Effects of Nicotine on the Development of Zebrafish Embryos

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Abstract

Nicotine can damage many vital conditions for life, such as the brain and arteries. To witness/observe/recognize the effects of nicotine on humans an experiment using zebrafish embryos was conducted. The zebrafish embryonic structure and nervous system are closely related to that of humans, resulting in zebrafish being an ideal embryo to utilize for experimentation in order to see chemical effects on the embryonic development and structure pertaining to humans. The experiment in question is meant to see the effects of varying amounts of nicotine on the developing zebrafish embryos with comparison, as it would pertain to humans. This was done by placing ten zebrafish embryos each into four wells filled with solutions of varying amounts of nicotine (0.0 mg/mL of nicotine (the control solution), 0.05 mg/mL of nicotine, 0.1 mg/mL of nicotine and 0.2 mg/mL of nicotine). The zebrafish were observed through a microscope over the course of five days with the solutions changed daily. The data collected represents observations made specifically the mortality rate of the embryos recorded daily over the course of the experiment. After five days, the results explicitly displayed how nicotine can severely damage the development of fetal and young fish as higher concentrations of nicotine caused more fatalities among the zebrafish. As zebrafish and humans have similar nervous systems, nicotine could have comparable effects on humans, developing children especially.

Introduction

According to the Centers for Disease Control and Prevention, approximately 480,000 people die per year in the US from smoking, and secondhand smoke exposure is responsible for over 41,000 deaths per year as stated by the Government of the Unites States of America (2007). Studies conducted by the Government of the Unites States of America (2007) have shown that the main chemical in tobacco products, nicotine, is the perpetrator because of its known effects on the brain, heart and arteries, eyes, metabolism, reproductive system and bones. This research could help lead to less deaths by tobacco and smoke exposure. To verify these studies, an experiment was conducted using zebrafish embryos. Zebrafish and humans are shown to be similar, as they have comparable development and nervous systems. Using zebrafish is also beneficial because the embryos are transparent, making it easy to see what is occurring inside them. They also develop quickly, keeping the length of the experiment to a minimum. This experiment was designed to answer the question, how does nicotine affect the nervous system? As stated by Jonathan Knight Institute of Neuroscience (2002), ". . . [by using zebrafish] it is possible to determine what genes are involved and then compare them to the equivalent genes within the human genome." The overall hypothesis for the experiment is: the zebrafish placed in a higher concentration of nicotine are more likely to die than those put in a lower concentration or in a solution without nicotine because studies show that nicotine has been responsible for thousands of deaths in the US.

Materials and Methods

For this experiment, the materials required include: 40 zebrafish embryos, a multi-well plate with at least four wells, tape, a marker of some kind, one transfer pipette, one large bore transfer pipette, a camera and four minimum bore pipettes that measure to at least 1 mm, one Bausch and Lomb dissecting microscope, one small beaker (50 mL), diluted solutions of nicotine that contain 0.05 mg/mL, 0.1 mg/mL, 0.2 mg/mL and one control solution with no nicotine present. The last item required is an incubator that is set to 28.5 degrees Celsius; the well-plates

containing the embryos will be placed in this over-night. As a safety precaution, wearing latex free rubber gloves is recommended to protect the hands from the nicotine solutions.

To begin the experiment, stretch a piece of tape across the cover of the lid of the multi-well plates and tear it off. Using the marker, under each separate well, write down 0.0 mg/mL, 0.05 mg/mL, 0.1 mg/mL, and 0.2 mg/mL. Place a different minimum bore pipette in each solution of diluted nicotine. Taking the lid off of the multi-well plate and using the large bore transfer pipette, place 10 zebrafish embryos into each well. Then, taking the pipette that is in the 0.0 mg/mL solution, measure 1 mm of the solution and place it in the corresponding well. Now using the pipette in the 0.05 mg/mL solution, measure 1mm again and place it in the corresponding well. Repeat this step for the 0.1 mg/mL and 0.2 mg/mL. Once finished, place the multi-well plate containing zebrafish embryos under the Bausch and Lomb dissecting microscope. Focus on one well at a time and record the coloring of the embryos in each well. Remove all dead (black) embryos and replace with clear (living) embryos to ensure that the starting sample of embryos is accurate and does not skew the data. Take a picture through the microscope focusing on one embryo and rename the picture to the date and well number. Do this for each well. When finished observing, place the multi-well plate in the incubator.

