Effects of Ethanol and Nicotine Concentrations on Zebrafish Embryo Development

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Abstract

The aim of this experiment was to examine the effects that different concentrations of ethanol and nicotine had on developing zebrafish embryos over a course of 96 hours. The results would then be used as a model for the health of other vertebrates, such as humans. The different concentrations of nicotine and ethanol were added to a multiwell plate, each well containing zebrafish embryos. Over a period of 96 hours, the zebrafish were closely observed for any birth defects and major trends in the amount of fatalities. It was observed that the highest concentration of both chemicals had the highest number of deaths. As well as this, it was found that high concentrations led to birth defects such as low pigmentation. There was also a correlation between the amount of deaths and time. These results demonstrated the negative effects of nicotine and ethanol on not just zebrafish, but all developing vertebrates. Thus, our investigation concluded that in humans (a vertebrae), the consumption of nicotine and ethanol during pregnancy risks birth defect for the newborn, as well as possible death.

Introduction

The direction of this experiment was to detect if zebrafish embryo development is affected when exposed to various concentrations of nicotine and ethanol. Since the development of zebrafish is similar to that of other vertebrates such as humans, this experiment could signal the effect of nicotine and ethanol in fetal development. Zebrafish also have a very high reproduction rate and are very easily maintained and manipulated, which makes it a convenient task to experiment with them (Badman,et.al., n.d.).

In this experiment, we used multiple concentrations of ethanol, or alcohol, a substance found in alcoholic beverages that are commonly consumed by humans. Ethanol, when consumed at high concentrations and amounts, can cause several issues in the human body. This includes issues in the liver such as Fibrosis, and in the heart, often causing Arrhythmias (an irregular heartbeat) and increasing one's blood pressure. Experimentation has also determined that over-consumption of ethanol can cause cancer in the mouth, esophagus, throat, liver, and breast Finally, alcohol overconsumption can risk the weakening of the immune system, allowing the body to become much more subject to disease (“Alcohol's Effects on the Body,” n.d.). Studies

have also shown that ethanol consumption in pregnant women has negative effects on the fetus. The alcohol the mother consumes is quickly also transported to the baby through the umbilical cord, thus all the negative effects such as cancer, heart problems, lung problems, and a weakening immune system that the mother might encounter, will also affect the baby. However, it has been found that there are even more problems that may arise from this scenario. Ethanol can lead the baby to have health conditions called fetal alcohol spectrum disorders, or FASD. Additionally, alcohol can cause the baby to have birth defects, intellectual disabilities, learning problems, speech and language delays, behavioral problems, vision and hearing problems, and to be born too soon ("Alcohol during pregnancy," 2012).

As well as ethanol, we experimented with different concentrations of nicotine. Nicotine is an addictive and harmful toxin commonly found in human consumed tobacco cigarettes (Mishra, 2015). It has been found that consuming tobacco during pregnancy can greatly put the developing fetus at risk. In fact, it can often result in an increased morbidity as well as mortality. Nicotine is a leading cause of the abnormalities in the brains of newborn children. Many different studies also found that tobacco exposure during pregnancy can lead to a great number of birth defects such as troubles in cognitive skill development as well as behavior in children and adolescents. Many parents choose to substitute smoking tobacco with smokeless cigarettes. However, this type of consumption of nicotine has also been found to create development issues in a child (Wickström, 2007).

We hypothesised that if developing zebrafish embryos are exposed to multiple concentrations of nicotine and ethanol, then they will experience birth defects, because nicotine and ethanol are harmful substances. We also predicted that the higher the concentration of nicotine and ethanol, the more deaths will occur.

Materials

The materials used in this experiment consisted of zebrafish (*Danio rerio*) embryos, a minimum bore 1.5mm pipettes, large bore pipettes, multi-well plate with lid, a sharpie for labeling, a dissecting microscope, a standard smartphone camera for data collection, a beaker for dead embryos and liquid disposal, Instant Ocean/Embryo Media Solution, an incubator set at

