The Effects of Vitamin E as a Counteractive Treatment for the Toxins Nicotine and Ethanol

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Abstract:

In the following experiment, we tested the counteractive effects of vitamin E on known toxins ethanol and nicotine, which negatively impact embryonic development. The goal of the experiment was to simulate what a human embryo, exposed to a harsh embryonic life, in combination with vitamin E, would experience. The results could help make decisions or provide new information to give to expecting mothers. We kept track of the abnormalities in each of the zebrafish embryos to record whether or not their chances of abnormalities decreased when treated with vitamin E. Toxic ethanol and nicotine were each tested individually on zebrafish embryos. The trials with ethanol and nicotine were repeated with the introduction of vitamin E, a vitamin thought to have counteractive effects on the damages caused by these harmful chemicals. At the conclusion of the experiment, it was determined that a significant difference did not occur when treated with vitamin E. However, the door of opportunity for further exploration of this treatment is wide open.

Introduction:

Toxins are easy to come by in today's society. Many women live in environments where they are surrounded by toxic chemicals that can decrease the viability of their embryos, and cause damage during pregnancy. Early exposure to toxins such as nicotine and alcohol can have damaging effects on the development of embryos. These toxins can lead to several chronic illnesses because of the oxidative damage created by oxidants and free radicals (5, 3). In some cases, exposure to nicotine and ethanol is unintentional or unavoidable: many human pregnancies are not detectable until after five weeks of conception, leaving over a month in which mothers may unintentionally expose their fetus to damaging toxins at a critical stage of

development. While the effects of known toxins like nicotine and alcohol on embryos are widely known, preventative measures are not commonly discussed. Antioxidants are able to combat the increase of free radicals by preventing their formation or promoting their decomposition (5). One example of an antioxidant that has shown promising evidence for its counteractive effects against toxins is vitamin E. According to an article about the effect of vitamin E and mouse liver, published in the National Medicine Library, "vitamin E can mitigate the toxic effects of alcohol and can be suitably used as a potential therapeutic agent for alcohol-induced oxidative damage in the liver" (3). In another study using mice brains instead of mice livers, similar results were yielded regarding vitamin E's reductive effects on alcohol-related oxidative damage (2). Not only is vitamin E proven to counteract the oxidative damage from alcohol, but there are also studies about the counteractive effect of vitamin E on nicotine. According to an article on Science Daily, "Vitamin E is one of the first lines of defense in human lung tissue against the ravages of cigarette smoke… If the body has adequate levels of vitamin E, this protective antioxidant can …prevent the destruction of lung membranes" (1). Furthermore, in a study testing the effects of vitamin E on oxidative stress in smokers, The Malaysian Journal of Medical Science concluded that daily vitamin E supplementation reduced oxidative stress smokers faced (4). If vitamin E is shown to reduce the damage of these toxins on embryos, women can use vitamin E supplements to protect themselves and their fetuses against nicotine and ethanol before, during, and after pregnancy.

In this experiment, the effects of vitamin E on ethanol and nicotine-exposed zebrafish embryos will be tested as a model for human fetuses. Zebrafish are close enough to make an appropriate model because 70% of human genes are found in zebrafish. Also, zebrafish share many of the same organs as humans, namely eyes, a mouth, brain, spinal cord, intestines, heart,

ears, nose, muscles, blood, bone, cartilage, and teeth. Since humans and zebrafish share so many of the basic elements, they are an appropriate model for human development. Because of the relationship between humans and zebrafish and the aforementioned studies, it is hypothesized that vitamin E will reduce the physiologically damaging impacts of nicotine and alcohol in zebrafish embryos as measured by physical deformities and survival rates.

Methods and Materials:

- 4 Falcon dishes
- 75-78 Zebrafish Embryos
- 0.25 mL liquid vitamin E per 50mL solution
- 50 mL each solution
    - Nicotine (0.05 mg/mL)
    - Ethanol (0.11 mg/mL)
    - Ethanol (0.11 mg/mL) and vitamin E (25 mg/L)
    - Nicotine (0.05 mg/mL) and vitamin E (25 mg/L)
- Instant Ocean solution (IOS) at a concentration of 200 mg/L
- 6 Pipettes
- 5 Flasks
- Incubator at 28 Degrees Celsius