After 24 hours, remove the multi-well plate from the incubator and remove the lid. Then place the well plate under the microscope and look at each well. Live embryos look clear/white, while dead embryos look opaque and black. Record the number of embryos present, hatched and dead, take notes of any features and/or deformities. Take a picture of one embryo (or fish if one hatched) in each well and rename the picture to the date and well number. Remove the dead embryos with a transfer pipette after recording and place into a small beaker to dispose of - do not replace the dead embryos. Next, using a transfer pipette, remove the excess solution from the 0.0 mg/mL, making sure that no live embryos/fish are removed with the solution, and put it in the same small beaker that the dead embryos were placed in. Once all of the dead embryos are removed, obtain more of the corresponding solution, following the previous steps of solution transfer from the day before, and put it into the well. Repeat this process for all of the wells. Place the multi-well plates back into the incubator for another 24 hours. Continue to repeat this process over the course of five days. If a well no longer has a single living embryo in it, do not replace the liquid. Instead, remove all of the contents in that well. What this procedure measures are the mortality rates of the zebrafish placed in different concentrations of nicotine.

There are some precautions to take during this procedure. Avoid touching the fish or embryos because they would have traces of nicotine that would transfer to the skin. To prevent this wear latex free gloves when handling the solutions. Handle the wells with care and avoid shaking them, otherwise it would alarm and cause distress to the embryos and fish. Be sure to use clean pipettes, as it is crucial to ensure the most accurate results. If a dirty pipette is used it will cross-contaminate nicotine solution concentrations and can cause inaccurate results.

Results

This experiment was intended to prove that nicotine at high concentration levels can kill what is exposed to it, in this case, zebrafish embryos. Zebrafish embryos have a similar developmental process and structures as developing human fetuses, allowing for experimentation to be theoretically comparable. On a larger scale of affiliation, the experiment is intended to show that women who smoke during pregnancy can and will do significant damage or even kill the developing fetus inside. The experiment was set up this way because it is the most efficient way to see how fast the nicotine exposure can affect the mortality rate of the fish embryos.

The independent variable in the experiment was the amount of nicotine in each well and the dependent variable is the number of living zebrafish embryos in each well. The independent and dependent variable relate to each other because if the amount of nicotine is increased, more of the fish embryos will die. The general trend found in the results supports this as shown in Figures One and Two. As the days go on, the line showing how many fish remain drops in Figure One as it measures the number of live zebrafish embryos. However the line rises in Figure Two because it shows how many zebrafish embryos are dead. The data observed during the course of the 5-day experiment included the amount of dead embryos versus the amount of living embryos. As seen in the figures one and two below, the mortality rate of the embryos grew each day. Many of the embryos that were in the wells containing high amounts of nicotine were discolored, almost black looking and the fish that hatched inside of the wells had black spots, this can be seen in figures four, six, and seven. A few of the fish that hatched were deformed, having misshapen bodies, as seen in figure six. The last well (well four) contained the highest concentration of nicotine (0.2 mg/mL), which had a large effect on the fish and embryos, seen in figure six and also portrayed in figure two. The first well contained no nicotine and had the highest amount of survival among the zebrafish in this experiment. The zebrafish in well one looked clear and healthy, as seen in figures five and one. As seen in the Chi Squares the results were significant. This is important because it means the hypothesis can be assumed as correct.

