

Title

How Nicotine Affects the Growth of Zebrafish

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Abstract

The development of zebrafish when growing in nicotine is terrible for the growth of the fish and embryos. The importance of developing embryos with the nicotine is to show that nicotine is unhealthy for them and also not good for people. The purpose of the experiment was to see exactly how well the embryos and fish grew in solutions of 0.0 mg/mL, 0.05 mg/mL of nicotine, 0.1mg/mL of nicotine, and 0.2 mg/mL of nicotine, and to correlate with human health, it also shows that nicotine is terrible for human and fetal development. Some of the methods were checking the fish growth every day and switching out the solutions as well. Some of the results were that at least half of the fish in each well had not survived and that nicotine is a non-beneficial growth factor. These results relate to human health by showing that nicotine might be a deteriorating factor in the health of humans.

Introduction

Nicotine is a drug that is very addictive and limiting to one's health. According to Felman (2018), "Nicotine also makes the pancreas produce less insulin, causing a slight increase in blood sugar or glucose." Also, according to Felman (2018), "When humans, mammals, and most other types of animals are exposed to nicotine, it increases their heart rate, heart muscle oxygen consumption rate, and heart stroke volume. These are known as pharmacologic effects." According to Felman (2018), "The heart can experience the following after taking in nicotine: changes in heart rate and rhythm an increase in blood pressure constrictions and diseases of the coronary artery and an increased risk of stroke." If the nicotine affects the embryos and fish growth negatively, then the nicotine is non-beneficial to their growth and development, because then if the nicotine is in their systems, they will not grow and develop and most likely will die. The question the group is trying to answer is, does nicotine positively or negatively affect the growth of zebrafish? The purpose of this question was to see how the nicotine affected the zebrafish during and after exposure to nicotine, and to see if the fish grew at all during the process, and they didn't grow very much.

Materials and Methods

Materials:

- 3 / Stock Solutions of Nicotine (0.0 mg/mL, 0.05 mg/mL, 0.1 mg/mL , 0.2 mg/mL)
- 3 / Beaker
- 40 zebrafish embryos
- 3 / transfer pipettes
- 1 / Multi-well plates

- 1 / 28.5°C Incubator
- 4 / disposable pipettes, minimum bore 1.5mm
- 4 / disposable pipettes, 1 mL, to transport embryos
- 1 / 50mL beaker for disposal of dead embryos and liquids
- Pencil
- Binder to hold all research papers
- 1 / Dissecting microscope
- 3 pairs of gloves for each day the solutions were switched

Methods:

Day 1:

The embryos were obtained by the group. Gloves were worn to avoid skin contact. The embryos were placed into the multi-welled plate. Within the plate, there were 12 wells, 4 of which were used for the experiment. The embryos were placed in the wells with the transfer pipette and natural solution that they were in. The liquids were very carefully switched out using a small pipette and the microscope to see exactly what was getting removed from the well, which should have just been the natural liquid that the embryos came in. Once the original liquid had been removed, the wells were filled with the stock solutions of nicotine (0.0 mg/mL, 0.05 mg/mL, 0.1 mg/mL, and 0.2 mg/mL).

Day 2:

Gloves were worn to avoid skin contact. The solutions were exchanged for fresh solutions, using a pipette, and no eggs were destroyed or taken out. The only eggs that were removed were already dead, and they were removed when the solutions were switched out

Day 3:

Gloves were worn to avoid skin contact. The shells from the eggs were removed by using the microscope to see and the pipette to remove the eggshells when the solutions were exchanged. Most of the fish were still alive, though some had died. The dead fish were also transferred out of the wells with the old solution and eggshells.

Day 4:

Gloves were worn to avoid skin contact. The group switched out the solutions and discarded any dead fish or eggshells using the microscope to see the dead fish and remaining eggshells and using a pipette to switch the solutions and remove the waste. The live fish were transferred to a bigger tank with the fish from the rest of the class.

A chi-square analysis was performed to ensure statistical significance.

Results

The reason for the research and experiment was to see how nicotine affects the growth of zebrafish embryos. The experiment was set up the way it was because to be able to see how the fish would react to the nicotine after multiple days and to see the way the fish grew. The hypothesis: If the nicotine affects the embryos and fish growth negatively, then the nicotine is non-beneficial to their growth and development because then if the nicotine is in their systems, they will not grow and

develop and most likely will die. The embryos had started to look as if they had fish inside of them. The embryos were looked at closer under the dissecting microscope to see if the eggs were still alive and healthy. The eggs had hatched but the zebrafish weren't moving around just yet because of the exhaustion it took to hatch. All of the fish had been hatched at this point, but a few more of the fish had died. The dependent variable was how much the embryos and fish grew. The independent variable was the amount of solution in each well (0.5 mg/mL, 0.1 mg/mL, 0.2 mg/mL, and the controlled amount of 0.0 mg/mL). These are related because it shows the amount of nicotine taken into the fish and embryos, and how the fish were affected by the nicotine. In the experiment the chi square value was 30.42, and when compared to the critical value, 7.82, it is more, showing that the null hypothesis was rejected. The degree of freedom to help get the critical value on the chi square, is 3. These results from the chi square show that the null hypothesis was rejected meaning the results were not due to change but nicotine did have an effect on the developing embryos mortality rate.

