The Harmful Effects of 5-Hour Energy to Zebrafish Survival Rate

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Abstract

5-hour energy will have negative results on zebrafish and their development. This is important to know because it will help educate others on the effects of caffeine on development, survival rate, and reaction time. Research was done to show the effects on 5-hour energy and prove its negative effects. Zebrafish are an important organism to do research studies upon because they share many similarities to humans, including the muscle, hearts, blood, kidneys, eyes and brains. The experiment took place over four days. Four different amounts of 5-hour Energy Extra strength (0%,1%, 5%, and,10%) were placed and each solution was replaced every day with a new solution to help compare sufficient and more accurate results. It was noticed that when placed in solutions with higher amounts of 5-hour Energy, there was a great increase in the death to survival ratio. Based on these results this supports the hypothesis that caffeine has negative effects on human and zebrafish development, reaction time, and survival rate. It's important to understand the effects of caffeine on zebrafish because seeing the effect on development and survival rates, people can relate that to the effect of caffeine on humans. Because zebrafish have many things in common with humans such as muscle, hearts, blood, kidneys, eyes and brains.

Introduction

Caffeine is something most humans drink daily, whether it be in your morning coffee or your red bull, it's something that everyone is familiar with. Too much caffeine isn't good for any human, and large doses can cause insomnia, nervousness, restlessness, stomach irritation, nausea, increased heart rate, and respiration, plus plenty of other side effects, and there have even been deaths from too much caffeine. According to Kuakini (2016), “Many people consume it though, and every day, about 90 percent of Americans consume caffeine in some form. More than half of the adults in the country consume 300 milligrams a day, making it America’s most popular drug.”

According to Heb (2007),“5-hour energy is one of the many things that uses caffeine. It consists of vitamin B6, folic acid, vitamin B12, sodium, taurine, glucuronolactone, malic acid, and N-Acetyl L-tyrosine, L-phenylalanine, caffeine, and citicoline” Basically, it's bad for your system and the question of what would happen if zebrafish embryos were exposed to 5-hour energy appeared. “Taurine is one of the key ingredients in the 5-hour energy and is the main thing that makes it bad for you” According” Biggers (2014), taurine has negative side effects on the human body when served in large doses. This means that

the zebrafish will have to develop in that. What will be tested on the zebrafish will be how many of them survive while living in the 5-hour Energy.The experiment was done to find the negative effects of 5-hour-energy on zebrafish embryos. When humans are developing and their mom drinks caffeine ragarly it can affect the developing human. Since zebrafish and humans have much in common. If the zebrafish are forced to develop in the 5-hour Energy, then they will have a low survival rate because the 5-hour Energy contains harmful chemicals

Materials

42 zebrafish embryos
2 containers of 5-hour Energy Extra strength
1 beaker for dead/damaged embryos
1 sharpie
1 Disposable pipette
Instant ocean solution
4 - 100mL beakers
Pipettes
Microscope
28.5 degree Celsius incubator
Rubber gloves

Methods

Day one: First, the mixtures were created. The control group consisted of 100% instant ocean water. The other three groups were 1% 5-hour extra strength, 5% 5-hour extra strength, and 10% 5-hour extra strength. Then the tray was labeled with the proper percentages. The plate was gathered and 10 zebrafish embryos were placed in each of the four containers. It's important the embryos that looked fuzzy were taken out because those embryos were dead and were not able to be placed. Dead and extra embryos, plus any other residual was taken out so a total of somewhere around 10 were in each group. The solutions were dropped into each of the plates for the zebrafish to develop. Then the zebrafish were observed and the observations were documented, and finally, the plate of zebrafish were placed into 28.5*C incubator and left them overnight.

Day Two, Three, and Four: The well plate was removed from the incubator. The dead/damaged embryos were removed using a thin-tipped pipette, then placed in a waste beaker. It was recorded for each day how many had survived, how many died, and how many had hatched. The solution was replaced every day so the zebrafish wouldn't die off in the polluted solution.