Zebrafish embryos were produced in tanks within Muskego High School and were placed in Instant Ocean solution at a concentration of 200 mg/L. After the embryos were produced, solutions of each concentration being tested were created. The first solution was the control and consisted of 50 mL of 200 mg/L Instant Ocean solution (IOS). The second solution was 50 mL

of IOS and 10 mL of liquid nicotine. The third solution was 50 mL IOS, 10 mL liquid nicotine and .25 mL liquid vitamin E. The fourth again had 50 mL IOS as well as 5.5 mL liquid ethanol. The final solution was once again 50 mL IOS in combination with 5.5 mL liquid ethanol and .25 mL liquid vitamin E. Three mL of each solution was added into each of 15 wells depending on the solution being tested. Shortly afterward, five zebrafish embryos were added to each well using a sterile pipet. We encountered an error during this step when eight embryos were added to the first well (trial) of the nicotine (0.05 mg/mL) solution. All other wells contained the appropriate number of embryos, for a total usage of 78 embryos. Data was collected at the same time daily for four days. Developmental stages as well as health issues like edemas, deformations, and death were consistently observed. Each day of the trial the number of dead versus living embryos was recorded in addition to the stage of development and any visible defects or abnormalities. After the recording was completed each day, the embryos were placed in an incubator at 28 degrees Celsius. On days two and three the solution in each well was carefully taken out by tipping the Falcon dishes to allow the embryos to collect on the bottom, and then carefully removing as much old liquid as possible utilizing one pipet per solution. After the liquid was taken out, a new liquid of each solution was added to the corresponding well. On the fourth day of the experiment, the total number of living embryos was recorded in addition to any malformations. The number of living embryos was subsequently used in completing a fischer test to determine if there was a significant difference in the number of living embryos in each solution compared to the control.

Results:

After exposing some embryos to nicotine, ethanol, and these toxins in combination with vitamin E, we concluded that there was not a statistically significant difference between the groups exposed to the toxin and those with the toxin and vitamin E. Our independent variable was the substance being tested: nicotine, ethanol, and vitamin E.. Our dependent variable was the number of living fish; however, developmental stages were also recorded. Our control group was not exposed to nicotine, ethanol, or vitamin E. While the results were not significantly different, further testing could yield interesting results.

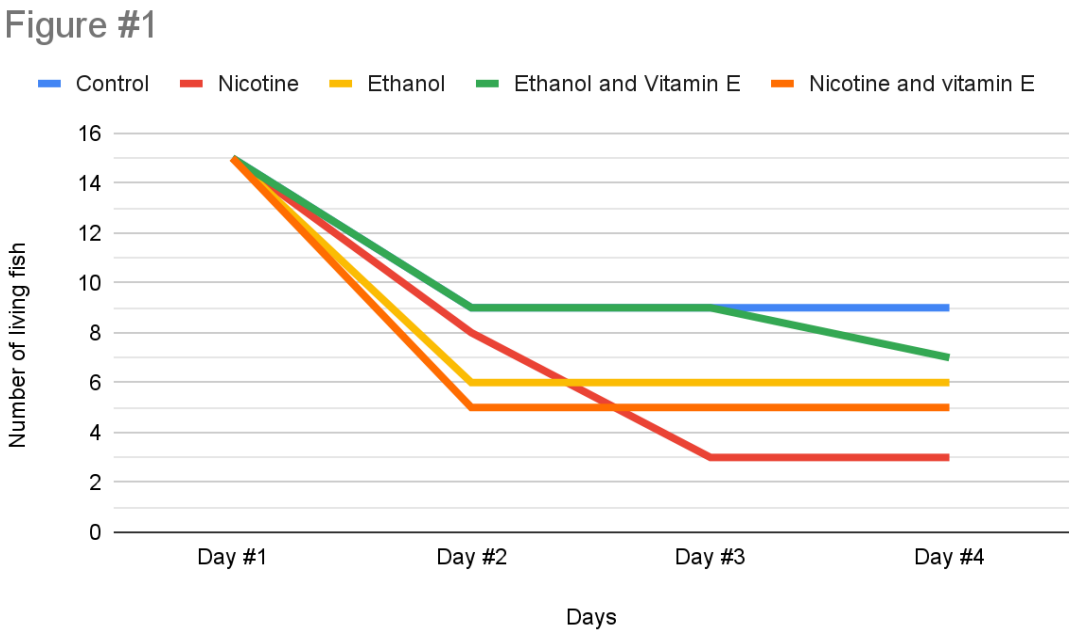

Figure 1 (above) shows the number of living zebrafish for each of the five different solutions. Compared to the control's results, nicotine showed the most fatalities; ethanol with vitamin E showed the least fatalities compared to the control. This graph shows no significant difference between substances mixed with vitamin E and substance without vitamin E.

