Effects of Nicotine on Zebrafish Embryos Hatch Rate

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Abstract

The purpose of this experiment is to show the effects of nicotine in relation to the hatch rate of zebrafish embryos. It is important to know the effect of nicotine on developing embryos because the same thinking can be applied to developing human babies. If a pregnant mother is getting nicotine in her system, it can affect the baby just like the nicotine effects zebrafish embryos. During this experiment, different concentrations of diluted nicotine were placed in different wells with 10 zebrafish embryos. The effects of nicotine on the zebrafish was measured through the number hatched. There were some sources of error during the experiment. The control group prematurely died due to a protozoa infestation. Otherwise, the data showed a decrease in the amount of embryos hatched due to the amount of nicotine. This data can show that nicotine has a negative effect on zebrafish, which then can be used to show that nicotine can also have a negative effect on humans as well.

Introduction

The purpose of this experiment is to demonstrate how nicotine affects zebrafish early development and correlating those results to human fetal development. Nicotine is a drug that's been known as a substance despised by most people for its effects on the human brain. The substance is known to get people hooked on the drug and sends them in a downward spiral of addiction that will harm their bodies.

According to Klee, Ebbert, Schneider, Hurt, & Ekker (2011). "Nicotine exposure alters muscle development and swimming behavior in embryonic zebrafish" This shows that the effect of nicotine on zebrafish can alter their development in the muscles which can make it harder for them to get out of their shell which in turn will slow down the hatch rate.

Also the study said, "Imaging results suggest that nicotine delays differentiation of spinal motoneurons and induced axon path-finding errors." (Klee, Ebbert, Schneider, Hurt, & Ekker, 2011.) This is another example of a way that the development of the zebrafish was slowed down due to nicotine exposure.

Some other things that nicotine can do to zebrafish according to Parker, & Connaughton (2007), “Results indicate that nicotine exposure significantly reduces notochord length and eye diameter (growth), adversely affects the startle responses (behavior), and decreases survival.“ This is showing ways that nicotine can negatively affect the growth and behavior in zebrafish, making them hatch too early or too late.

Based on the research knowledge, the hypothesis is that if nicotine is put in zebrafish embryos, then the hatch rate of the zebrafish embryos will slow down tremendously because the nicotine will affect their nervous system and their ability to hatch as fast as a normal zebrafish embryo would.

Materials and Methods

Materials

- 40 zebrafish embryos
- 1 well tray
- Diluted Nicotine (0.0 mg/mL, 0.05 mg/mL, 0.1 mg/mL, 0.2 mg/mL)
- 4 fine bore pipettes
- 5 large bore pipettes
- 4-100ml beaker for nicotine
- 5-50 ml beaker for waste
- 1 microscope
- 1 roll of tape for labeling
- 1 incubator that was 28.5 ℃

Methods

Day 1:

The first thing that was done was the well tray was labeled with increasing amounts of nicotine solutions for the corresponding wells. The amounts were (0.0 mg/mL, 0.05 mg/mL, 0.1 mg/mL, 0.2 mg/mL). Then 10 zebrafish embryos were placed using a large bore pipette into the top row of the well tray, there were 10 embryos in each well. Any residual liquid remaining after transfer was removed using a small bore pipette. Next, the amount of diluted nicotine needed for each corresponding well was placed using a large bore pipette. The well tray was placed in an incubator for 24 hours.

Days 2-3:

The next day, the tray was taken out and observed. Once the number of zebrafish hatched and living was recorded, the existing nicotine was extracted using a fine bore pipette and dumped into a 50 mL beaker for waste. Any dead zebrafish were found by observation. If they were black, then they were dead. The dead zebrafish were extracted using large bore pipettes. Then, the large pipette was used for getting the diluted nicotine into the well tray. This was repeated for the next three wells with their individual amounts of the diluted nicotine. Once this was done, the lid was put on to the well tray, and it was placed in the incubator for 24 hours everyday at 28.5 degrees Celsius. This was repeated for days 3.

