Danio rerio Lab: A Study Into the Effects of Chlorpheniramine on Zebrafish Embryo.

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Abstract:

The purpose of this experiment was to determine the effects of chlorpheniramine on human fetal development through the study of the effects of chlorpheniramine on zebrafish embryonic development. Fertilized zebrafish eggs were exposed to various concentration of chlorpheniramine and observed over five days, replacing the solution daily. No effects were found, which may be due to the small number of eggs tested in the experiment. This may indicate chlorpheniramine is safe to use by pregnant women.

Introduction:

The purpose of this experiment was to study the effects of chlorpheniramine on zebrafish embryos by varying the medication concentrations in the embryos' environments. Currently there isn't any supported evidence of harmful effects of chlorpheniramine on human fetal development, although other antihistamines are known to cause birth defects, which is why it was chosen for this experiment. Zebrafish share seventy percent of their genomes with humans, which results in eighty-four percent of human disease causing genes to have a zebrafish equivalent. Also, zebrafish are quick developers, maturing in as much in a day as a human fetus would in thirty, which is why zebrafish will be used in place of human embryos, not to mention they're cheap to maintain. If the concentration of the medication increases, then the number of malformations and embryo deaths will increase because other antihistamines are known to cause malformations in rat embryos.

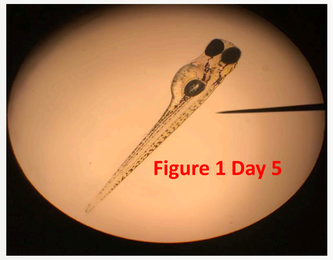


Figure 1 Day 5

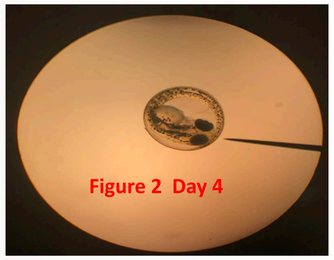


Figure 2 Day 4

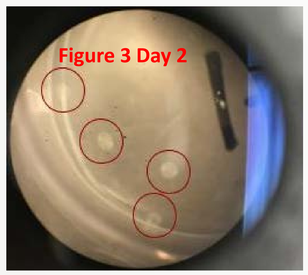


Figure 3 Day 2

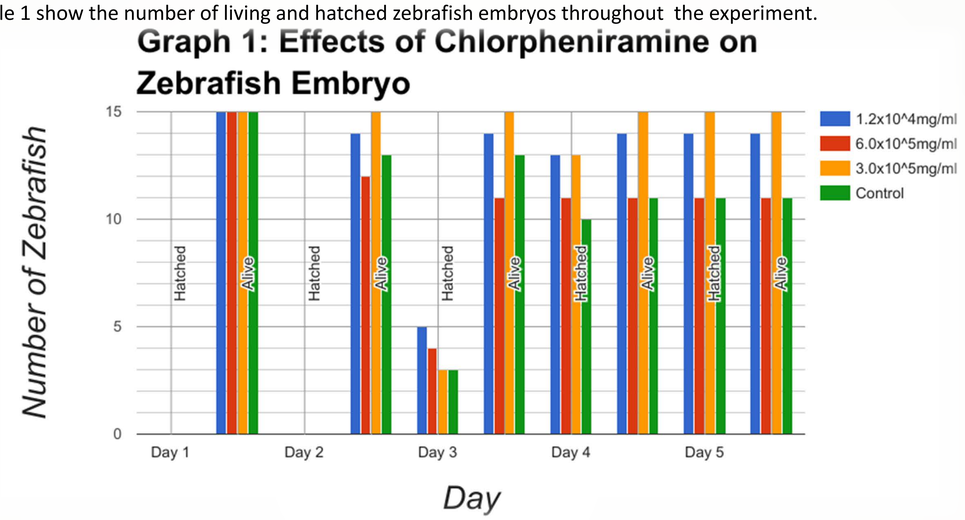
Materials and Methods:

Sixty fertilized Zebrafish were distributed across twelve seven milliliter wells.1.2104mg/ml, 6.0105mg/ml, 3.0105mg/ml, and 0 mg/ml solutions of chlorpheniramine, from crushed ChlorTabs, and instant ocean were made. Each concentration of chlorpheniramine was given three wells of five zebrafish. The zebrafish were observed under a stereoscopic scope and left in an incubator at 28 degrees celsius for 24 hours. Each of the next 4 days the living, dead, and hatched zebrafish embryo were counted under a stereoscope. A few of the embryo were observed under a compound microscope for abnormalities. The dead were removed and the solutions were replaced depositing both the dead and old solutions into a waste beaker for disposal. The collected data was checked for significant patterns on an online unpaired t-test after the fifth day.

