Dechampsia antarctica CRUDE EXTRACT PROTECTS AGAINST Hg(II) INDUCED TOXICITY IN *Drosophila melanogaster*

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Abstract: In this study the potential protective activity of *Dechampsia antarctica* crude extract (DaE) in the fruit fly *Drosophila melanogaster* exposed to mercury (HgCl_2 ; 100 μM) during 48hrs was evaluated. The survival rate and locomotor activity were evaluated as well as signs of toxicity. Flies (both genders; 1-4 days old) were exposed to HgCl_2 100 μM dissolved in 1% sucrose in the presence or absence of different concentrations of DaE (1% and 5% in sucrose). After 48hrs of exposure, the live flies were counted. The negative geotaxis behavior was determined as an index of locomotor activity. After treatments, the live flies were transferred to glass tubes and gently tapped to the bottom of the tube. The number of flies that climbed 6 cm during 6 seconds was counted. The exposure of flies to mercury caused a significant increase in mortality (25%) when compared to controls (treated with 1% sucrose solution only). In parallel, mercury exposure induced a significant decrease in locomotor activity. The co-administration of DaE at all concentrations tested completely abolished the mercury-induced signs of toxicity. In conclusion, our results show, for the first time, the protective effects of *Dechampsia antarctica* against the deleterious effects of mercury *in vivo*.

Keywords: *Dechampsia antarctica*, Mercury, Fruit Fly, Toxicity

Introduction

Mercury has been recognized as a ubiquitous environmental toxicant whose toxicity is associated to neurological and developmental deficits in animals and humans. Environmental hazards such as those in Japan and Iraq between the 50s and 70s, marked the toxicological importance of this environmental contaminant (Clarkson *et al.*, 2003). Anthropogenic sources of mercury still pose high risk to human and environmental health in present times. Furthermore it is important to note that it has been shown that mercury transport from more densely populated regions (lower latitudes) results in the accumulation of methylmercury in the food chain of Arctic and Antarctic environments (Barkay & Poulain, 2007). Due to its potential bioaccumulation in fish, as well as its intensive applications in industry, coal fired power plants and mining, intoxication episodes are mainly related to

diet and occupational exposures (Clarkson *et al.*, 2003; Honda *et al.*, 2006).

Despite being extensively studied in the last decades, mercury is still considered a major human and environmental health problem (Clarkson & Magos, 2006), in this aspect, there is no effective treatment available for mercury intoxication associated damage. The mechanisms underlying mercury toxicity are not well understood. However, it has been shown that oxidative stress plays a central role in this process (Farina *et al.*, 2011). Thus, compounds that possess antioxidant activity may be potential candidates in the treatment of mercury poisoning. Studies in literature have shown that plant extracts and natural compounds are able to block the toxic actions of mercury in animal models (Farina *et al.*, 2005; Franco *et al.*, 2007).

D. antarctica are native vascular plants of Antarctica, occurring extensively from the South Orkney and South Shetland Islands in the north, to Terra Firma Islands, in the southwestern Antarctic Peninsula (Alberdi *et al.*, 2002). Previous studies have reported extracts obtained from *D. antarctica* protected from DNA damage and mutation triggered by UVC radiation in yeast (Betina *et al.*, 2009). Considering its promising potential, there is a lack of studies focused on its biological and biotechnological properties.

The fruit fly *Drosophila melanogaster* is one of modern genetics premier model systems, with an extensive literature ranging from classical and modern genetics to biochemistry to physiology and complex phenotypes. Although humans and *D. melanogaster* are only distantly evolutionarily related, almost 75% of disease-related genes in humans have functional orthologs in the fly (Pandey & Nichols, 2011), making the fly a reasonable model system for humans and other vertebrate. *D. melanogaster* have a rapid reproductive cycle and are easily maintained and handled in the lab making them ideal organisms for the use in *in vivo* bioassays. They also have been proven to be a powerful model system for the study of development and functioning of the nervous system and for the study of fundamental cellular pathways responsible for metal and insecticide toxicity (Ahamed *et al.*, 2010).

Considering (i) the absence of effective treatments for mercury poisoning (Tchounwou *et al.*, 2003), (ii) the protective effects of plant extracts against mercury-induced toxicity in animal models *in vivo* and *in vitro* (Farina *et al.*, 2005) and (iii) the observed protective effect of *Dechampsia antarctica* against UV toxicity (Betina *et al.*, 2009), the present study was aimed to investigate the potential protective effects of *Dechampsia antarctica* (DaE) crude extract against mercury-induced mortality and locomotor deficits in *Drosophila melanogaster*.