Day 4:

On day 4, the final results were recorded. The zebrafish were examined through the microscope for the last time. Once the results were recorded, any dead fish were removed and placed into the 50 mL beaker. Then the zebrafish were moved to the 50 mL beaker and were extracted through large pipettes because they were dead.

The following safety precautions taken during this experiment were wearing safety gloves and washing our hands many times when dealing with nicotine and the zebrafish. A chi square analysis was performed to test for statistical significance of data.

Results

The purpose of this experiment was conducted to see how the zebrafish would react to being exposed to increasing concentrations of nicotine. This data can be used to show the correlation to human embryonic development. The hypothesis was that the nicotine would affect the hatch rate and the growth of the fish because it is a harmful chemical and has been shown to do similar things to humans, especially infants. As a note, the control well was infected and killed by protozoans, which are a type of eukaryotes.

The independent variable is the amount of nicotine (0.0 mg/mL, 0.05 mg/mL, 0.1 mg/mL, 0.2 mg/mL) and the dependent variable is the hatch rate. They are related to each other because when the concentration of nicotine increases over time the zebrafish have a higher mortality rate and a decrease in hatch rate. The control variable was the 0.0 mg/mL solution. The controlled variables in the experiment were the temperature in the incubator, the well size, and the amount of solution in each well.

As you can see in figure 1, the embryos are unhatched 24 hours post fertilization. This is showing that the nicotine has not yet begun to affect the hatch rate of the embryos. In figure 2, the zebrafish is more developed and preparing to hatch. Then in figure 3 and figure 4, they are finally hatched. In figure 4, more growth can be seen, and their physical features begin to become more pronounced. Table 1 and figure 5 show the overall trend of decreasing hatch rate with increasing nicotine.

After the fish would hatch, they would spend time on their sides to gain energy. This behavior lasted longer in the fish with higher concentrations of nicotine. After they were turned upright, the zebrafish exposed to the nicotines reaction speed would be slower when they reacted to movement. This can explain the correlation between the amount of nicotine exposure and the zebrafish's behavior.

A chi-square value was used to test for independence of the nicotine solution and the hatch rate. The experimental chi-square value was 22.49. The degree of freedom was 3. The critical value was 7.82. From this data, the null hypothesis was rejected. This showed that nicotine did have an effect on hatch rate.

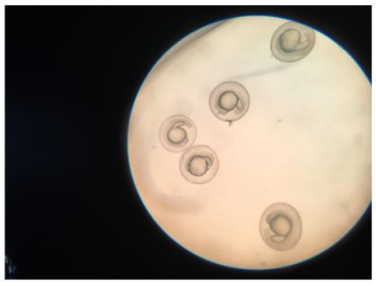

Figure 1: Zebrafish 24 hours post fertilization

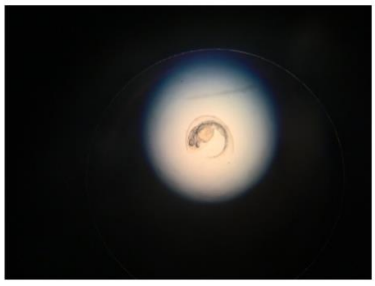

Figure 2: Zebrafish 48 hours post fertilization

Figure 3: Zebrafish 72 hours post fertilization

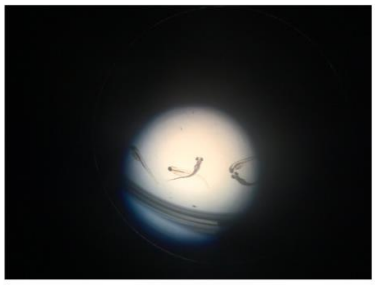

Figure 4: Zebrafish 96 hours post fertilization

Table 1: Amount of Zebrafish Embryos Hatched and Living Per Day, in Relation to the Amount of Nicotine Used

Table 1 shows that there was a problem in the control group, it was infected by protozoans. Which were killing the fish. Other than that the chart shows some correlation between the hatch rate and the amount of nicotine.