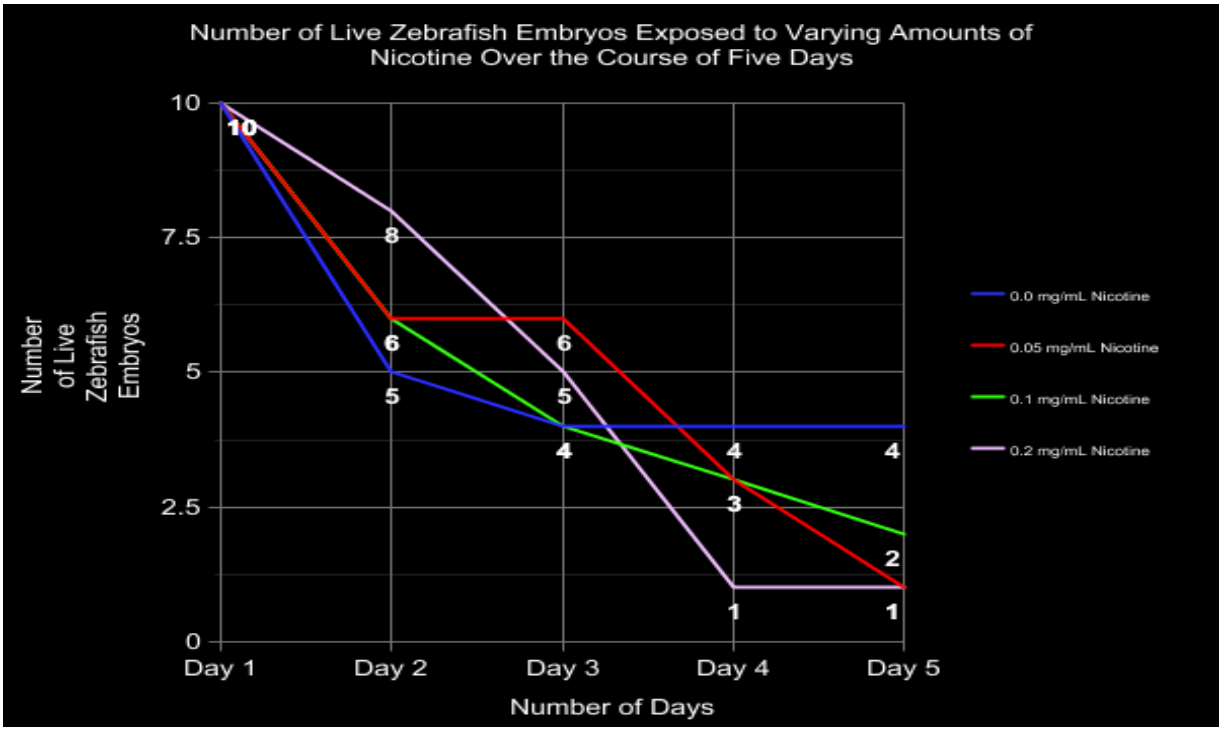

Figure 1: Number of Live Zebrafish Exposed to Nicotine

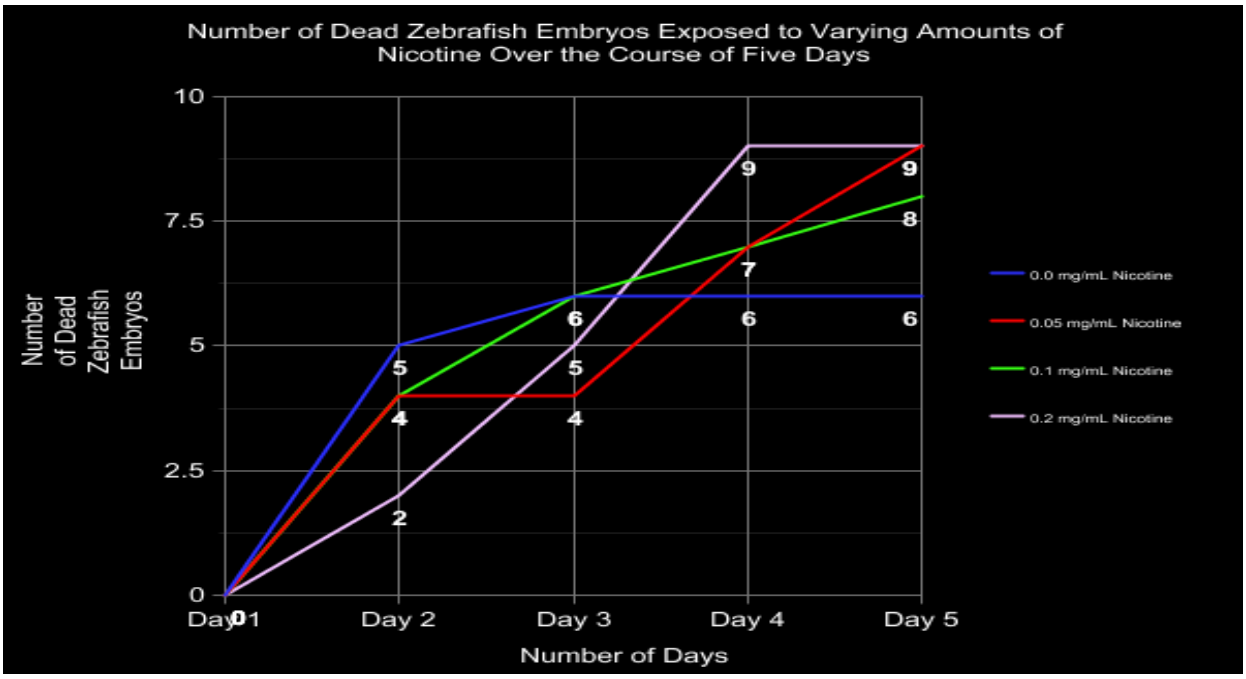

Figure 2: Number of Dead Zebrafish Exposed to Nicotine

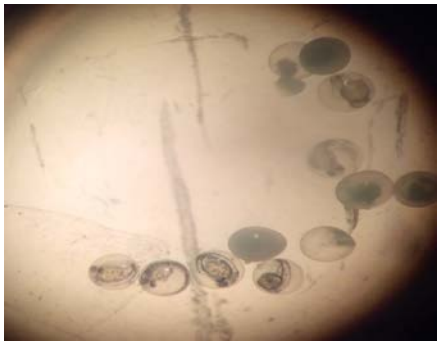

Figure Three: Well 1, Day 1- Healthy Zebrafish Embryo

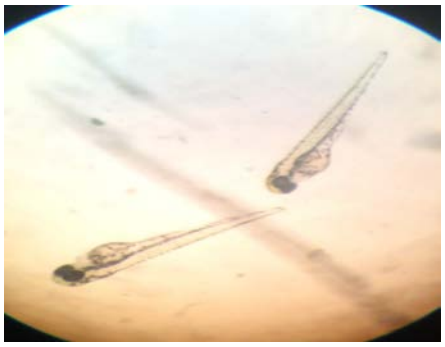

Figure Four: Well 3, Day 3- Zebrafish Deformed

Figure Five: Well 1, Day 4, Healthy Zebrafish

Figure Six: Well 4, Day 5, Dead/Deformed Zebrafish

Figure Seven: Well 4, Day 4

Day Five Chi Square Charts

Treatment	Live	Dead	Total For Rows
Control (0.0 mg/mL)	4	0	4
0.05 mg/mL	1	2	3
0.1 mg/mL	2	1	3
0.2 mg/mL	1	0	1
Total For Columns	8	3	Total For Table: 11

Live

Treatment	O	E	(O-E)	(O-E)*2	(O-E)*2/E
Control (0.0 mg/mL)	4	2.90	1.10	1.1900	0.41037
0.05 mg/mL	1	0.72	0.27	0.07458	0.1033
0.1 mg/mL	2	1.45	0.55	0.2975	0.20518
0.2 mg/mL	1	0.72	0.27	0.07458	0.1033

X*2 = 3.3202

Dead

Treatment	O	E	(O-E)	(O-E)*2	(O-E)*2/E
Control (0.0 mg/mL)	0	0	0	0	0
0.05 mg/mL	2	1.45	0.55	0.2975	0.20518
0.1 mg/mL	1	0.72	0.27	0.07458	0.1033

0.2 mg/mL	0	0	0	0	0

X*2=0.30848

Table Total: 3.62868

Discussion

Figures one and two from the results section show that as the days went on significantly less zebrafish embryos, let alone hatching of said embryos, were alive by the end of the experiment. The biggest decline of zebrafish life was seen in the well that contained 0.05 mg/mL of nicotine and the well that contained 0.2 mg/mL of nicotine. This somewhat supports the hypothesis because the prediction was that the more nicotine that was in the well equaled more death. Possible errors could have arisen during the procedure, as four of the zebrafish in the second well died within 24 hours, and only two died in the last well throughout the duration of the experiment. Some of the dead embryos may have been mistaken for living embryos and were accidentally left in the well until there was a chance to look under the microscope again. As a result, the well containing 0.1 mg/mL Nicotine had the second most live fish embryos. Another error that may have occurred is that only one person was doing the chi square math and could've easily made an error do to all of the small decimals. The control, shown in figure one, had more fish embryos survive. The number of zebrafish that are alive in the control is well above the fish alive in the other wells thus proving part of the hypothesis right.

By checking the embryos under the microscope to dispose of the dead ones, the results would have been much more accurate and increase the chance of making a solid statement on whether or not the hypothesis is true. However, when consulting the Chi Squares, the degree of freedom was 3.6, which means the hypothesis can be accepted, and it can be assumed the experiment was overall a success in proving the hypothesis. Some of the embryos that had hatched in the nicotine solution wells were deformed, having black in them and what appeared to be a bump compared to those that had hatched in the control. Thus concluding that nicotine is not only responsible for fetal deaths, but is also responsible for birth deformities. Through additional research the hypothesis could be proven and the data could be solidified, the question still remains, if nicotine does these observable things to zebrafish, what does it do to the mind?

References

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