Figure 1:

Days of Live Zebrafish

The Amount of Eggs Changes

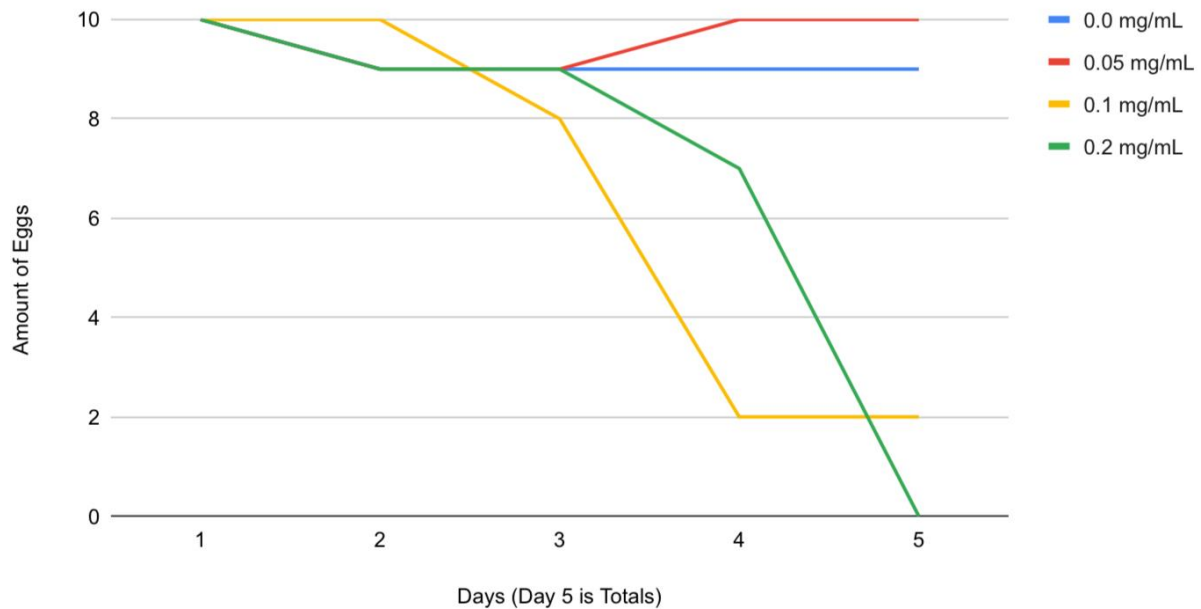


Figure 1. Showing a decrease in the number of embryos and zebrafish.

Days of Live Zebrafish

Table 1. Showing the exact amounts of fish and embryos left after each day.

Days of Live Zebrafish	Day 1	Day 2	Day 3	Day 4	Day 5
0.0 mg/mL	10	9	9	9	9
0.05 mg/mL	10	9	9	10	10
0.1 mg/mL	10	10	8	2	2
0.2 mg/mL	10	9	9	7	0

Discussion

The results of the experiment showed that nicotine is a non beneficial thing to the zebrafish, which could end up making the zebrafish population decrease. The results proved the hypothesis to be true. In the experiment, it showed that nicotine is bad for developmental health and growth. There have been studies on pregnant women showing that the women who smoked during pregnancy had more problems with a healthy developing fetus and baby, while the non-smoking women had healthier and more developed babies. These results also showed that when embryos in a nicotine solution didn't develop as well as the embryos in the control solution, which was normal water, and that the zebrafish weren't as responsive to their natural environment as a normal zebrafish. The hypothesis, stating the nicotine is terrible for the embryos and fish, was true because looking at the results, the fish and embryos were dying in higher concentrations of nicotine compared to the controlled and smallest amounts. The question the group is trying to answer is, does nicotine positively or negatively affect the growth of zebrafish? The purpose of this question was to see how the nicotine affected the zebrafish during and after exposure to nicotine and to see if the fish grew at all during the process, and they didn't grow very much. From this experiment, what had been learned was that nicotine is a very terrible drug and stunts the growth and development of embryos and zebrafish and which shows that, if a pregnant woman ingests nicotine it will affect fetal growth and development, and could possibly kill the fetus. This experiment also shows that if nicotine is ingested in large amounts, like cigarettes, it will end up killing the invertebrate faster. In the experiment the chi square value was 30.42, and when compared to the critical value, 7.82, it is more, showing that the null hypothesis was rejected. The degree of freedom to help get the critical value on the chi square, is 3. These results from the chi square show that the null hypothesis was rejected. The null hypothesis is, if nicotine was good for the embryos and zebrafish, then it is a positive benefit to the growth and development of embryos and zebrafish, showing that nicotine doesn't kill the fish off. Some of the limitations of the experiment were not having more embryos and zebrafish to experiment on, or having more stock solutions to test. Some things that could have been improved were making sure to count correctly and to steadily and carefully watch the solutions, instead of being slightly sloppy with the work. The logic to accepting the hypothesis is that the hypothesis had said that nicotine would negatively impact the growth and development of the embryos and zebrafish, and so with the hypothesis being true, it shows that the experiment had gone correctly. There was only one error in the experiment, which was on the first day the control group of embryos didn't have the water to hatch and grow in, because the person transferring them to the

well didn't realize there was no water in the well, but otherwise there weren't any errors in the experiment. This experiment has shown that nicotine is terrible for development and growth, and that when the zebrafish were in higher concentrations of nicotine, they died off faster than the ones in the lower concentrations or the controlled concentration. The development of the fish was limited because of the fact that nicotine basically kills.

References

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