Treatment	**# of starting fish**	**24 hours post fertilization**		**48 hours post fertilization**		**72 hours post fertilization**		**96 hours post fertilization**	
		# Hatched	# Live	#Hatched	# Live	# Hatched	# Live	# Hatched	# Live
0.0mg/mL	10	0	10	0	10	4	9	0	0
0.05mg/mL	10	0	10	0	10	9	10	9	9
0.1mg/mL	10	0	10	0	10	1	10	1	2
0.2mg/mL	10	0	10	0	10	5	10	6	8

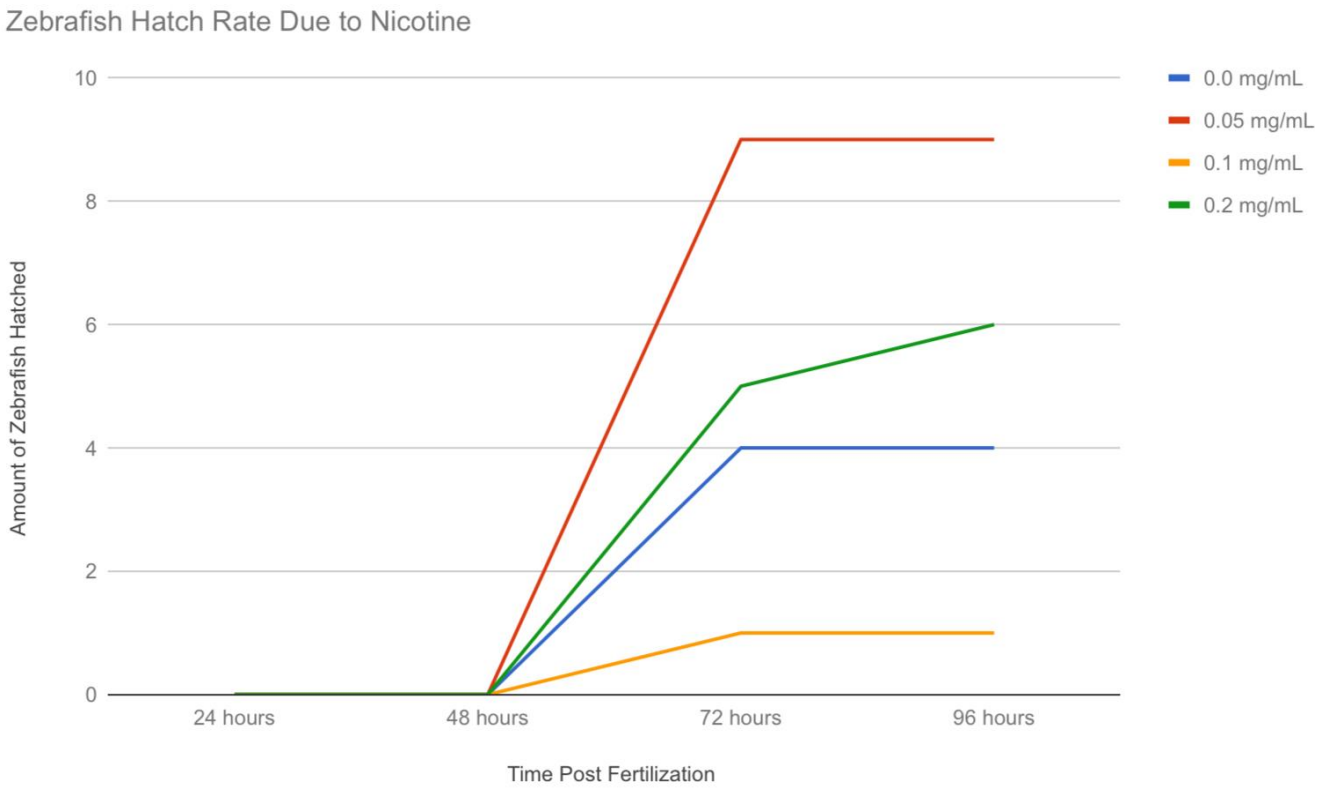

Figure 5: Zebrafish Hatch Rate Due To Nicotine Exposure

Figure 5 shows the information on the chart in a more visual fashion. As you can see once the time hits 48 hours, the hatch rate takes a dramatic rise. The control group had less of a rise due to the fact that there were protozoans in that specific well.