Materials and Methods

Drosophila stock and culture: *Drosophila melanogaster* (Harwich strain) was obtained from the National Species Stock Center, Bowling Green, OH, USA. The flies were reared in 2.5 cm × 6.5 cm glass bottles containing 5 mL of standard medium (1% w/v brewer's yeast; 2% w/v sucrose; 1% w/v powdered milk; 1% w/v agar; 0.08% v/w Nepagin) at constant temperature and humidity (25°C ± 1°C; 60%

relative humidity, respectively). All experiments were performed with the same strain.

Mercury Chloride exposure: Adult flies, 1- to 4- days post eclosion, were exposed to HgCl₂ using the following protocol: 120 adult flies (both genders) were starved in empty vials for 3 h at 25°C. Following starvation, groups of 30 flies were placed for 48 hours in vials containing a filter paper saturated with 500 µl of sucrose 1% (control); 500 µl of HgCl₂ (100 µM) solution diluted in 1% sucrose; 500 µl of HgCl₂ (100 µM) + different concentrations of DaE; different concentrations of DaE diluted in sucrose 1%. The concentration of HgCl₂ used in this work was based on a previous study of our group (Paula *et al.*, 2012).

Survival rate analysis: To quantify fly survival rates, three replicates of groups of 30 adult flies were exposed to HgCl₂ for 48 hours and the number of live and dead was counted every 24 hours. Results were analyzed and plotted as percent live flies (Paula *et al.*, 2012).

Locomotor assay: Locomotor ability was determined using the negative geotaxis assay as described previously (Paula *et al.*, 2012) with some modifications. Briefly, for each assay, 20 flies (1-4 days old; both genders) were immobilized on ice for 1-2 min and placed separately in vertical glass columns (length, 25 cm; diameter, 1.5 cm). After 30 mins recovery, the flies were gently tapped to the bottom of the column and the time required for flies to climb up to 6 cm in the columns during 6 seconds was registered.

Statistical Analysis

Data were analyzed using one way analysis of variance (ANOVA) followed by Tukey's post hoc test. Differences were considered significant when $p < 0.05$.

Results

In the present study, flies (*D. melanogaster*) were exposed to Hg(II) toxicity for 48 hours using a concentration of 100 µM of HgCl₂ dissolved in a sucrose solution and survival and locomotor activity was quantified. Hg(II) exposure resulted in significant fly mortality (Figure 1), which is in agreement with our previous publication (Paula *et al.*, 2012). Hg(II) exposure also had a significantly deleterious impact on locomotor behavior. Flies treated with 100 µM Hg(II) for 48 h took longer to achieve 6 cm in the vials (Figure 2) and significantly more flies remained at the base of the flasks

Figure 1. Analysis of survival rate after exposure to Hg (II) and/or DaE concentrations. We calculated percent survival after flies were exposed to HgCl_2 100 μM for 48 hours in the presence or absence of DaE. Bars represent the mean $\pm$ SEM of three experiments performed individually and are expressed as percent live flies in relation to control group (absence of Hg). * $p < 0.05$ in comparison to control.

Figure 2. Negative geotaxis behavior after exposure to Hg (II) and DaE concentrations. We quantified negative geotaxis after flies were treated with 100 μM HgCl_2 for 48 hrs in presence or absence of DaE. Results are expressed as mean $\pm$ SEM of three independent experiments and represents the number of flies that reached the top of a column and those that remained at the bottom after 6 s. * $p < 0.05$ in comparison to control.

when tapped to the bottom of tubes when compared to control groups (Figure 2). Both assays indicated that Hg(II) exposure significantly impacts complex phenotypes, such as survivorship and locomotor behavior.

Discussion and Conclusion

The co-administration of Hg(II) and concentrations of DaE was undertaken to investigate the potential of this plant extract to revert the phenotypes induced by Hg (II) exposure. As shown in Figures 1 and 2, the two DaE concentrations tested (1 and 5%) were able to block Hg (II)-induced mortality and locomotor behavior impairment. In conclusion, our results show for the first time the protective effects of *Dechampsia antactica* extract against Hg(II) toxic effects *in vivo*. The observed protective effects of DaE may be related to the presence of secondary metabolites

with antioxidant activity. Further studies are necessary to elucidate the exact mechanisms of protection and identify the chemical components of the extract that are responsible for its protective actions.