Figure 2

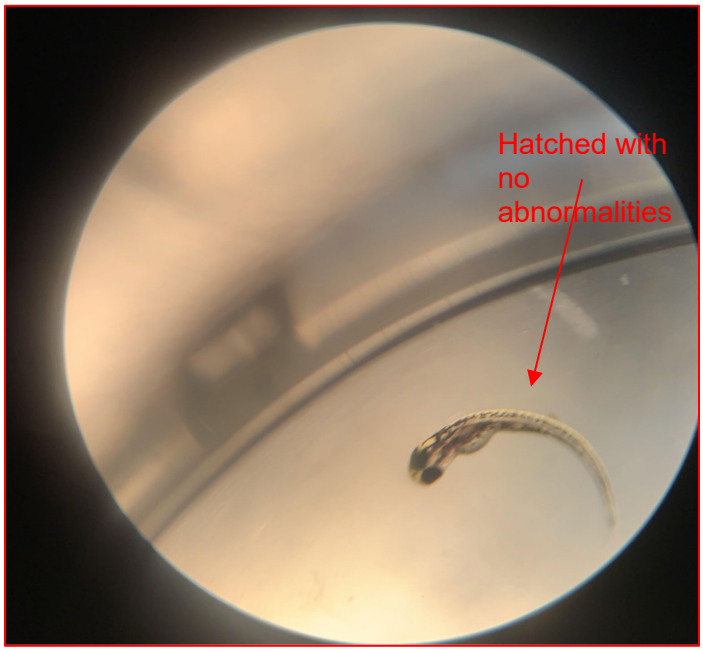

Figure 2 (above) shows one newly hatched zebrafish from the control group on day 4. Our control group was fully hatched on the 4th day of the experiment, and the zebrafish developed at a normal rate. The zebrafish showed no sign of abnormalities.

Figure 3

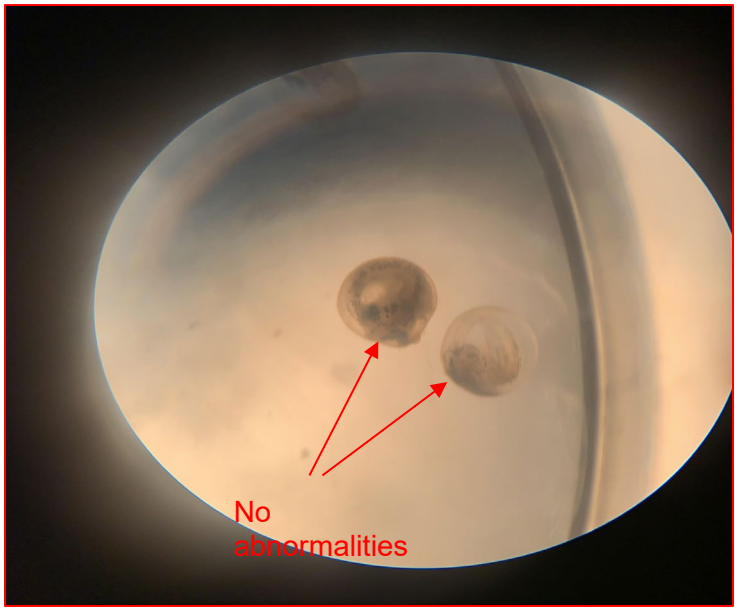

Figure 3 (above) shows the nicotine group on day 4. Our nicotine group showed slow development as well as a low survival rate. However, it did not show any sign of abnormalities.

Figure 4

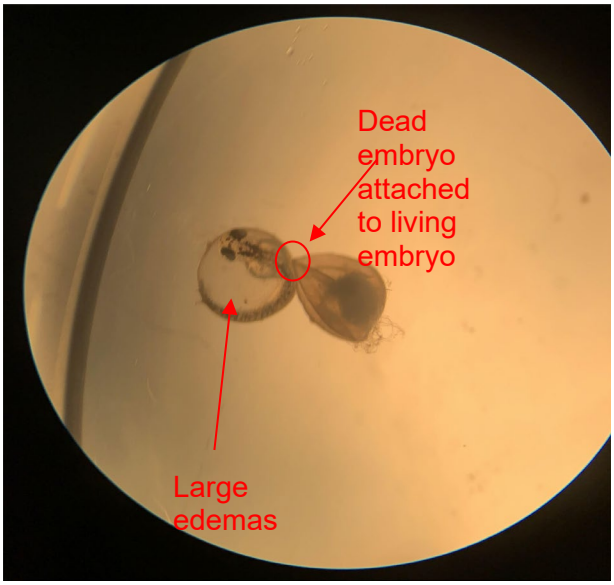

Figure 4 (above) shows the ethanol group on day 4. The ethanol group showed slow development and a fairly poor survival rate. Moreover, the group showed many signs of abnormalities that included embryos stuck to other deceased embryos, large edemas, and vibrating movement.