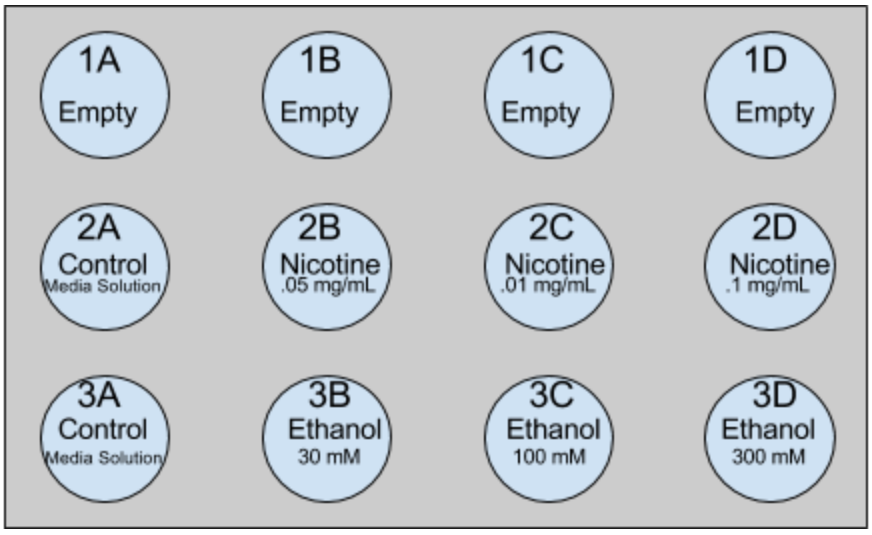
around 28℃ for storage of the multiwell plate, Nicotine solution at concentrations of 0.01, 0.05, and 0.1 mg/mL, and ethanol solution at concentrations of 30, 100, and 300 mM.

Method

First, 1 mL of each concentration of Nicotine solution (0.01, 0.05, and 0.1mg/mL), 1 mL each concentration of the ethanol solution (30, 100, and 300 mM), and 1 mL of Instant Ocean/ Embryo Media Solution was added to its assigned well. To that, 8 zebrafish (*Danio rerio*) embryos were added to each well consisting of solution. Following this, data was collected by using a standard smartphone camera to take pictures of fish from each well.

After 24 hours, the wells were examined for any deaths and birth defects that may have taken place using a dissecting microscope, and the quantitative data was then recorded. Next, additional data was collected by using a smartphone camera to take photos of Zebrafish from each well. Next, each dead zebrafish embryo was collected using a 1.5mm pipette and disposed of. Then the solution from each well was drained and clean solution was added. The well was placed in the incubator at around 28℃. This process was repeated 48 and 72 hours after the fertilization of the embryos. Finally at 96 hours, the the wells were examined for any deaths and/or birth defects, and the remaining zebrafish, as well as the chemicals, were disposed.

Diagram 1: The diagram below shows the multiwell plate used in this experiment, and the concentrations of each solution in each well.

