The Effect of *Arnica montana* on Zebrafish Embryonic Development

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Abstract

Arnica montana is a flowering plant that is moderately toxic. It is used as a homeopathic drug to treat bruises, heal tissues, and is anti-inflammatory. Pregnant women use products with *Arnica montana* to help prevent stretch marks; however, research about the side effects of *Arnica* is slim. For this experiment, we observed the embryonic development of zebrafish in different concentrations of *Arnica montana* for three days. We observed that those exposed to *Arnica montana* had more deformities than those not exposed. Due to continued use of *Arnica* during pregnancy, the data collected on zebrafish embryos indicate probable embryonic consequences.

Introduction

Arnica montana, a homeopathic drug with few studies showing its validity, has become widely used to accelerate healing of skin redness and bruises. However, there is little to no research observing its effects on embryonic development. *Arnica* functions by increasing the speed of macrophages, accelerating the elimination of cell waste and foriegn bodies (1). Although *Arnica* is predicted to have said function, there are still many questions on its efficacy and its safety (3) as it has not been approved by the FDA. Women who used *Arnica* to treat local trauma prior to pregnancy likely continue to use it during pregnancy, so the safety of *Arnica*, especially in embryonic development, is extremely important. Most studies evaluate the efficacy of *Arnica*; however, there is a lack of information discussing its safety (2). For pregnant women, it is important to understand not only the effects of *Arnica* as a skin care product, but also the effects of *Arnica* in fetal development. Although there are not many studies displaying negative effects of *Arnica*, it is hypothesized that if zebrafish embryos are exposed to different concentrations of *Arnica*, the embryos which are exposed to more *Arnica* will have a lower survival rate and more deformities than those with no exposure.

Methods

Four small jars were each filled with 100 ml of Instant Ocean solution (200 mg/L). The *Arnica montana* 30c tablets were crushed and measured into three groups: 0.25g, 0.75g, and 1.5g. Then each group was converted into concentration by mass percentages. Next the groups of crushed tablets were dissolved in the separate jars of Instant Ocean solution. Each experimental group was designated a Falcon dish where six wells were filled with 3 ml of solution and five zebrafish embryos. The Falcon dishes were placed in an incubator at 34°C for 24 hours. For the next three days, the embryos were observed under a microscope, and data was collected about their development. Each day the dead embryos were removed and the 3 ml of solution was replaced. Finally, a Fisher Test was performed to determine whether or not there was a significant difference in the number of deformities of the embryos in different concentrations.

Figure 1

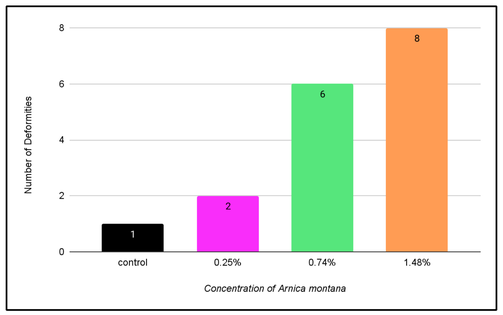
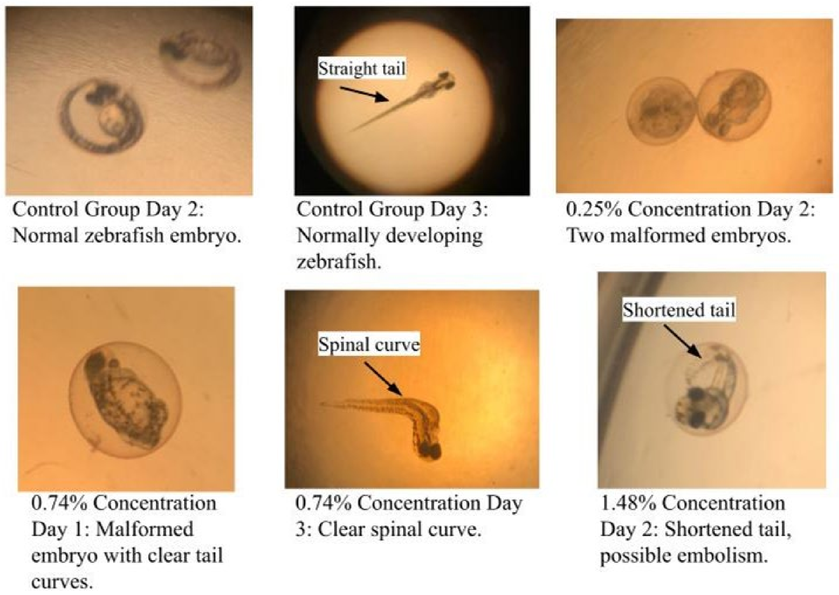