Figure 5

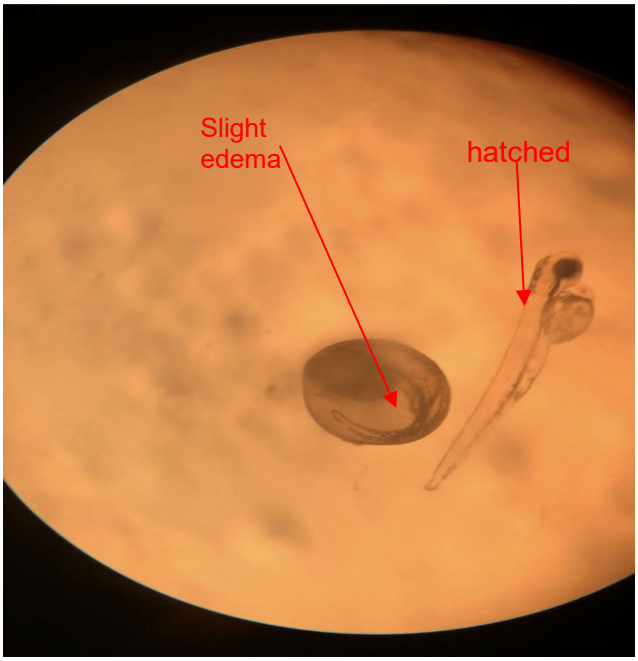

Figure 5 (above) shows ethanol with a vitamin E group on day 4. The ethanol and vitamin E group showed normal development and good survival rates. Furthermore, the group showed no abnormalities except for slight edemas.

Figure 6

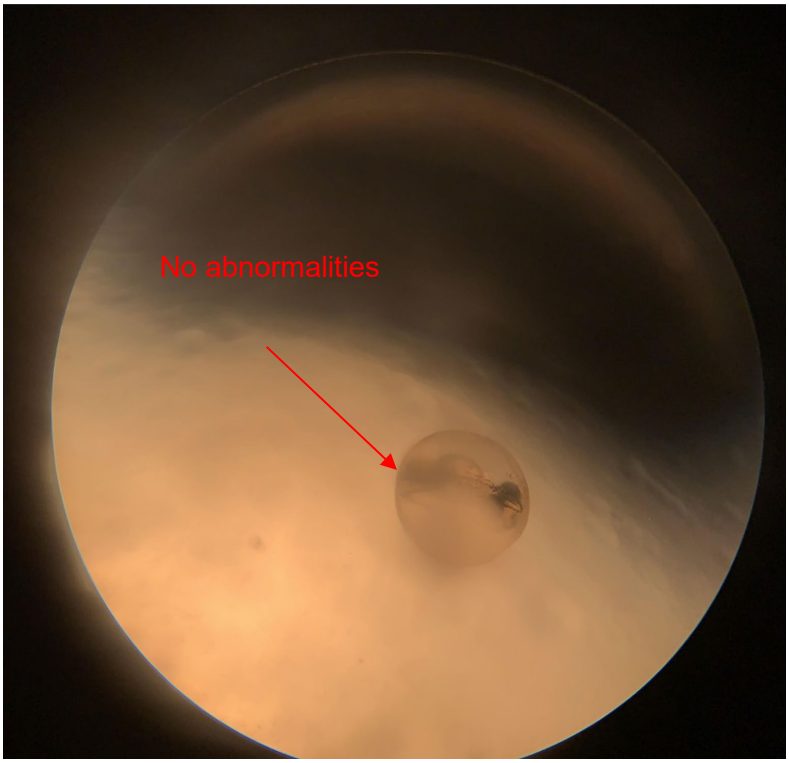

Figure 6 (above) shows the nicotine and vitamin E group on day 4. The nicotine and vitamin E group showed slow development and a low survival rate. However, the group showed no signs of abnormalities.

Discussion:

At the end of our experiment, after our Fischer test calculations were complete, we failed to reject the null hypothesis for our vitamin E involved trials regarding survival of the embryos. Our p-values for these two tests were 0.6968 for the nicotine and vitamin E solution and 1.000 for the ethanol and vitamin E solution. Therefore, the differences in the results are not considered significant. One source of error could be that the liquid vitamin E was in an oil form, thus failing to fully dissolve into the water-based solutions. While the vitamin was added to the solutions, it is possible that its lack of total incorporation into the solution could have caused an error. Another error was encountered when eight zebrafish embryos were placed in trial one (well one) of the nicotine solution, and five were placed in each of the other cells. The only significant difference in survival, with a p-value of .0377, came from the nicotine solution compared to the control. This may have a correlation to the larger sample size used in this comparison. The toxins we used in this experiment, nicotine and ethanol, are known to cause defects in embryos and fetuses. The goal of this experiment was to predict the impact of a human embryo's exposure to vitamin E as a substance with counteracting effects to known toxins. At the conclusion of our experiment, we have determined that while no significant difference occurred, we feel that further experimentation with larger sample sizes may help to determine the actual validity of the experiment. Continually, further experimentation utilizing larger concentrations of vitamin E could yield different results. Furthermore, testing on mammalian animals such as mice may help to determine whether vitamin E could be a reliable substance for counteracting some of the negative effects associated with the consumption of nicotine and ethanol early in pregnancy.

Works Cited

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