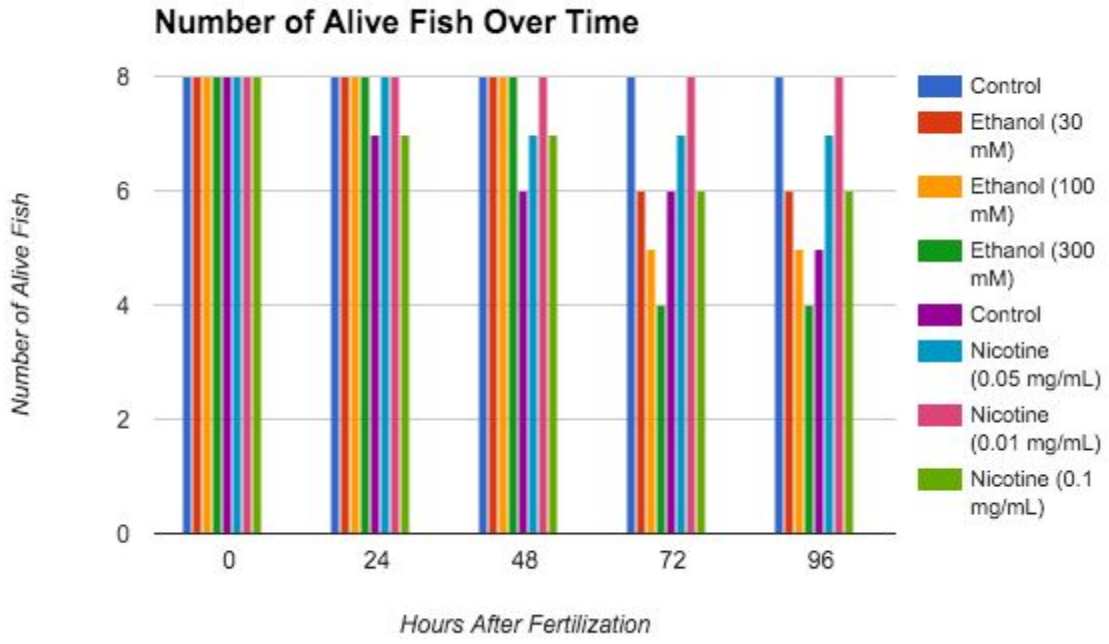


Results

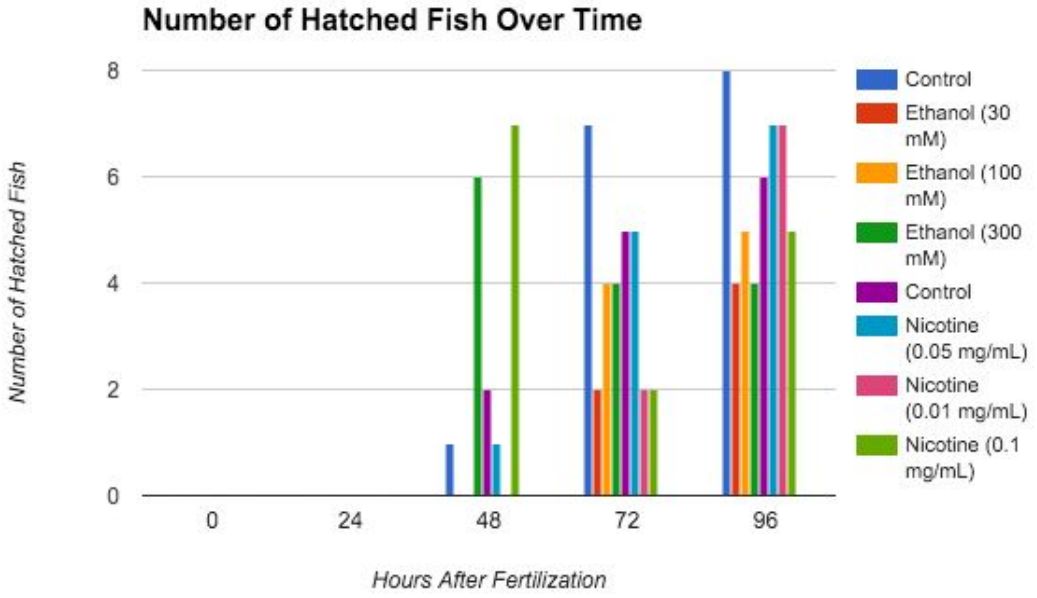
Table 1: The number of alive and hatched embryos after the given amount of type.

Number of Alive and Hatched Embryos Over Time										
Treatment	Well	# of Starting Fish	24 Hours post Fertilization		48 Hours post Fertilization		72 Hours post Fertilization		96 Hours post Fertilization	
			# hatched	# alive	# hatched	# alive	# hatched	# alive	# hatched	# alive
Control	2A	8	0	7	2	6	5	6	6	5
Nicotine (0.05 mg/mL)	2B	8	0	8	1	7	5	7	7	7
Nicotine (0.01 mg/mL)	2C	8	0	8	0	8	2	8	7	8
Nicotine (0.1 mg/mL)	2D	8	0	7	1	7	2	6	5	6
Control	3A	8	0	8	1	8	7	8	8	8
ethanol (30 mM)	3B	8	0	8	0	8	2	6	4	6
ethanol (100 mM)	3C	8	0	8	0	8	4	5	5	5
ethanol (300 mM)	3D	8	0	8	6	8	4	4	4	4

Graph 1: The graph below depicts the number of surviving fish over 0, 24, 48, 72, and 96 hours post fertilization.



Graph 2: The graph below depicts the number of hatched fish over 0, 24, 48, 72, and 96 hours post fertilization.



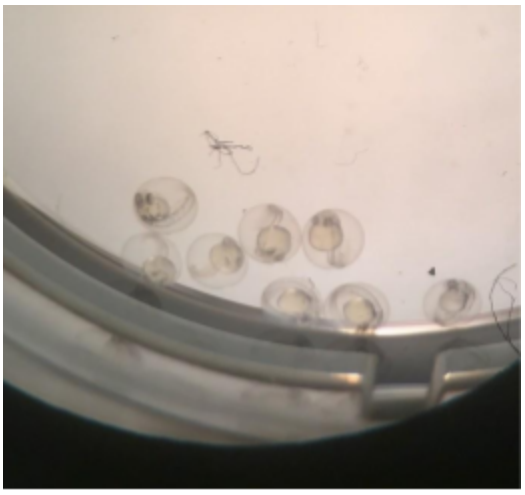


Figure 1: Zebrafish embryos from well 2B in 0.05 mg/mL of nicotine solution at 24 hours post fertilization. Development seems normal.

