The Effect of Nicotine on Development of Zebrafish Embryos
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Abstract:
More than 22 million American women smoke and approximately 15-20% of these women continue to smoke during pregnancy, this leads to the inhalation of nicotine. This affects embryonic development negatively because nicotine is harmful to various processes of development. Nicotine exposure is known to cause many problems. It ranges from premature births to an increase in heart rate. The purpose of this experiment was to show the negative effects of the chemical nicotine to zebrafish embryos so that people are more aware. To test the effects of nicotine, zebrafish embryos were placed in a multiwell container and four of the wells were filled. Then various concentrations of nicotine were added, exposing the embryos to the harmful chemical. Throughout the four days, the development of the embryos were recorded, some embryos were deformed and some had died. Since zebrafish shared some similar characteristics to human, zebrafish were an ideal subject to test. The concept of this experiment is to determine the developmental delays, diseases and deaths caused by the exposure of nicotine in humans.

Introduction:
Over the past weeks, the effects of nicotine were studied on zebrafish. The effects of nicotine were tested on zebrafish because zebrafish develop fast and they possess similar characteristics of humans, this allows the effects of nicotine to be shown in a safe way. According to the Kimmel, C. B. (1995, January). Stages of embryonic development of the zebrafish. The zebrafish have seven developing stage of embryogenesis; “zygote, cleavage, blastula, gastrula, segmentation, pharyngula, and hatching.” Each stage of embryogenesis is extremely important for the developing zebrafish embryos because the end result will dictate how the zebrafish will live and whether or not it will reproduce. In the zygote stage, the zebrafish is fertilized and encoded in the DNA. In the cleavage stage, the YSL (yolk syncytial layer) is formed. In the blastula stage, the embryos form the blastocoele. In the gastrula and segmentation stage, the term ring is formed which dictates where bodily organs will be formed, the dermis, vertebrae and skeletal muscles are formed, the beginning of the primary organs also develop along with the neural cord and the notochord, the body also moves for the first time. During the Pharyngula and hatching stage the body axis begins to straighten, the nervous system nervous system is hollow and is expanding, the circulatory system develops and the heart starts to beat causing blood to circulate. At the end of the seventh stage, the zebrafish will begin to hatch and mature to a fully healthy zebrafish adult. The effect of nicotine can affect any of these stages causing the zebrafish not to develop right. This experiment tests the effects of nicotine on the development of the embryo.

According to the Mishra, Aseem, et al. “Harmful Effects of Nicotine.” Indian Journal of Medical and Paediatric Oncology : Official Journal of Indian Society of Medical & Paediatric Oncology, Medknow Publications & Media Pvt Ltd, 2015., humans can be affected by the
nicotine just like the zebrafish, which makes it important for younger generations to test and understand the effect of nicotine. Nicotine is a toxic stimulant that is the main ingredient in tobacco. 15 to 20 percent of pregnant women smoke tobacco and through research smoking tobacco during pregnancy was found to cause delayed skeleton growth, premature births, underdevelopment, and behavior issues to the baby. Nicotine was also found to cause a large increase of heartbeat, and many diseases such as lung cancer, emphysema, chronic bronchitis, cancer, especially in the respiratory system, leukemia, heart disease, stroke, diabetes. Nicotine also causes about ⅓ of cancer deaths.

The purpose of the experiment is to demonstrate the effects of nicotine on the development of zebrafish embryos relating to the development of human embryos. This experiment is extremely important in today's society because so many women smoke during pregnancy, and if woman know about this study they will be informed with the negative outcomes of inhaling nicotine and how it affects their child's development.

The experimental hypothesis was if embryos were exposed to high amounts of nicotine then the embryos would have slow development, underdevelopment, and premature births because nicotine interferes with the stages of early embryonic development.

**Materials and Methods**

**Materials:**
- (1 bottle) Stock solution of Nicotine (0.05, 0.1, 0.2 mg/mL nicotine)
- (1) Beaker for dead embryos and liquid disposal
- (1) Sharpie
- (1 bottle) Instant Ocean/Embryo Media Solution
- (4) Large bore transfer pipette, minimum bore (1.5 mm for transferring eggs to observation container and manipulating then in the container)
- (4) Transfer pipette
- (1) Multi-well plates
- (1) 28.5 °C incubator
- (1) Dissecting microscope
- Student data sheet