Figure 1 shows the total number of deformities for the embryos in the different concentrations of *Arnica montana* at the end of the experiment. As the concentration of *Arnica* increased, the number of deformities also increased.



Control Group Day 2: Normal zebrafish embryo.

Control Group Day 3: Normally developing zebrafish.

0.25% Concentration Day 2: Two malformed embryos.

0.74% Concentration Day 1: Malformed embryo with clear tail curves.

0.74% Concentration Day 3: Clear spinal curve.

1.48% Concentration Day 2: Shortened tail, possible embolism.

Results

For this experiment the independent variable was the different concentrations of *Arnica*, and the dependent variable was the number of deformities after three days of observation. The control group was the embryos in solution with no *Arnica.* All groups were placed in the same incubation environment with the same Instant Ocean solution. A Fisher Test was performed to calculate the statistical significance between the dependent and independent variables. Because standard error of the mean could not be found and there were no expected values for this experiment, a Fisher Test was the most appropriate. As a result, the difference in number of deformities between the control group, 0.25%, and 0.74% concentration proved insignificant. However, the Fisher Test proved statistically significant between the control group and the highest concentration with a p-value of 0.0142.

Discussion

After three days of observation, the zebrafish embryos in higher concentrations of *Arnica* had more deformities. Many hatched zebrafish in the two highest concentrations had spinal curvatures. Several unhatched embryos had edemas and appeared malformed. We conclude that higher concentrations of *Arnica* cause more developmental defects in the embryo stage. This supports our hypothesis that exposure to *Arnica montana* would cause developmental defects. Our experiment did have limitations that could have affected the results. For instance, the tablets used to dissolve *Arnica montana* into the solution also contained small amounts of lactose and sucrose, which could have contributed to the observations. Additionally, we were limited in time with only three days of observations. If we had conducted this experiment for a longer period of time, more data about defects and fatality would have been collected. Overall, the significance of the deformities caused by high concentration of *Arnica* suggests that more research is needed to determine the health effects on humans using products with *Arnica montana,* especially pregnant women, as the detrimental effects on zebrafish embryos may indicate similar effects on human embryonic development.

Figure 2

Fisher Test			
Treatments	Control vs .25%	Control vs .74%	Control vs 1.48%
P-value	p=1.0000	p=0.0801	p=0.0142
Significant?	No	No (.03 away from being significant)	Yes

Figure 2 shows the p-values calculated from performing our Fisher Test. The number of deformities was compared between each experimental group and the control group (α=.05).

References

1. "Impact of Ethanol and Heat Stress–Dependent Effect of Ultra-Diluted Arnica montana 6 CH on in Vitro Embryo Production in Cattle - ScienceDirect." *ScienceDirect.Com | Science, Health and Medical Journals, Full Text Articles and Books.*
2. "Thieme E-Journals - Homeopathy / Abstract." *Home - Thieme Connect*.
3. Marzotto, Marta, et al. "Arnica montana Stimulates Extracellular Matrix Gene Expression in a Macrophage Cell Line Differentiated to Wound-Healing Phenotype." *PLoS ONE*, vol. 11, no. 11, Nov. 2016, pp. 1–25.