Discussion

It can be seen in the data that the zebrafish's development was slowed tremendously due to nicotine exposure. Based on the background research, it can be concluded that the slow zebrafish development can be used to show the correlation to human fetal development due to nicotine. It also showed that nicotine had a negative effect on specific parts of the fish. These parts consist of its eyes, spine, and muscles. In the experiment, the zebrafish were having more trouble hatching depending on the amount of nicotine. It is assumed that this is because the development of those parts worsened their ability to hatch.

The development slowed down at the 72 hour mark (fig. 5), and the mortality of the fish was increased when in the 0.05 mg/mL to 0.1 mg/mL mixture of nicotine. The

research found was related to the results, because it said that the development of the zebrafish's muscles were slower, making it harder to escape from its shell. This can be used to speculate why the fish hatch rate went down at a specific point (72 hours).

The data recorded while doing the experiment showed that over time, nicotine can have a negative impact on the fish's hatch rate. In the first 48 hours, nothing was happening to the zebrafish. Finally, at 72 hours the zebrafish almost all hatched but then the hatch rate stayed the same from there. In the control, the zebrafish were eaten by protozoa and none of the zebrafish survived. An assumption that can be made is that if the control group wasn't infected with protozoans, then the control would have hatched faster because it didn't have any nicotine exposure.

Protozoans, which are small eukaryotes, ended up infecting and eating the zebrafish in the control group, making it harder to find specific correlation to hatch rate based on nicotine exposure. However, like what was stated before, it can be assumed based on the research, that the hatch rate would have been faster in that group. This is because of the fact that there was no nicotine exposure.

Ways to improve how the experiment was run would be being more careful when removing the zebrafish when replacing the nicotine in the well tray. This could have been improved by taking more time to properly transport the zebrafish without damage. Another way to improve is by doing the experiment more than once. This would improve the data significance and accuracy. Another thing that could have been done is to use a larger sample size and a variety of different amounts of nicotine mixtures, this would help get more precise results. An error that occurred in the experiment (stated before) is that protozoa killed all the zebrafish in the control group. An unanswered question is how all of the protozoa got into the control group.

In conclusion, it was harder to notice the zebrafish's heartbeat with the ones that swam in the nicotine mixture than the control fish's heartbeat. This is because the heartbeat in the nicotine mixture fish was observed to be weaker than the control fish's heartbeat. This is another example of how nicotine can affect the zebrafish's behavior. The 0.05 mg/mL nicotine mixture wasn't enough to affect the zebrafish, but as soon as you add more you could see a drastic change in the time it takes to hatch and the behaviors of the fish. A question for further study could be to test fish at additional lower concentrations to determine how much nicotine is required before it becomes statistically significant.

References Cited

Parker, B., & Connaughton, V. P. (2007). Effects of nicotine on growth and development in larval zebrafish. *Zebrafish*, *4*(1), 59–68. https://doi.org/10.1089/zeb.2006.9994 https://www.ncbi.nlm.nih.gov/pubmed/18041943

Klee, E. W., Ebbert, J. O., Schneider, H., Hurt, R. D., & Ekker, S. C. (2011). Zebrafish for the study of the biological effects of nicotine. *Nicotine & Tobacco Research*, *13*(5), 301–312. https://doi.org/10.1093/ntr/ntr010 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3145391/#__sec8title

Eddins, D., Petro, A., Williams, P., Cerutti, D. T., & Levin, E. D. (2008). Nicotine effects on learning in zebrafish: The role of dopaminergic systems. *Psychopharmacology*, *202*(1), 103. https://doi.org/10.1007/s00213-008-1287-4 https://link.springer.com/article/10.1007/s00213-008-1287-4