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Hormesis and longevity with tannins: Free of charge or cost-intensive?

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HIGHLIGHTS

- Tannin building blocks can extend the lifespan of *C. elegans.*
- Longevity partly appears in a hormetic manner.
- Each lifespan extension was accompanied by side effects in other life traits.
- Longevity and hormetic benefits might be dearly bought advantages.

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ABSTRACT

Hormetic lifespan extension is, for obvious reasons, beneficial to an individual. But is this effect really cost-neutral? To answer this question, four tannic polyphenols were tested on the nematode *Caenorhabditis elegans*. All were able to extend the lifespan, but only some in a hormetic fashion. Additional life trait variables including stress resistance, reproductive behavior, growth, and physical fitness were observed during the exposure to the most life extending concentrations. These traits represent the quality of life and the population fitness, being the most important parameters of a hormetic treatment besides lifespan. Indeed, it emerged that each life-extension is accompanied by a constraining effect in at least one other endpoint, for example growth, mobility, stress resistance, or reproduction. Thus, in this context, longevity could not be considered to be attained for free and therefore it is likely that other hormetic benefits may also incur cost-intensive and unpredictable side-effects.

1. Introduction

Plant secondary metabolites (PSMs), such as tannins and other polyphenols, are not metabolic waste products or evolutionary remnants without current function, nor are they merely metabolic end products that are toxic to plants and are therefore stored away in vacuoles; rather they are produced to protect plants against abiotic stressors as well as against herbivores (Close and McArthur, 2002). To fulfill their deterrent function, PSMs must possess a toxic potential; they are natural xenobiotics and induce a variety of stress defense responses (Rattan, 2012; Demirovic and Rattan, 2013). Due to the long period of co-existence between PSMs and herbivores, the latter have developed biochemical and molecular biological strategies that aim to convert the adverse stress to benefit their individual integrity and health. Consequently, there is still no concrete consensus whether PSMs cause adverse or beneficial effects on the consuming animals (Singleton, 1981; Koleckar et al., 2008). This seemingly contradiction may be due to the chemical nature of the PSMs and, in particular, on the concentrations/dosages applied. Often, low concentrations/dosages appear to stimulate, whereas high concentrations/dosages are toxic. This dose–response is termed "hormesis".

Hormesis is often described as a stimulatory effect in response to (minor) stress exposure and is characterized by a U-shaped or rather J-shaped dose–response curve (Calabrese and Baldwin, 2001; Douglas, 2008). Moreover, it is considered to be a universal effect, which is independent of stressor, endpoint or organism (Calabrese, 2010). Hormesis is surprisingly omnipresent, a notion that is supported by numerous reports covering a wide range of organisms (from microbes, plants to mammals) including diverse chemical, biological, and physical stressors, as well as different endpoints (such as lifespan, growth, and reproduction). In fact, the concept of hormesis is an inherent part for the aging research and for longevity interventions and its relevance is nicely summarized in Calabrese et al. (2012). But it remains insufficiently understood how the hormetic modulation of one endpoint affects another endpoint within the same organism. Is hormetic stimulation linked to a "cost" or does it induce detrimental side effects? It has been hypothesized that the energy-intensive prolongation of lifespan negatively affects other life-parameters. This hypothesis

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is supported by Forbes (2000), who states that hormesis may not stimulate simultaneously and equally all life-history traits.

From an ecological perspective, true hormetic response stimulations elicited by biological, chemical, or physical stressors must lead to an increase in population fitness. In nature, however, one hormetic life trait response often takes place at the expense of another life trait variable (LeBlanc et al., 2000; Weltje et al., 2005). The embryotoxicity and reproductive toxicity reported in these papers represent rather severe adverse effects. However, we are convinced that more subtle adverse manifestations occur and that the single, unambiguous, streamlined definition of hormesis as "... a dose–response relationship for a single endpoint that is characterized by reversal of response between low and high doses of... stressors" (Kendig et al., 2010) is a zero-sum game and does not meet ecological requirements.

To uncover the potential cost of hormetic stimulation, we selected tannic acid (TA), ellagic acid (EA), gallic acid (GA) and catechin (CT), four polyphenolic tannin building blocks collectively referred to as tannins. They are abundant secondary metabolites in plants and components of humic substances (Leenheer, 2009). Since these polyphenols are known to exert stimulatory (Chung et al., 1998; Buzzini et al., 2008; Koleckar et al., 2008) as well as detrimental effects on organisms (Arhelger et al., 1965; Singleton, 1981; Zhu and Filippich, 1992; Wauters et al., 2001), the existence of a hormetic mode of action seems plausible. We investigated changes in lifespan upon chronic tannin exposure using the nematode *Caenorhabditis elegans.* This popular invertebrate model organism enables the rapid study of bioassays and numerous hormetic effects have, in the past, been reported (Cypser and Johnson, 2002; Cypser et al., 2006).

The aim of this study was not restricted to defining the hormetic life-extending concentrations of polyphenols, but rather to explore the cost of hormesis and longevity. Therefore, other fitness parameters were assessed at the life-extending dose, including stress resistance, growth and reproduction.