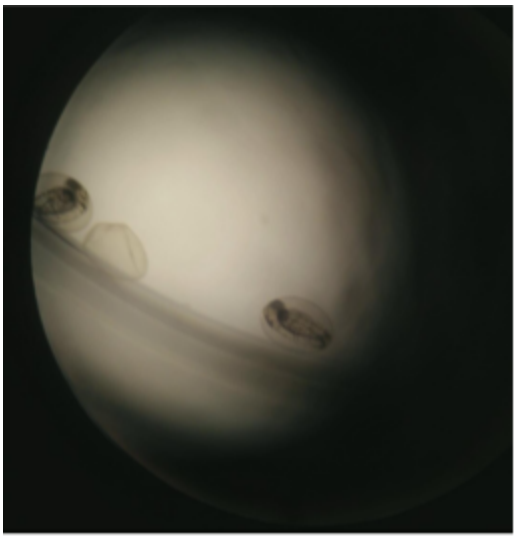


Figure 2: Zebrafish embryos from well 2A in the control Ocean/Embryo Media Solution at 48 hours post fertilization. Development seems normal, and few have hatched. Distinct features such as the heart and eyes are becoming noticeable.

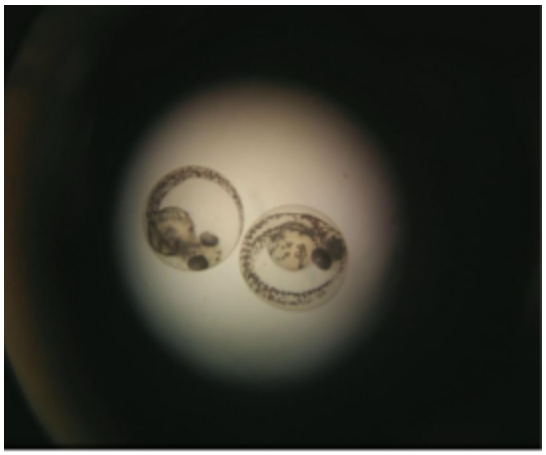


Figure 5: Zebrafish embryos from well 3B in 30 mM concentration of ethanol at 48 hours. Development seems to be normal.

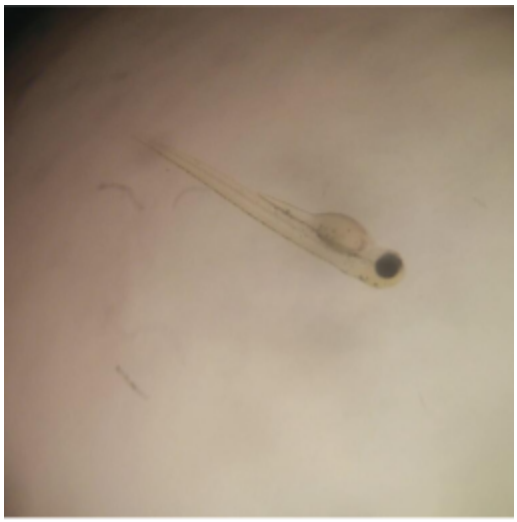


Figure 6: Little to no pigment is observable in this zebrafish from well 3D in 300 mM of ethanol solution at 72 hours.

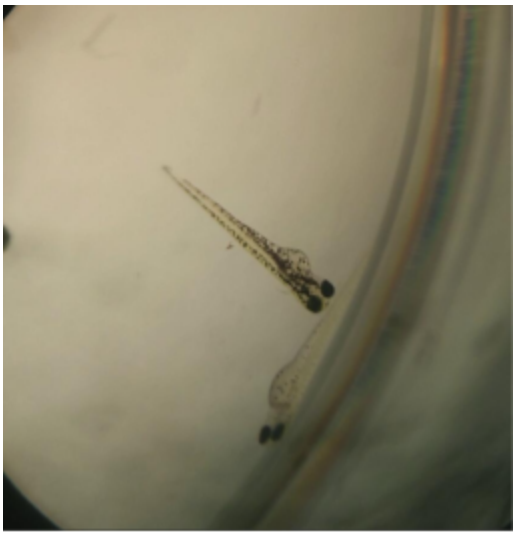


Figure 7: The fish with little to no pigment (Fish 1) can be easily compared to another fish in the same well with pigment (Fish 2). This is from well 3D in 300 mM of ethanol solution at 96 hours.