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References

- Ahamed, M.; Posgai, R.; Gorey, T.J.; Nielsen, M.; Hussain, S.M. & Rowe, J.J. (2010). Silver nanoparticles induced heat shock protein 70, oxidative stress and apoptosis in *Drosophila melanogaster*. *Toxicology and Applied Pharmacology*, 242(3):263-269.
- Alberdi, M.; Bravo, L.A.; Gutierrez, A.; Gidekel, M. & Corcuera, L.J. (2002). Ecophysiology of Antarctic vascular plants, *Physiologia Plantarum*, 115 (4): 479–486.

- Aschner, M.; Syversen, T.; Souza, D.O.; Rocha, J.B. & Farina, M. (2007). Involvement of glutamate and reactive oxygen species in methylmercury neurotoxicity. *Brazilian Journal Of Medical and Biological Research*, 40(3): 285-291.
- Barkay, T. & Poulain, A.J. (2007). Mercury (micro)biogeochemistry in polar environments. *FEMS Microbiology Ecology* 59(2):232-41.
- Clarkson, T.W.; Magos, L. & Myers, G.J. (2003). The toxicology of mercury—current exposures and clinical manifestations. *The New England Journal of Medicine*, 349(18): 1731-1737.
- Clarkson, T.W. & Magos, L. (2006). The toxicology of mercury and its chemical compounds. *Critical Reviews in Toxicology*, 36(8):609-62.
- Farina, M.; Cereser, V.; Portela, L.V.; Mendez, A.; Porciúncula, L. O.; Fornaguera, J.; Gonçalves, C.A.; Wofchuk, S.T.; Rocha, J.B. & Souza, D.O. (2005). Methylmercury increases S100B content in rat cerebrospinal fluid. *Environmental Toxicology and Pharmacology*, 19(2): 249–253.
- Farina, M.; Rocha, J.B. & Aschner, M. (2011). Mechanisms of methylmercury-induced neurotoxicity: evidence from experimental studies. *Life Sciences*, 89(15-16):555-63.
- Franco, J.L.; Braga, H.C.; Stringari, J.; Missau, F.C.; Posser, T.; Mendes, B.G.; Leal, R.B.; Santos, A.R.; Dafre, A.L.; Pizzolatti, M.G. & Farina, M. (2007). Mercurial-induced hydrogen peroxide generation in mouse brain mitochondria: protective effects of quercetin. *Chemical Research in Toxicology*, 20(12): 1919-1926.
- Honda, S.; Hylander, L. & Sakamoto, M. (2006). Recent advances in evaluation of health effects on mercury with special reference to methylmercury-A minireview. *Environmental Health and Preventive Medicine*, 11(4):171-6.
- Pandey, U.B. & Nichols, C.D. (2011). Human Disease Models in *Drosophila melanogaster* and the Role of the Fly in Therapeutic Drug Discovery. *Pharmacological Reviews*, 63(2):411–436.
- Paula, M.T.; Zemolin, A.P.; Vargas, A.P.; Golombieski, R.M.; Loreto, E.L.S.; Saidelles, A.P.; Picoloto, R.S.; Flores, E.M.M.; Pereira, A.B.; Rocha, J.B.T.; Merritt, T.J.S, Franco, J.L. & Posser, T. (2012) Effects of Hg(II) exposure on MAPK phosphorylation and antioxidant system in *D. melanogaster*. *Environmental Toxicology*, doi: 10.1002/tox.21788.
- Pereira, B.K.; Rosa, R.M.; Silva, J.; Guecheva, T.N.; Oliveira, I.M.; Ianistcki, M.; Benvegnú, V.C.; Furtado, G.V.; Ferraz, A.; Richter, M.F.; Schroder, N.; Pereira, A.B. & Henriques, J.A.P. (2009). Protective effects of three extracts from Antarctic plants against ultraviolet radiation in several biological models. *Journal of Photochemistry and Photobiology B: Biology*, 96(2):117–129.
- Tchounwou, P.B.; Ayensu, W.K.; Ninashvili, N. & Sutton, D. (2003). Environmental exposure to mercury and its toxicopathologic implications for public health. *Environmental Toxicology*, 18(3): 149-175.