**Methods:**

The multiwell plate was labeled to show the concentration of nicotine in the water. Using latex gloves, four of the wells were filled with four different solutions of nicotine. The first well was a control group and it was filled with instant ocean. The second well was an experimental group, it was filled with a solution that was 0.05 mg/mL nicotine. The third well was an
experimental group, it was filled with a solution that was 0.1 mg/mL nicotine. The fourth well was also an experimental group, it was filled with a solution that was 0.2 mg/mL nicotine. Using latex gloves ten embryos were transferred into each of the solutions with a transfer pipette. Residual solution was removed from transfer, and nicotine solutions were added and the data was collected. The number of embryos were double checked by using a dissecting microscope. The multiwell plate was stored in an incubator overnight at 28.5 degrees Celsius. Twenty-four hours later or on day 2 the well plate was removed from the incubator. With a dissecting microscope the embryos were checked for the amount that died or hatched. Any blackish colored (dead) embryos were removed from the multiwell, the amount of live and dead embryos were recorded in a data table. Using a transfer pipette the nicotine solutions were removed from each well and replaced with the solution, the same amount of nicotine was replaced. The multiwell plate was placed under a dissecting microscope and each well was observed, and the observations were recorded on a student data sheet. The observations were recorded on a student data sheet. The multiwell plate was moved back to the incubator. Twenty four hours later or on day 3 everything on day 2 was repeated and new data was recorded. Twenty four hours later or on day 4 repeated day 3 recording all data. Then the live fishes were separated from the dead fishes and other organisms. The live fishes were placed into an incubator with all the other fishes from the different groups. The dead organisms were disposed down the sink.

A chi-square analysis was performed to test for statistical significance of data.

**Results:**
Embryos Alive in a Different Nicotine Concentrations

Amount of Live Embryos

0 24 48 72 96

Hours

0 2.5 7.5 10

Control
0.05 mL Nicotine
0.1 mL Nicotine
0.2 mL Nicotine

Embryos Hatched in Different Nicotine Concentrations

<table>
<thead>
<tr>
<th>HOURS</th>
<th>Control</th>
<th>0.05 mL Nicotine</th>
<th>0.1 mL Nicotine</th>
<th>0.2 mL Nicotine</th>
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<tr>
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<td>9</td>
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</tbody>
</table>

Embryos Hatched in a Different Nicotine Concentrations

Amount of Embryos Hatched

0 24 48 72 96

Hours

0 2.5 7.5 10

Control
0.05 mL Nicotine
0.1 mL Nicotine
0.2 mL Nicotine

0.05 mg/mL of nicotine

0.2 mg/mL of nicotine
The zebrafish lab was conducted to test the effects of different levels of nicotine on zebrafish. It was hypothesized that if zebrafish were placed in water with nicotine, then the zebrafish would have many effects including premature birth, delayed growth and slowed skeletal growth because of the strong effects of nicotine. For the experiment, the embryos were separated into four wells with anywhere from 0 mL of nicotine to 0.2 mL nicotine. The independent variable was the concentration of nicotine and the dependent variable was the number of live and hatched embryos.

The embryos in the highest nicotine concentration hatched a day earlier than the controlled group showing premature birth. The zebrafish with nicotine developed faster but had more deformations. The zebrafish that were exposed to nicotine had a noticeable increase in their heartbeat compared to the control group. During the experiment, it was observed that little parasites called protozoa were eating the zebrafish that had already died. It was also observed that the zebrafish in the wells with nicotine were moving slowly or were struggling to move. It was found that the higher the concentration of nicotine the higher chance the zebrafish was to hatch early and die. Although many side effects of nicotine were shown there were many things that could not be shown because of lack of technology such as; cognition, mental disabilities, societal issues, and behavioral issues.
Discussion:

After four days of recording the development of zebrafish it has been proven that the higher solutions of nicotine negatively affects their health. The results support the initial claim, the higher level of nicotine in the well, will cause more zebrafish to die, have birth defects, slowed skeletal growth, premature births, underdevelopment, and an increased heart rate. Due to the exposure of nicotine, the zebrafish prematurely hatched, some developed deformities and some just died. Since they were born premature the developments of the zebrafishes were delayed, causing them to struggle with something like swimming because the lack of strength. It was also recorded that the zebrafishes with the most percentage of nicotine in their well had a higher heart rate. As a result in an increased heart rate, it is speculated the zebrafish can suffer from a heart attack and since they have similar characteristics as humans it can be correlated to human health. This experiment showed many side effects of nicotine but further research should be conducted to test the things we cannot test, because of the lack of tools, such as mental health, depression, domain effects, and nervous system effects. If the nervous system is affected by nicotine, the fish might have the following symptoms, not eating, increased blood pressure or low activity. Further testing could have included the number of deformed zebrafishes in each well.

The results supported the hypothesis and expanded the knowledge of the effects of nicotine during embryonic developmental stages. How hypothesis was supported more at higher levels of nicotine. Further experiments should occur to find out the effects nicotine on mental health by using better technology and a better experimental group. This could be concluded by having a bigger dependent group (Zebrafish).

References

Effect of Nicotine on Chick Development,

Kawakita, Atsuyo, et al. “Nicotine Acts on Growth Plate Chondrocytes to Delay Skeletal Growth through the α7 Neuronal Nicotinic Acetylcholine Receptor.” *PLOS ONE*, Public Library of Science, journals.plos.org/plosone/article?id=10.1371%2fJournal.pone.0003945 (link doesn’t work—doesn’t get me to any article)


Mrs. Wickham’s citations are below:

