: This is a picture taken from one of the control wells. All of the concentrations looked like this on day one except for the highest concentration.
Figure 6: This is a picture taken on day one in one of the high concentration wells. All of the high wells looked like this with no survivors. The embryos are black and unraveled.

Figure 7: This is a picture taken on day 2 of well C-2 a low concentration well. This picture shows two deformities in the spines of the hatched fry. The spines of these two fry are bent like a C. This is thought to be a result of the aspirin solution they have developed in.
Figure 8: This is a picture taken on day 3 in one of the medium concentration wells. This picture of the dead embryo shows that there is yet another spine deformity. This fry may not be alive but it still gives the information needed to support the hypothesis.

Discussion:

The results of this experiment supported the hypothesis that aspirin does negatively affect embryos. The most significant result of the embryos being exposed to the aspirin was the hatch rate. It was clear throughout the experiment that the aspirin slowed the development of the embryos. This experiment only lasted 5 days which means it was unknown whether or not the fry would eventually hatch or if they would die. The highest concentration of aspirin also had a significant impact on the mortality rate of the zebrafish. This also supports the hypothesis and demonstrated the negative effect on embryos. Another observation found that two zebrafish fry found in the low concentration had spine deformities along with a dead fry in the medium concentration with a spine deformity. These deformities are thought to be a result of the aspirin however, this could be coincidental and have nothing to do with this experiment. But if these deformities are a result of the aspirin this would also support the hypothesis and would also show how aspirin can impact a zebrafish embryo or a human embryo.

Some errors that may have been conducted throughout this experiment may have been miscounting, also the chance of an embryo being sucked up while doing a water change. One thing that could possibly better the experiment could have been extending the number of testing days to for example see if the non hatched fry end up hatching or dying. Another improvement to this could be finding a more precise and efficient instrument for doing water changes.

Is aspirin really that beneficial for all the negative effects it may have on a human? Aspirin is a very convenient and overlooked medicine and hopefully this experiment will stimulate more studies to see the impacts it has on embryos and possibly humans. This experiment questions whether aspirin should be such a readily available product and if there should be more restrictions on the use of this medicine. The results of this experiment may help further the study of aspirin and they strongly supported the hypothesis of aspirin negatively affecting the development of embryos.
References