A chi-square analysis was performed to ensure statistical significance.
*note you should wear rubber gloves when completing this experiment to protect the skin for chemical exposure

Results

This experiment was conducted to see the harmful effects of the 5-hour Energy Extra strength solution on zebrafish, specifically their survival rate. Results of this lab experiment were directly compared to zebrafish in our control group, which were put in conditions where they would have a 100% survival rate. Zebrafish were used due to the sheer resemblance in how they grow and develop in relation to humans. 5-hour Energy solutions were measured with water to create something the zebrafish could properly grow and survive in. The independent variables were the 1% group, the 5%, and the 10%, and the instant ocean solution (0%). The dependent variable was the survival rate in which the different solutions affected. The incubator keeped the zebrafish at a consistent 28.5°C. As stated earlier, zebrafish in control had a survival rate of 100%, unlike the fish in the experimental groups. Zebrafish dying off early was something creating a roadblock in the experiment, but despite that, the data that was able to be collected from remaining zebrafish embryos in the experimental groups had lower survival rates, which can relate to the 5-hour Energy solution *(see table 1.)* lower survival was a reoccurring result in groups in 5-hour Energy solutions. Zebrafish were not able to survive in any amounts of 5-hour Energy, *(see figure 2.)* Which along with everything else shows us clearly how harmful 5-hour Energy Extra strength can be. This supports the hypothesis that 5-hour Energy negatively impacts zebrafish survival rate *(see figure 1.)* The chi-square analysis was used to test for the independence of different caffeine solutions and the survival rate of zebrafish. The chi-square value of this data set was calculated to be 34.47. Using 3 degrees of freedom and a predetermined level of significance of .23, the critical value was found to be 9.65. Using the chi-square analysis, 34.47 is greater than the critical value of 9.65, which rejects the null hypothesis and proves that the results were not by chance alone.

Table 1 Survival and hatching rates in different solutions over time.
This table shows that as time in any 5-hour energy solution increases the survival rate will decrease. As the control (0%) stays the same as 100%

5-hour energy (extra strength)	**# of starting fish**	**24 hours past fertilization**		**48 hours past fertilization**		**72 hours past fertilization**		**96 hours past fertilization**	
		Hatched	**Live**	**Hatched**	**Live**	**Hatched**	**Live**	**Hatched**	**Live**
0%	**10**	**0**	**10**	**5**	**10**	**3**	**10**	**1**	**10**
1%	**12**	**0**	**3**	**2**	**2**	**0**	**2**	**0**	**2**
5%	**10**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
10%	**12**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**