Table 1: Effects of Chlorpheniramine on Zebrafish Embryo Development After Fertilization.

	Day 1		Day 2		Day 3		Day 4		Day 5	
Concentration Chlorpheniramine	Hatched	Living	Hatched	Living	Hatched	Living	Hatched	Living	Hatched	Living
1.2x10^4mg/ml	0	15	0	14	5	14	13	14	14	14
6.0x10^5mg/ml	0	15	0	12	4	11	11	11	11	11
3.0x10^5mg/ml	0	15	0	15	3	15	13	15	15	15
0 mg/ml	0	15	0	13	3	13	10	11	11	11

Table 1 show the number of living and hatched zebrafish embryos throughout the experiment.



Graph 1 show the number of living and hatched zebrafish embryos throughout the experiment.

Analysis:

Table 2: Zebrafish Chlorpheniramine Concentration Experiment Analysis Results

Unpaired t-Test (Compared to Control)		Calculated by GraphPad (2017)	
	3.0x10^5mg/ml	6.0x10^5mg/ml	1.2x10^4mg/ml
Number of Zebrafish alive Data			
P Value	0.0327	0.5927	0.0727
Significant?	Yes	No	No
Confidence Interval	-4.48 to -0.32	-1.88 to 3.08	-3.39 to 0.19
Standard Error	0.748	1.077	0.775
Number of Zebrafish Hatched Data			
P Value	0.7376	0.9105	0.6904
Significant?	No	No	No
Confidence Interval	-10.70 to 7.90	-8.35 to 7.55	-10.53 to 7.33
Standard Error	4.035	3.447	3.873

An analysis of each experimental trial with the control using an unpaired t-test showed that only the 3.0105mg/ml of chlorpheniramine trial number alive data was calculated to have significant data variance. Considering the non-significance of the other data and the small data pool the 3.0105mg/ml of chlorpheniramine trial number alive data is likely an outlier that is part of the five percent whose confidence interval does not contain the population mean and insignificant. The unpaired t-test was used because the groups were independent of one another. According to GraphPad (2017), this experiment passes the analysis checklist for when to use an unpaired t-test. As for the results, it shows that the variance in the data of each trial was insignificant. In other words, no chlorpheniramine effect was found.

Results:

The experiment involved placing five fertilized zebrafish embryo in twelve seven-milliliter well in a twelve-well plate and subjecting them to various concentrations of chlorpheniramine from ChlorTabs over five days, replacing the solution daily. During these days, the embryos were observed as they developed for effects of the medication. In this experiment the independent variable was the concentration of medication and the dependent variable was the development of the fish and survival rate. The control was the instant ocean solution with 0 mg/ml chlorpheniramine. The experimental groups, the other concentrations, were compared to the control group to see the effects of chlorpheniramine. As the figures and data show the concentrations of chlorpheniramine had no effect on the development of the zebrafish.

Discussion:

As the analysis and data show the experiment was inconclusive on the effects of chlorpheniramine. More data is required to test the effects of chlorpheniramine on the fish, but based on the current data, the hypothesis, if the concentration of the medication increases, then the number of malformations and embryo deaths will increase, was rejected. The results of the experiment showed that chlorpheniramine has little to no impact on the zebrafish embryos' development and hints that chlorpheniramine may have been beneficial. This, of course, is subjective without additional experiments.

As for sources of error in this experiment, the method of calculating the equivalent human dosage for zebrafish embryos was erroneous being based on variable and unreliable volumes instead of the relatively consistent mass comparison. To amend this equivalent dosage should be calculated via mass comparison. Also despite dosages being doubled and quadrupled, there was little to no difference in developmental progress. Greater variance in dosages should be considered, perhaps ten times or a hundred time the equivalent dosage keeping in mind osmotic effects of increased solute concentrations. Also, small data pool of this experiment brought about uncertain results. In repeating this experiment more zebrafish need to be tested per trial, a hundred per concentration trial should suffice for future experiments. In increasing the number of zebrafish in one volume of solution, equivalent dosages will need to be recalculated to correspond the collective mass of the embryos. In future studies of the effects of chlorpheniramine, replacing the zebrafish as subjects with pregnant mammals which more closely represent pregnant women should be considered to better test the effect of chlorpheniramine, antihistamines, and other drugs on fetal development.

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