Control: At 24 and 48 hours, all zebrafish appeared to be developing normally. However, in well 2A, there was a varying rate of death of the zebrafish embryos submerged in the control Ocean/Embryo Media solution. At 72 hours, a fish in well 2A developed pericardial edema, or pericardial effusion. In well 3A, which also contained the control Ocean/Embryo Media solution, at 96 hours, all zebrafish were still alive and had hatched. There were also no signs of any birth defects.

Nicotine: At 24 hours, as shown in *Figure 1*, and 48 hours, as shown in *Figure 2*, development of the zebrafish embryos seemed normal at all concentrations. At 48 hours in all wells, some zebrafish had already hatched, and almost all were still alive. Their hearts as well as eyes were becoming observable. However once it got to 72 hours, the effects of the nicotine became increasingly noticeable. The amount of deaths increased; especially at a concentration of 0.1 (the highest concentration) where only 6 zebrafish remained alive. From those 6, only 2 hatched, signaling that the embryos left unhatched had trouble hatching. Finally, at 96 hours, it was observed that there was still one embryo that had not yet hatched in well 2D. As well as this, one of the fish in well 2D had barely, if at all, developed any pigment as shown in *Figure 4*.

Ethanol: Similar to the results of nicotine, at 24 hours as well as at 48 hours, development of the zebrafish embryos seemed normal at all concentrations. At 48 hours in all wells, some zebrafish had already hatched, and almost all were still alive. Their hearts as well as eyes were becoming observable. However, at 72 hours the effects of the ethanol became noticeable. Similar to well 2D, one fish had barely, if any, development of pigment in well 3D. The higher the concentration of ethanol, the more dead zebrafish there were. In fact, in well 3D at a concentration of 300 mM, the zebrafish population had decreased with only 50% of the zebrafish still alive.

Conclusion

It was clearly noted that as time went on, there were more and more deaths. As well as this, it was noticed that the higher the concentration of nicotine and ethanol, the higher the amount of deaths. For example, the number of living fish for 0.1 mg/mL concentration of

nicotine started at 8, and went down to 6 living zebrafish. Similarly, the number of living fish for the 300 mM concentration of ethanol started at 8 fish, and then dramatically went down, decreasing to only 4 living fish at 72 hours. As well as this, there were very low hatching rates in high concentrations of both solutions, making it easily concluded that the higher the concentration, the less the fish hatch. Finally, it can be concluded that high levels of nicotine and ethanol cause birth defects. This was especially noticed in our experiment at 72 hours in wells 2D and 3D where the zebrafish had barely, if not at all, developed pigmentation.

Our data displayed that there were negative effects on zebrafish embryos over time, in higher concentrations of nicotine and ethanol. This correlation can be tied very easily to other vertebrates, since zebrafish (which are vertebrates) are excellent models. Humans, who are vertebrates, many times consume ethanol (alcohol) and nicotine during pregnancy. Many women do not know the significant effect these substances can have on their baby. For example, as shown by our investigation, zebrafish, and vertebrates alike, obtain birth defects and are more prone to die when in they are exposed to nicotine and ethanol in the development stage. This could be a crucial study which may help decrease greatly decrease the infant mortality rate.

Before conducting this investigation, we hypothesised that if developing zebrafish embryos are exposed to multiple concentrations of nicotine and ethanol, then they will experience birth defects. We also predicted that the higher the concentration of nicotine and ethanol, the more deaths will occur. The results of our experiment support this hypothesis. As predicted, nicotine and ethanol had negative effects, including birth defects, on the developing zebrafish. As wells as this, the correlation between high concentrations and higher likelihood of deaths and birth defects was also observed during our investigation.

A possible source of error was when replacing the the solutions of Ocean/Embryo Media, ethanol, and nicotine; the embryos could have been subject to contamination of the solutions. This error could be improved by using a different pipette for every well so that there would be no cross-contamination. Another possible source of error included harming the fish by vacuuming them out of the well, thinking they were dead. As well as this, since our experiment only consisted of 8 fish per concentration of solution, it could mean that our results may be considered purely chance. The experiment could be improved by using a larger sample of zebrafish embryos

to increase the accuracy of the data. And finally, the heartbeat of the fish could have been recorded to examine the data at another angle, for more accurate results and conclusions.

Questions

This investigation led to questions regarding following experiments that could be conducted to further expand knowledge about this topic. What would the results be if our sample size was much bigger than 8 fish per concentration? How would the results vary if extra concentrations of ethanol and nicotine were used for experimentation? Would it have been more beneficial to experiment with more than one well per concentration of ethanol and nicotine? What other ways can we experiment the effect of nicotine and ethanol on human health?

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