2. Materials and methods

The detailed descriptions of the methods are described in (Saul et al., 2010, 2011), thus only a short overview of the experimental setup is given here. The *C. elegans* wild-type strain N2 (var. Bristol) was cultivated at 20 °C according to (Brenner, 1974), maintained on nematode growth medium (NGM) and fed with the *Escherichia coli* strain OP50. The worms, as well as the bacteria, were obtained from the Caenorhabditis Genetics Centre (University of Minnesota). The tannins (Sigma–Aldrich, Taufkirchen, Germany) or only the solvent DMSO (Applichem, Darmstadt, Germany) for the control plates, respectively, were added to the NGM and the OP50 bacteria.

For all assays, except for the initial reproduction trials, pre-exposed L4 larvae were selected and frequently transferred to fresh plates. At the 6th day of adulthood, the nematodes were moved to 35 °C or 0.8 mM H_2O_2 (thermal and oxidative stress resistance test, respectively) for 8 h and the surviving fraction was determined thereafter. For the body length assessment, heat-killed worms were sized under a microscope. For the pharyngeal activity measurement, the pumping frequency was determined in 15 s intervals. To determine the lifespan, dead and surviving animals were counted daily, starting with the first day of adulthood until all animals were dead. In order to count the total offspring of each single nematode, L4 larvae were transferred individually to fresh plates each day until reproduction was completed. The offspring of each worm was summated. For the mobility assay, the nematodes were transferred for 25 and 30 s to fresh plates at the 6th and 9th day of adulthood, respectively. The animals were removed and only straight crawler lanes were measured. To determine the initial reproductive capacity, nematodes were synchronized and the offspring per nematode was counted 85 h after the egg stage.

Statistical significance in the lifespan trials was calculated by means of a log-rank test (Bioinformatics at the Walter and Eliza Hall Institute of Medical Research; http://bioinf.wehi.edu.au/software/russell/logrank). The chi-square test was applied for the stress resistance experiments, and one-way ANOVA for all remaining tests (SigmaStat 3.5; SPSS Inc., Chicago, IL). At least two trials were conducted for all measurements.

3. Results and discussion

The polyphenols TA, EA, GA, and CT were tested (over a dose range of five to six concentrations) for their ability to elicit longevity in the nematodes. As seen in Table 1, each tannin was able to significantly extend the mean lifespan in at least one concentration (EA), four concentrations (TA and GA) or all concentrations (CT). Furthermore, life-shortening properties were observed at higher concentrations of TA and EA. The comparative concentration-responses are shown in Fig. 1, which offers an overview of the relationship between tannin concentration and mean lifespan. GA and CT exposure resulted in a relatively stable life-extension with no apparent concentration-response effect, however inverted "J" curves were observed for TA and EA, with a clear toxic potential at higher concentrations. TA was notably the most potent elicitor of longevity. More detailed data regarding the lifespan trials can be found in (Saul et al., 2009, 2010, 2011).

Regarding the previously stated hypothesis that hormesis is independent of the organism, the endpoint and the stressor, the data presented here suggests that at least the latter (the stressor) is not universally applicable. Indeed, whilst TA and EA displayed classical hormetic patterns, concentration dependency was not observed with the CT-mediated longevity. In addition, also GA might not be a convincing hormetic substance. The highest tested concentration of GA was not able to extend the lifespan in *C. elegans*, and it is likely that higher concentrations may result in toxic effects. However, compared to TA and EA, the hormetic effect was weak, possibly even negligible.

More interesting perhaps is the investigation into the "cost" and knock on effects of hormesis. In the context of this paper one may argue that the quality of life is equally, possibly more, important than pure longevity. Thus, a body-fitness test was conducted (Fig. 2) to explore whether the nematode's mobility changes during the exposure to a life-extending concentration of tannins (100 μM TA, 50 μM EA, 300 μM GA, and 200 μM CT). Moreover, to differentiate between direct and indirect effects, the mobility was also determined following the transfer of previously exposed nematodes to control plates (defined as "/0" in the legend of Fig. 2). All conditions resulted in an equal or enhanced locomotory behavior, with the exception of TA. Worms exposed to TA-spiked bacteria moved more slowly, an effect that was lost, even reversed, once returned to control plates. It is conceivable that it is not the animal that suffers from reduced mobility, but rather is the consequence of the tannin-containing bacteria being more viscous than control bacteria, thereby affecting the movement. If fitness was impaired due to a change in physical properties or quality of the environment, then this should not be considered a cost of hormesis.

Nevertheless, Fig. 3 reveals more convincing evidence regarding the real cost of hormetic longevity. Although the pharynx pumping frequency, the thermal stress resistance, and the total reproductive capacity were impervious to tannin exposure, the initial reproduction was significantly delayed (for TA, EA, and GA) (Saul et al., 2011). Hercus et al. (2003) previously described the inhibiting effect of heat-stress induced hormesis in *Drosophila melanogaster*