RESPONSE OF GLUTATHIONE S-TRANSFERASE TO ALUMINUM IN SIGNAL CRAYFISH (*PACIFASTACUS LENIUSCULUS*)

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Introduction

Aluminum is the most abundant metal within the earth's crust [1]. Organisms can be exposed to aluminum in the environment at toxic levels due to both anthropogenic sources and natural sources. Under acidic conditions, aluminum is forced into solution and can end up in aquatic environments [2]. Aluminum exposure can also be due to its use in household materials, consumer products, and medications [2]. Chemical pollutants like aluminum can interact with the detoxification pathways of organisms [3]. Signal crayfish (*Pacifastacus leniusculus)* serve as optimal biological model organisms because of their abundance in SW Idaho and their trophic interactions in aquatic and terrestrial environments [5, 6, 7]. Crayfish hepatopancreas accumulates high levels of metals via chemical exposure [8]. The functions of the hepatopancreas include absorption and storage of nutrients, digestion, and ovarian formation [9]. GST activity has been detected in hepatopancreas tissue of signal crayfish [3]. In this study, GST levels were measured in the hepatopancreas of signal crayfish after being treated with increasing doses of aluminum to characterize the response of GST after aluminum exposure. This will help us understand how and if aluminum modulates the phase II detoxification pathway.

Goal The purpose of the study was to examine the effect of aluminum on glutathione S-transferase, an enzyme involved in the phase II detoxification pathway.

Hypothesis Based on reports in the literature, we expect aluminum to elicit a dose dependent inhibition of GST due to competitive, non-competitive binding or the altering of gene expression for GST [3, 10].

Phase II Detoxification Enzyme

Glutathione S-transferase is a phase II detoxification enzyme which catalyzes the conjugation of glutathione to toxic and polar substrates which are then excreted via urine or bile [4]. Toxins removed by glutathione S-transferases include pesticides, carcinogens, acetaminophen, and xenobiotics [4]. Levels of GST may serve as a biomarker of induction or inhibition due to interactions with xenobiotic chemicals or heavy metals [9].

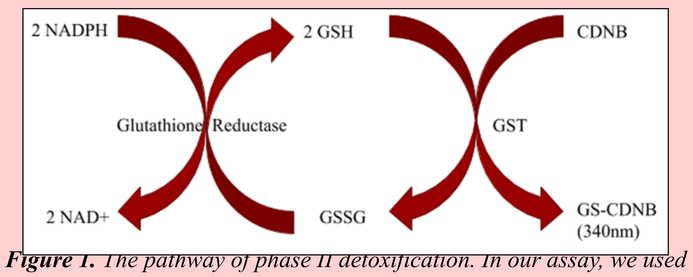


Figure 1. *The pathway of phase II detoxification. In our assay, we used CDNB as a xenobiotic toxin to initiate the reaction.*

Results

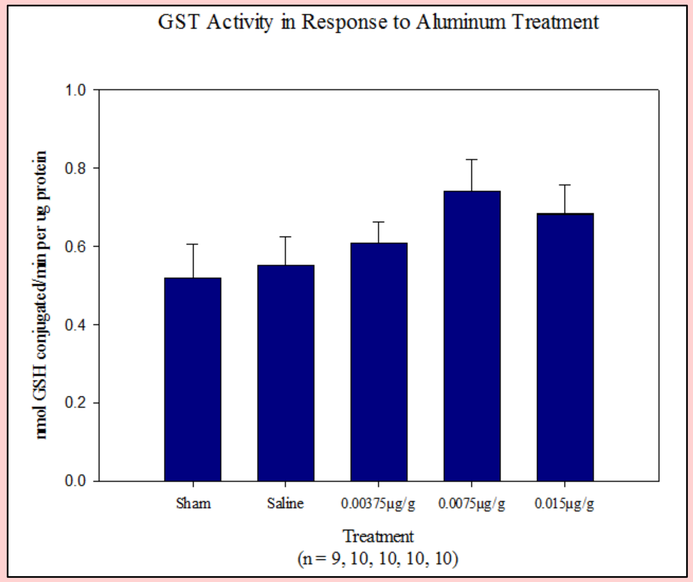
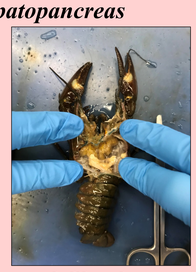