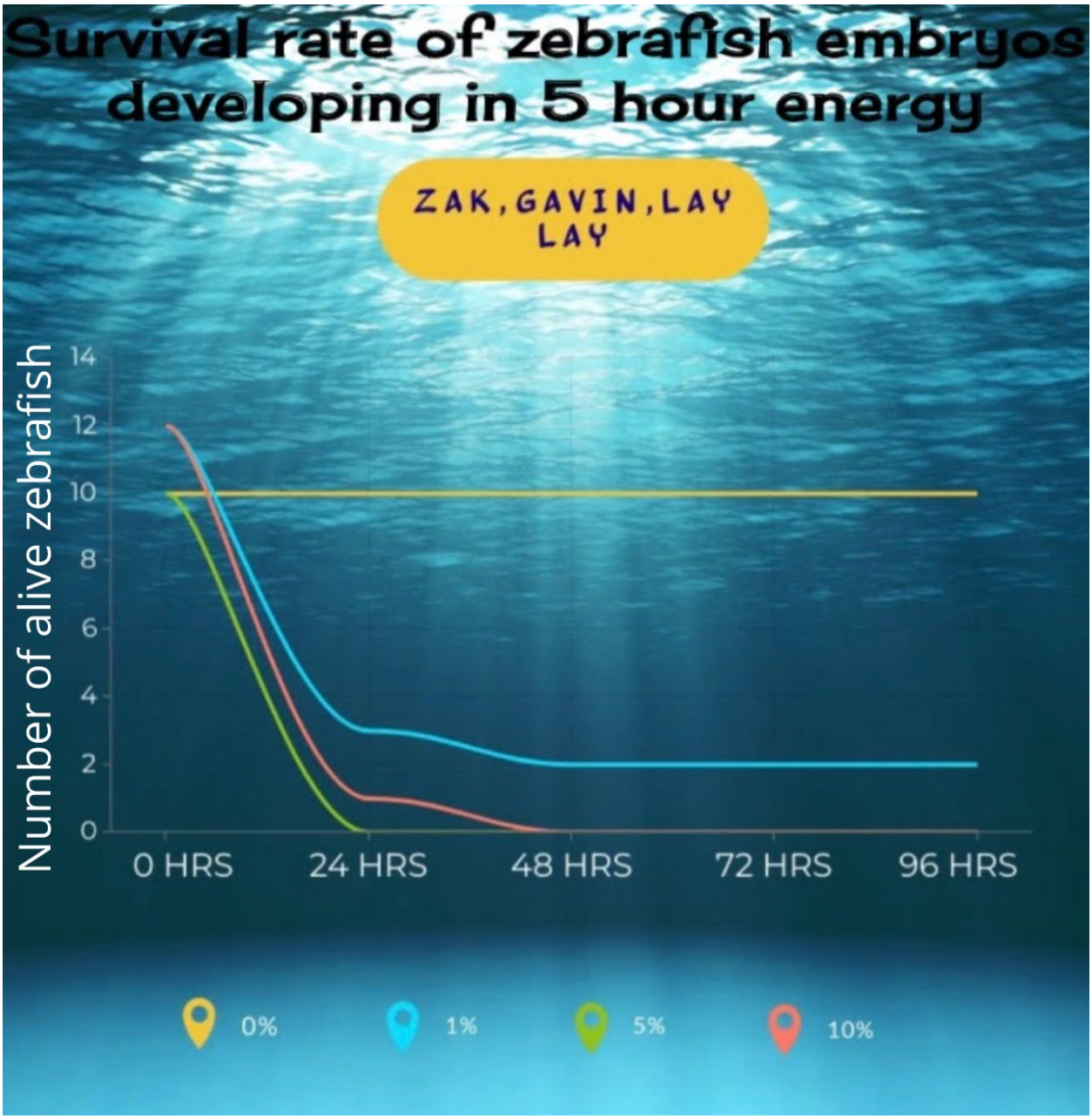


Figure 1 Survival rate of zebrafish embryos developing in 5-hour Energy

This graph shows that as the time in any 5-hour energy solution increases the survival rate will decrease. As the control (0%) stays the same as 100%

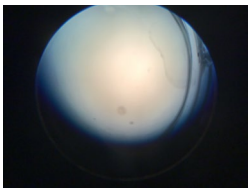
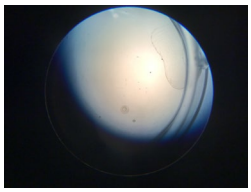
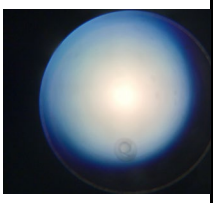
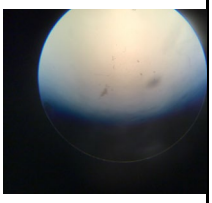
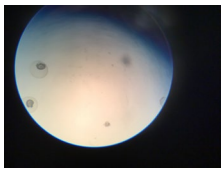
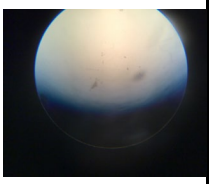
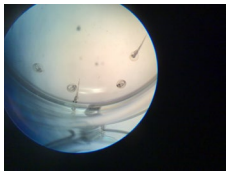
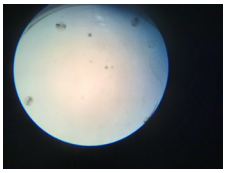
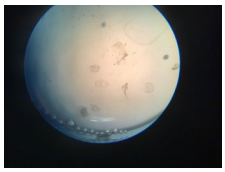
Zebrafish development as seen through microscope	Control (0%)	1%	5%	10%
Day 1				
Day 2			None living	
Day 3			None living	None living
Day 4			None living	None living

Figure 2 Development of zebrafish over time
This table shows the decreasing survival rate as time increases in the 5-hour Energy solutions. As the control (0%) stays the same.

Discussion

Zebrafish, when exposed to 5-hour Energy extra strength appears to have negative results when developing. The hypothesis was that 5-hour Energy would decrease the survival rate on the zebrafish. The zebrafish placed in 5-hour Energy had trouble developing and even in very little amounts of the 5 hour solution they didn't survive. It can be concluded that 5-hour Energy has a negative impact on the development of embryos. Sources stated that 5-hour Energy was bad for human development and provides more than the average amount of B12, having negative effects on your well being. As shown in the experiment, 5-hour Energy had negative results on the zebrafish. The data showed that all the groups with the 5-hour Energy solution

died off rapidly and fast, while the control group had a constant survival rate. It was concluded that the hypothesis was correct because trends were seen in the data where any group where zebrafish were placed

in the 5-hour Energy solution died off, while the ones developing in the control were just fine. It can be stated with confidence that 5-hour Energy provides negative effects on development. This isn't something that should be taken with a grain of salt, as the experiment conducted shows how bad the 5-hour Energy is and it should be taken into consideration before deciding whether to consume 5-hour Energy especially for pregnant women. If conducted again, what would be changed would be the concentrations of the solution amounts. Instead of the 1%, 5%, and 10%, it would be changed to .5%, 1%, and 2%, because as shown, five and ten percent was too strong for developing embryos to handle. It would be interesting to see how they would react to normal 5-hour Energy, and not the extra strength kind.

Literature Cited

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