antioxidants

MDPI

Supplement Material: Rebaudioside A Enhances Resistance to Oxidative Stress and Extends Lifespan and Healthspan in *Caenorhabditis elegans*

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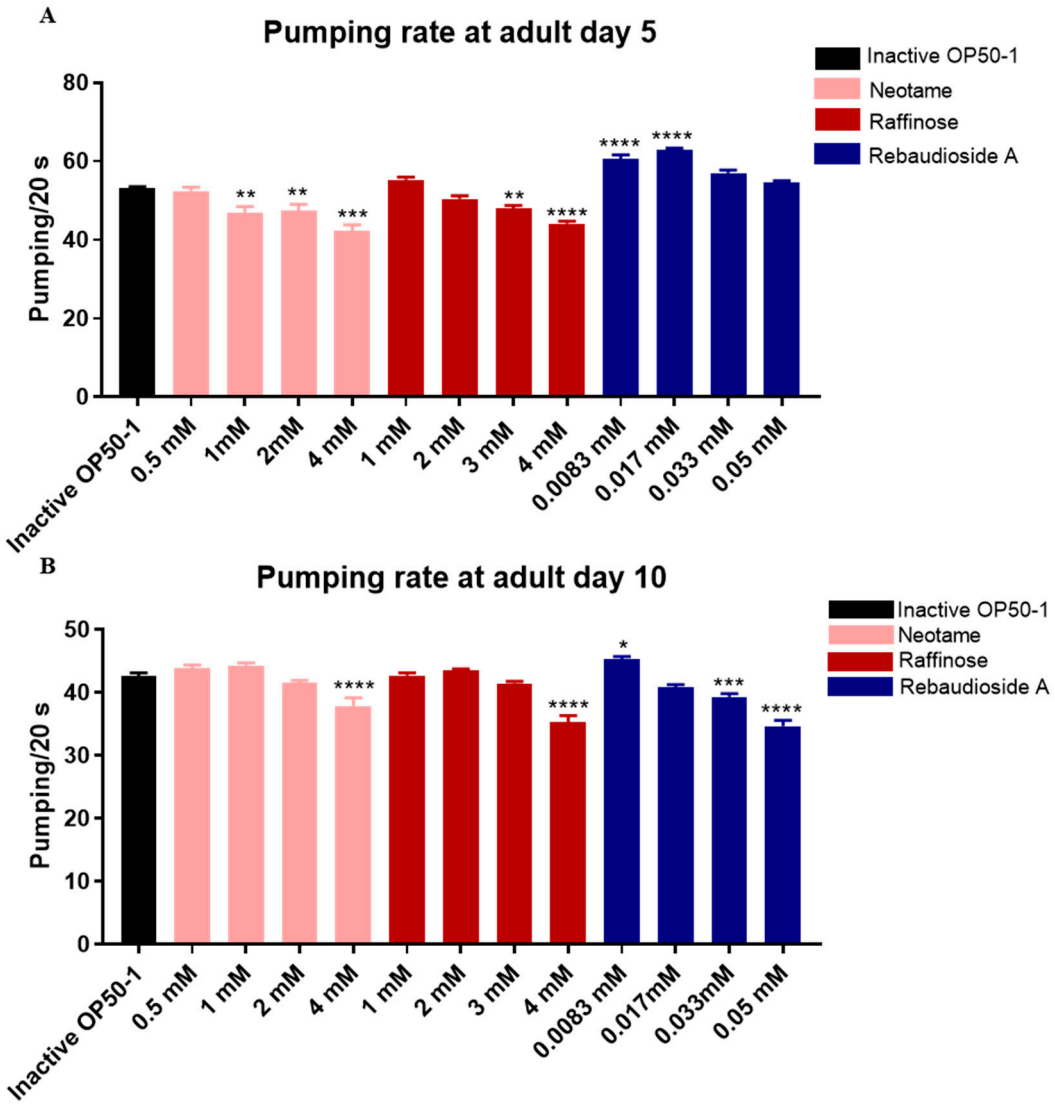
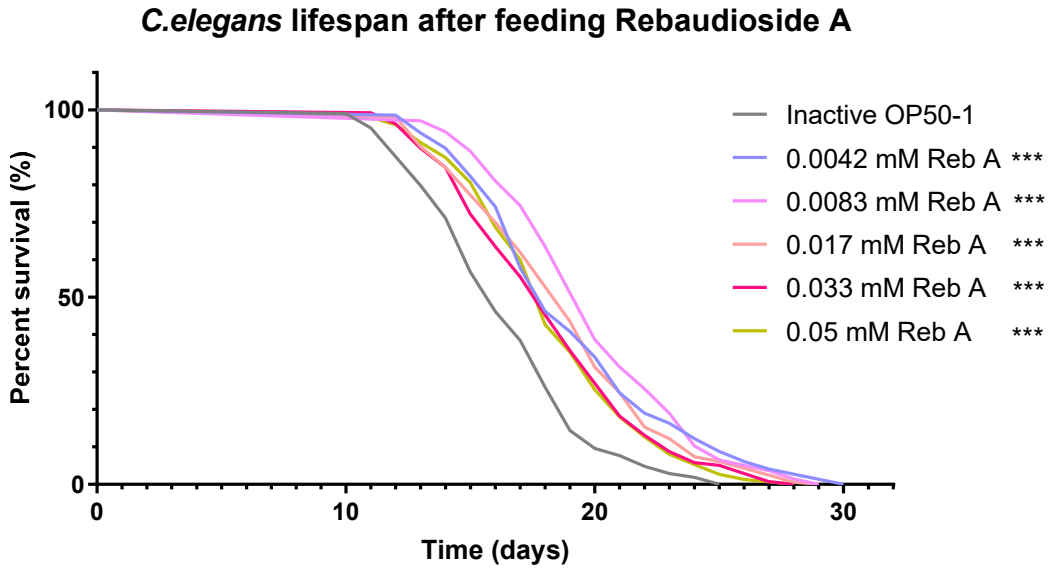
In addition to natural NNS Reb A, we also tested pharyngeal pumping rate after treating worms with artificial NNS neotame and low-caloric oligosaccharides raffinose. Neotame- (1 mM, 2 mM, 4 mM) and raffinose- (3 mM, 4 mM) treated worms showed significantly lower pharyngeal pumping rate in the 5th day adults relative to control groups (Supplement Figure S1A), whereas for the 10th day adults, 4 mM neotame and 4 mM raffinose supplementation significantly reduced pharyngeal pumping rate (Supplement Figure S1B). Therefore, Reb A was subsequently chosen for further investigation of its potential anti-aging effect on *C. elegans.*



Supplement Figure S1. NNSs pumping rate in *C. elegans*. (**A**) Pumping rate in worms (wild-type N2 strain) at the 5th day of adulthood on control NGM, as well as NGM supplemented with designated concentrations of neotame, raffinose and Reb A, respectively. (**B**) Pumping rate in worms (wild-type N2 strain) at the 10th day of adulthood in on control NGM and NGM supplemented with designated concentrations of neotame, raffinose and Reb A. One-way ANOVA with post-hoc Dunnet's test was used for evaluation of statistical significance.

We had set five different concentrations to investigate the effect of Reb A on lifespan extension. While all concentrations tested extended lifespan in *C. elegans* to varying extents, treatment with 0.0083 mM Reb A brought about the greatest expansion in lifespan. Therefore, we adopted the treatment concentration of Reb A at 0.0083 mM for all subsequent experiments.



Supplement Figure S2. The effects of Reb A supplementation on lifespan in *C. elegans*. Survival plot and lifespan assay comparing worms grown on control NGM and that supplemented with varying concentrations of Reb A (0.0042 mM–0.05 mM). Statistical significance was calculated by Log-rank (Mantel-Cox) test, *** $p < 0.001$.