Figure 2. *Average GST activity in signal crayfish hepatopancreas 72 hours after treatments with different doses of aluminum chloride. None of the aluminum treatment groups differed from the saline controls (p=0.22).*

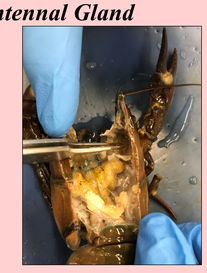
Other Relevant Results

- A sham group was tested in order to make sure the needle prick did not affect GST activity.
- A t-test was run between the sham and the saline controls, and was found to not be statistically significant *(p=0.39).*
- A Kruskal-Wallace One Way Analysis of Variance was run between the saline control and treatment groups and was found to not be statistically significant *(p=0.28).*

➢ ***Hepatopancreas***



➢ ***Antennal Gland***



Discussion and Conclusion

There was not a statistically significant difference in GST between the control groups and the groups treated with aluminum *(p=0.22).* Therefore, we cannot conclude that GST is sensitive to aluminum exposure.

It is important to note that our highest dose of aluminum was limited by both the solubility of $AlCl_3$ at pH 7 and by an injection volume of 1% of the total body weight of the animal.

Future

- Measure GST activity in crayfish antennal gland
- Measure GST activity after higher doses of aluminum
- Examine other detoxification and endogenous antioxidants in aluminum treated animals
  - Cytochrome P-450 enzymes
  - Glutathione

Methods

Treatments/Injections

- Sham
- Saline (1% body weight)
- 0.015µg/g, 0.0075 µg/g, 0.00375 µg/g $AlCl_3$ (1% body weight)

End Points Measured

- GST activity

Tissues Extracted
Hepatopancreas

Sample Collection Male and female crayfish were collected from C.J. Strike Reservoir in SW Idaho in May & June, 2019 and were allowed to acclimate in captivity for at least one week.

Aluminum Concentration The aluminum concentration of the saturated $AlCl_3$ solution (in 400mM NaCl, pH 7) was determined using Atomic Absorption Spectroscopy (AA).

GST Assay GST activity was measured using a colorimetric assay as described in an earlier study [3].

Statistical Analysis All statistical analyses were performed on Sigma Plot 13.0 using a Kruskal-Wallace One Way Analysis of Variance ($p < 0.05$).

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References

1. Nayak, P. (2002). Aluminum: Impacts and Disease. *Environmental Research*, *89*(2), 101-115.
2. Frumkin, H., Gerberding, J.L. (2008). Toxicological Profile for Aluminum. *U.S. Department of Health and Human Services*, Public Health Service.
3. Gunderson, M.P., et al. (2018). Response of phase I and II detoxification enzymes, glutathione, metallothionein, and acetylcholine esterase to mercury and dimethoate in signal crayfish (*Pacifastacus leniusculus*). *Chemosphere* 208: 749-756.
4. Hayes, J.D., Flanagan, J.U., Jowsey, I.R. (2005). Glutathione transferases. *Annual Review of Pharmacology and Toxicology* 45, 51-88
5. Kelly, C.I. (2019). Examining the phase II detoxification of signal crayfish (*Pacifastacus leniusculus*). *Department of Biology*, The College of Idaho.
6. Lodge, D.M., et al. (1994). Effects of an omnivorous crayfish (*Orconectes Rusticus*) on a freshwater littoral food web. *Ecology*, *75*(5), 1265-1281.
7. Larson, E.R., Olden, J.D. (2011) The state of crayfish in the Pacific Northwest. *Fisheries*, *36*(2), 60-73.
8. Faria, M., Huertas, D., Soto, D.X., Grimalt, J.O., Catalan, J., Riva, M.C., Barata, C. (2010). Contaminant accumulation and multi-biomarker responses in field collected zebra mussels (*Dreissena polymorpha*) and crayfish (*Procambarus clarkii*), to evaluate toxicological effects of industrial hazardous dumps in the Ebro river (NE Spain). *Chemosphere* 78, 232-240
9. Wang, W., Wu, X., Liu, Z., Zheng, H., Cheng, Y. (2014). Insights into hepatopancreatic function for nutrition metabolism and ovarian development in the crab *Portunus trituberculatus*: gene discovery in the comparative transcriptome of different hepatopancreas stages. *PLoS One*, *9*(1).
10. Ezaki, B. et al. (2004) Mechanism of gene expression of Arabidopsis glutathione S-transferase, AtGST1, and AtGST11 in response to aluminum stress. *Plant Physiology*, *134*(4), 1672-1682.